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(54) **Title:** METHODS FOR SELECTIVELY MODULATING THE ACTIVITY OF DISTINCT SUBTYPES OF CELLS

(57) **Abstract:** The present invention relates to pseudotyped retrovirus-like particles or retroviral vectors comprising both engineered envelope glycoproteins derived from a virus of the Paramyxoviridae family fused to a cell targeting domain and fused to a functional domain. The present invention also relates to the use of said pseudotyped retrovirus-like particles or retroviral vectors to selectively modulate the activity of specific subsets of cells, in particular of specific immune cells. These pseudotyped retrovirus-like particles or retroviral vectors are particularly useful for gene therapy, immune therapy and/or vaccination.

WO 2017/182585 A1

METHODS FOR SELECTIVELY MODULATING THE ACTIVITY OF DISTINCT SUBTYPES OF CELLS

Field of the invention

The present invention relates to methods for selectively modulating the activity of distinct subtypes of cells.

Background

Cells of the immune system are involved in many types of pathologies. Enhancing or reducing their activity is therefore in focus of many therapeutic strategies. Many types of immune cells with very distinct functions can be distinguished by the expression of certain cell surface proteins. There is so far no technology available that allows the selective activation or deactivation of only a distinct cell subtype, especially *in vivo*.

The use of cytokines to induce or promote the generation of a desirable immune response is an attractive approach in immune therapy against cancer. Cytokines are typically used as unspecific auxiliary substances to support other immune therapies or are administered additionally to chemotherapy. But up to now, the cytokine-based treatments are rarely used because of unsolved systemic toxicity. Besides, cytokines are usually applied systemically, thereby hitting all cell types expressing the relevant cytokine receptor. A fine tuned modulation at the disease relevant site is therefore not possible.

To overcome this drawback, cytokines may be fused to an anti-tumor antibody or connected to micro- or nano-particles with an anti-tumor antibody, thereby targeting the cytokines to the tumor and reducing the systemic side effects. However, these fusion proteins or cytokine-antibody loaded particles are less stable and cleared fast *in vivo*, leading to a relative low cytokine concentration at the disease sites. In addition, the release of the cytokines by cytokine-antibody loaded particles is dependent on pH. Moreover, cytokines (such as IL-2) delivered to the tumor by these previous approaches non-selectively stimulate various types of different immune cells in the complicated tumor microenvironment, including both immune effective and suppressive cells, thus limiting their applications for cancer treatment.

With respect to gene therapy, important target cells such as resting human T cells, B cells and HSCs (hematopoietic stem cells) are refractory to transduction with lentiviral vectors. The transduction mediated by conventional VSV-LV occurs only when T cells have been activated. In the gene therapy trials performed to date, T cells have been activated via cognate antigen receptors, which usually induce phenotypic and functional changes in T cells and eventually lead to a reduced T cell persistence and anti-tumor effect *in vivo*.

To promote gene transfer in resting T cells, several chimeric lentiviral vectors have been generated. For example, Verhoeyen et al. (*Blood, 2003, vol.101, n°6*) disclose HIV-derived vectors pseudotyped with two types of envelope glycoproteins: a chimeric MLV (*Murine Leukemia Virus*) envelope glycoprotein (gp) N-terminally fused to IL-7 and VSV-G (*Vesicular Stomatitis Virus glycoprotein*). These IL-7 vector particles can efficiently transduce resting T cells and induce T cell activation, but equally transduce CD4+ and CD8+ T cells. Thus, they cannot discriminate between the different T cells subsets. Moreover, the fact that a non-targeted envelope gp, such as VSV-G gp, is needed will possibly allow transduction of unwanted hematopoietic or endothelial cells. One exception is measles virus (MeV) glycoprotein pseudotyped lentiviral vectors (MV-LV). MV-LV can mediate the transduction of resting T lymphocytes without changing the G0/G1a cell cycle status. Zhou et al. *(J Immunol, 2015, vol. 195, n°5)* also disclose that CD4-targeted LV can transduce freshly isolated resting T cells, but with very low efficiency (less than 10%) at high particle dose.

Finally, current strategies for generating less differentiated tumor specific T cells for adoptive T cell therapy rely on optimizing the T cell stimulation and cultivation protocol. For example, to produce more T_{SCM} cells (*Stem memory T cells*) or T_{CM} cells (*Central memory T cells*), combinations of IL-15 and IL-7 or IL-21 and IL-7 are used for T cell stimulation and expansion. This cultivation system is very expensive since routine cytokine re-supplement (every two days) is required. Afterwards, several cell sorting steps are usually implemented to obtain the desirable cell types.

There is therefore still a need to provide methods for selectively and efficiently modulating the activity of distinct subtypes of immune cells.

Description of the invention

The Inventors have surprisingly found that it is possible to selectively modulate the activity of distinct subtypes of immune cells by using pseudotyped retrovirus-like particles or retroviral vectors (for example lentivirus-like particles (VLP) or lentiviral vectors (LV)) carrying both a cell-specific targeting domain (for example specific for CD4+ T cells or for CD8+ T cells) and a functional domain, for example a cytokine, that are each fused to a glycoprotein of a virus of the Paramyxoviridae family. Said retroviral vectors also specifically and efficiently deliver a packaged gene into the targeted cells.

As a matter of fact, it was unexpectedly found that combining a functional domain with a cell-specific targeting domain on pseudotyped retrovirus-like particles or retroviral vectors greatly improved the selective activity modulation of the targeted subtypes of immune cells by comparison to the corresponding particles or vectors comprising only the cell-specific targeting domain.

For example, the Inventors have shown that stimulatory cytokine-displaying T cell targeted particles selectively activate the targeted T cell subsets in cultures of the mixed cell types and *in vivo* in a human blood system mouse model and also deliver the packaged gene into the targeted T cell subtype, without any need for stimulatory culture conditions. IL-7 (Interleukin-7)-displaying CD4-targeted particles based on MeV (measles virus) glycoproteins (4^H/IL7^H-VLP) indeed specifically activate and promote the survival of cultivated primary CD4+ cells, whereas IL7-displaying CD8-targeted particles based on NiV (Nipah virus) glycoproteins (8^G/IL7^G-VLP) selectively activate and promote the survival of CD8+ T cells. Besides, 4^H/IL7^H-LV efficiently and specifically delivers GFP transgenes into CD4+ T cells in the cell mixture, in a dose-dependent manner. Compared to the parental CD4-targeted LV (not co-displaying IL-7), 4^H/IL7^H-LV is more efficient in delivering the therapeutic ErbB2CAR transgene selectively into resting CD4+ T cells. Similar exclusive gene transfer and stimulation of CD8+ T cells has also been observed with the CD8/IL-7 co-displaying NiV glycoprotein pseudotyped lentiviral vectors (8^G/IL7^G-LV). Indeed, 8G/IL7G-LV is more efficient in selective CD8+ T cell activation and targeted gene delivery than 8^G-LV (*see examples*).

The retrovirus-like particle and retroviral vector according to the invention present many advantages, such as:

- providing a high local concentration of cytokines at the cell target sites, thereby improving the efficiency and preventing severe side-effects of cytokine therapy,
- providing a more stable and constant stimulation of the target cells compared to soluble cytokines, thereby improving the efficiency of cytokine therapy,
- providing a very flexible system allowing to combine different types of cytokines and different types of targeting domains on a single particle or retroviral vector,
- allowing coupling of cell stimulation with cell-specific gene transfer *in vitro* or *in vivo*, which results in a more efficient transduction of the specific resting T cell subsets,
- allowing coupling of a T cell phenotype induction with gene transfer *in vitro* or *in vivo*, due to the interaction with the displayed cytokines on the particle or vector surface, the differentiation of the transduced cells being controlled,

and more generally:

- allowing delivery of biological material and/or therapeutic drug(s), including but not limited to gene, mRNA, shRNA, microRNA, peptide, protein, protein fragment and theirs combinations to specific cells or subset of cells, *in vitro* or *in vivo*,
- allowing an easier cell manufacturing process (for example T cell or hematopoietic cell manufacturing process) for adoptive therapy,
- preventing problems of resistance *in vivo* when using retrovirus-like particle or retroviral vector pseudotyped with glycoproteins from Nipah virus, thanks to low pre-existing antibodies in human beings.

The present invention thus relates to a pseudotyped retrovirus-like particle or retroviral vector comprising:

a) at least one cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G or H and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H, or a transmembrane domain and (ii) at least one cell targeting domain,
b) at least one modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G or H and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H, or a transmembrane domain and (ii) at least one functional domain, and
c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family and preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein F,

wherein said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

Said virus of the Paramyxoviridae family may be a virus of the *Morbillivirus* genus, for example selected in the group consisting of measles virus (MeV), canine distemper virus, Cetacean morbillivirus, Peste-des-petits-ruminants virus, Phocine distemper virus, and Rinderpest virus, or a virus of the *Henipavirus* genus, for example selected in the group consisting of Nipah virus (NiV), Cedar virus and Hendra virus.

The protein of a) and/or b) preferably comprises at least two mutations by comparison to the sequence of said envelope glycoprotein G or H, said at least two mutations resulting in the at least partially inability to bind at least one natural receptor of said envelope glycoprotein G or H.

Said cell targeting domain may be selected in the group consisting of a DARPin, a scFv, targeting peptide and their combinations and/or said functional domain may be selected in the group consisting of a cytokine, growth factor, hormone, neurotransmitter, apoptosis ligand and their combinations.

The target cells may be selected in the group consisting of cells of the hematopoietic system (including T cells, B cells, monocytes, Th1 cells, Th2 cells, Treg cells, mast cells, dendritic cells (DCs), natural killer (NK) cells, natural killer T (NKT) cells, macrophages, hematopoietic stem cells, progenitor T and/or B cells, erythroblasts, platelets and/or neutrophils), stroma cells, endothelial cells, liver cells, muscle cells, cells of the nervous system, diseased cells and their combinations.

The present invention also relates to the use of a pseudotyped retrovirus-like particle or retroviral vector as defined above, to selectively modulate the activity of target cells and/or selectively transduce target cells, the target cells being for example as defined above.

The present invention also relates to a method for selectively modulating the activity of target cells and/or selectively transducing target cells, wherein said method comprises contacting a pseudotyped retrovirus-like particle or retroviral vector as defined above with cells comprising said target cells, the target cells being for example as defined above.

The present invention also relates to a pseudotyped retrovirus-like particle or retroviral vector as defined above for use as a medicament, preferably in immune therapy gene therapy and/or vaccination, for example in the prevention and/or treatment of an immune disease (for example an auto-immune disease), cancer, genetic disease, allergic disease, inflammatory disease, infectious disease, metabolic disease, neurological disease (for example, neural dystrophy, Parkinson disease, Huntington disease, Alzheimer disease), muscular disease and their combinations.

The present invention also relates to a nucleic acid comprising a sequence encoding a cell targeting fusion protein and/or a sequence encoding a modulating fusion protein, wherein:

- said cell targeting fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain, and
- said modulating fusion protein comprises (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain.

The present invention also relates to a vector comprising a nucleic acid as defined above.

The present invention also relates to a method for producing a pseudotyped retrovirus-like particle or retroviral vector as defined above, wherein said method comprises co-transfecting a packaging cell line with:

(i) at least one nucleic acid encoding a cell targeting fusion protein, said cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain,

(ii) at least one nucleic acid encoding a modulating fusion protein, said modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain,

(iii) at least one nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family,

(iv) at least one vector comprising a nucleic acid encoding core proteins from said retrovirus and

(v) optionally, at least one vector comprising a packaging competent retroviral derived genome,

wherein said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

<u>Pseudotyped retrovirus-like particle or retroviral vector</u>

A “retrovirus-like particle” as used herein refers to a particle comprising retroviral proteins Gag, Pol and Env (envelope), but not comprising any retrovirus derived genetic information.

The Gag, Pol and Env proteins are derived from the retrovirus and are provided in trans by means of a packaging cell line.

As used herein, a “retroviral vector” comprises the Gag, Pol and Env (Envelope) proteins and a RNA molecule. Said RNA molecule does not contain any gag, env or pol gene, but comprises the psi element and LTRs which are required for an effective packaging of the RNA molecule into the resulting particles. Said RNA molecule may further comprise a gene of interest that is under the control of a suitable promoter and is thus expressed upon integration of said gene into the genome of the host or target cell.

A retrovirus-like particle or retroviral vector is a virus-like particle or viral vector, respectively, whose core proteins, i.e. the proteins encoded by the Gag and Pol genes, are derived from a retrovirus.

By the term “retrovirus”, it is herein meant a virus whose genome consists of a RNA molecule and that comprises a reverse-transcriptase.

A retrovirus is a member of the *Retroviridae* family. The retrovirus may be an Oncovirus, Lentivirus or Spumavirus.

The oncovirus may be an alpharetrovirus, a betaretrovirus, a deltaretrovirus, an epsilonretrovirus or a gammaretrovirus.

When the retrovirus is an oncovirus, said retrovirus may be MLV (Murine leukemia virus), ASV (Avian sarcoma virus), Feline leukemia virus, Bovine leukemia virus, RSV (Rous sarcoma virus), MPMV (Mason-Pfizer monkey virus), HTLV I (Human T-cell leukemia virus-I) or HTLV II (Human T-cell leukemia virus-II).

When the retrovirus is a lentivirus, said retrovirus may be HIV (Human Immunodeficiency Virus), preferably HIV-1 or HIV-2, SIV (Simian Immunodeficiency Virus), EIAV (Equine Infectious Anemia Virus), FIV (Feline Immunodeficiency Virus) or CAEV (Caprine Arthritis Encephalitis Virus).

When the retrovirus is a spumavirus, said retrovirus may be HFV (Human Foamy Virus).

For example, the retroviral vector may be a lentiviral vector, an alpha retroviral vector, a murine retroviral vector or a FIV vector.

Genomes of such retroviruses are readily available in gene databases.

In a preferred embodiment, said retrovirus is a lentivirus, more preferably HIV, such as HIV-1 or HIV-2.

The present invention thus preferably relates to a lentivirus like particle (LVP) or a lentiviral vector (LV), preferably a HIV-derived LVP or LV.

The term “pseudotyped” in the expression “pseudotyped retrovirus-like particle or retroviral vector” herein means that the retrovirus-like particle or retroviral vector bears envelope glycoproteins that are derived from at least another virus than said retrovirus and/or that are engineered envelope glycoproteins, for example chimeric and/or mutated envelope glycoproteins.

The retrovirus-like particle or retroviral vector according to the invention is for example pseudotyped with engineered glycoproteins derived from a virus of the Paramyxoviridae family, preferably from a virus of the *Morbillivirus* genus or of the *Henipavirus* genus glycoproteins, as detailed below.

Envelope glycoproteins of the Paramyxoviridae family

The modified envelope glycoproteins used for pseudotyping the retrovirus-like particle or retroviral vector according to the invention are derived from envelope glycoproteins of a virus of the Paramyxoviridae family.

The virus of the Paramyxoviridae family is preferably a virus of the *Morbillivirus* genus or of the *Henipavirus* genus.

The viruses of the *Morbillivirus* genus and of the *Henipavirus* genus use two types of glycoproteins to enter into a target cell: an attachment protein (called glycoprotein G in a virus of the *Henipavirus* genus or glycoprotein H in a virus of the *Morbillivirus* genus) and a glycoprotein F (also called fusion protein or protein F). The protein F mediates the fusion of viral membranes with the cellular membranes of the host cell. The glycoprotein G/H recognizes the receptor on the target membrane and supports the F protein in its membrane fusion function. Both glycoprotein G/H and glycoprotein F are used in a modified form for pseudotyping the retrovirus-like particle or retroviral vector according to the invention.

A virus of the *Morbillivirus* genus is for example selected in the group consisting of measles virus, Canine distemper virus, Cetacean morbillivirus, Peste-des-petits-ruminants virus, Phocine distemper virus and Rinderpest virus.

A preferred virus of the *Morbillivirus* genus is a measles virus (MeV).

A virus of the *Henipavirus* genus is for example selected in the group consisting of Nipah virus, Cedar virus and Hendra virus.

A preferred virus of the *Henipavirus* genus is a Nipah virus (NiV).

In a preferred embodiment, the modified enveloped glycoproteins are derived from the envelope glycoprotein H and the glycoprotein F of a measles virus or from the envelope glycoprotein G and the glycoprotein F of a Nipah virus.

An example of sequence of Nipah virus envelope glycoprotein G is sequence SEQ ID NO: 9.

An example of sequence of Nipah virus envelope glycoprotein F is sequence SEQ ID NO: 11.

An example of sequence of measles virus envelope glycoprotein H (called glycoprotein H) is sequence SEQ ID NO: 10.

An example of sequence of measles virus envelope glycoprotein F is sequence SEQ ID NO: 12.

Protein derived from an envelope glycoprotein G/H

The pseudotyped retrovirus-like particle or retroviral vector according to the invention comprises at least one cell targeting fusion protein and at least one modulating fusion protein. The cell targeting fusion protein and/or the modulating fusion protein comprise a first protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

The envelope glycoprotein G or H is as defined above in the section “Envelope glycoproteins of the Paramyxoviridae family”.

The virus of the Paramyxoviridae family is as defined above in the section “Pseudotyped retrovirus-like particle or retroviral vector”.

By the expression “protein derived from an envelope glycoprotein G or H”, it is herein meant that the protein comprises at least one modification by comparison to the sequence of the envelope glycoprotein G or H.

A preferred envelope glycoprotein G is a Nipah virus envelope glycoprotein G, also referred to as NiV envelope glycoprotein G, NiV G glycoprotein, or NiV-G.

A preferred envelope glycoprotein H is a measles virus envelope glycoprotein H, also referred to as MeV glycoprotein H, MeV hemagglutinin, or MV-H.

The envelope glycoprotein G or H is a wild-type or vaccine strain virus envelope glycoprotein or a variant thereof, provided that said variant retains the capacity of said wild-type or vaccine strain glycoprotein of recognizing the receptor on the target membrane and to support the F protein in its membrane fusion function.

A reference sequence for a NiV envelope glycoprotein G is sequence SEQ ID NO: 9.

The NiV envelope glycoprotein G may be of a sequence at least at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 9. For example, the NiV envelope glycoprotein may be of sequence SEQ ID NO: 9.

A reference sequence for MeV hemagglutinin is sequence SEQ ID NO: 10.

The MeV hemagglutinin may be of a sequence at least at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 10. For example, the MeV hemagglutinin may be of sequence SEQ ID NO: 10.

In a preferred embodiment, the protein derived from the envelope glycoprotein G lacks at least one part of the cytoplasmic region of said envelope glycoprotein G.

The protein lacking at least one part of the cytoplasmic region of said envelope glycoprotein G is also referred to as a protein truncated in its cytoplasmic region.

The expressions “protein lacking x amino acids of the cytoplasmic region”, “protein truncated by x amino acids in its cytoplasmic region” and “protein Δcx” are herein synonymous and may be used interchangeably.

Using an envelope glycoprotein G truncated in its cytoplasmic region greatly improves its incorporation into retrovirus-like particles and retroviral vectors, thereby allowing generating pseudotyped retrovirus-like particles or retroviral vectors with higher titers and production yield.

The cytoplasmic region of the envelope glycoprotein G is located at the N-terminus.

Thus, when ascertaining the location of the truncated part of a glycoprotein Δcx by reference to the non-truncated glycoprotein G, one begins counting at the second amino acid residue of the N-terminal end of the envelope glycoprotein G, i.e. omitting the first methinonine residue.

As an example, a protein that lacks X amino acids in the cytoplasmic region of the glycoprotein G of sequence SEQ ID NO: Z differs from said glycoprotein G in that it lacks amino acids 2 to 1+X of sequence SEQ ID NO: Z.

The localisation of the cytoplasmic region in the envelope glycoprotein G sequence can be easily determined by the skilled person.

The cytoplasmic region of the MeV H glycoprotein for example consists of the amino acids 1 to 34 of sequence SEQ ID NO: 10.

The cytoplasmic region of the NiV G glycoprotein for example consists of the amino acids 1 to 45 of sequence SEQ ID NO: 9.

For example, the protein derived from the envelope glycoprotein G or H may lack at least 10 amino acids, at least 15 amino acids, at least 18 amino acids, at least 20 amino acids in the cytoplasmic region.

For example, the protein derived from the measles virus envelope glycoprotein H may lack at least 10 amino acids, at least 15 amino acids, at least 18 amino acids, at least 20 amino acids, at least 24 amino acids in the cytoplasmic region. In a preferred embodiment, the protein derived from the measles virus envelope glycoprotein H lacks 15, 18, 20 or 24 amino acids.

For example, the protein derived from the Nipah virus envelope glycoprotein G may lack at least 10 amino acids, at least 15 amino acids, at least 18 amino acids, at least 20 amino acids, at least 25 amino acids or at least 30 amino acids in the cytoplasmic region.

In a preferred embodiment, the protein derived from the Nipah virus envelope glycoprotein G lacks 34 amino acids.

In a preferred embodiment, the protein derived from the envelope glycoprotein G or H is at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H.

The at least partially inability to bind at least one natural receptor of said envelope glycoprotein G or H may be obtained by at least one mutation introduced in the sequence of said envelope glycoprotein G or H .

For example, the protein derived from the envelope glycoprotein G or H may comprise at least one point mutation, preferably at least two point mutations, by comparison to the sequence of said envelope glycoprotein.

The point mutation may be an amino acid deletion, addition or substitution.

In a preferred embodiment, the point mutation is a substitution.

In a preferred embodiment, the protein derived from the envelope glycoprotein G or H is unable to bind, ie completely unable to bind, at least one natural receptor of said envelope glycoprotein G or H.

The inability of the protein derived from the envelope glycoprotein G or H to bind to its natural receptor greatly increases cell targeting efficiency.

Assessing the ability to bind to at least one natural receptor of an envelope glycoprotein G may be assessed by any method well known by the skilled person.

The natural NiV receptors are Ephrin-B2 and -B3 NiV receptors.

The protein derived from the NiV envelope glycoprotein G is thus preferably at least partially unable to bind Ephrin-B2 receptor and/or Ephrin-B3 NiV receptor, preferably both Ephrin-B2 receptor and Ephrin-B3 NiV receptor.

For example, the envelope glycoprotein NiV-G may comprise at least two or at least three point mutations by comparison to sequence SEQ ID NO: 9, wherein said point mutations are selected in the group consisting of E501A, W504A, Q530A and E533A.

In a preferred embodiment, the glycoprotein NiV-G comprises or consists of the point mutations E501A, W504A, Q530A and E533A, thus leading to the inability to bind Ephrin-B2 receptor and Ephrin-B3 NiV receptor.

The natural MeV receptors are SLAM, nectin-4 and CD46.

The protein derived from the MeV envelope glycoprotein H is thus preferably at least partially unable to bind SLAM, nectin-4 and/or CD46, preferably at least both SLAM and CD46.

For example, the envelope glycoprotein MV-H may comprise at least two or at least three point mutations by comparison to sequence SEQ ID NO: 10, wherein said point mutations are selected in the group consisting of Y481A, R533A, S548L and F549S.

In one preferred embodiment, the envelope glycoprotein MV-H comprises or consists of the point mutations Y481A, R533A, S548L and F549S, thus leading to the inability to bind SLAM and CD46.

Thus, the protein derived from the envelope glycoprotein G or H preferably lacks at least one part of the cytoplasmic region of said envelope glycoprotein G or H and/or is at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H.

In a more preferred embodiment, the protein derived from the envelope glycoprotein G or H both lacks at least one part of the cytoplasmic region of said envelope glycoprotein G or H and is at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H.

By “a sequence at least x% identical to a reference sequence”, it is herein intended that the sequence is identical to the reference sequence or differ from the reference sequence by up to 100-x amino acid alterations per each 100 amino acids of the reference sequence.

The alignment and the determination of the percentage of identity may be carried out manually or automatically using for instance the Needle program which is based on the Needleman and Wunsch algorithm, described in Needleman and Wunsch (1970) J. Mol Biol. 48:443-453, with for example the following parameters for polypeptide sequence comparison: comparison matrix: BLOSUM62, gap open penalty: 10 and gap extend penalty: 0.5, end gap penalty: false, end gap open penalty = 10, end gap extend penalty = 0.5; and the following parameters for polynucleotide sequence comparison: comparison matrix: DNAFULL; gap open penalty = 10, gap extend penalty = 0.5, end gap penalty: false, end gap open penalty = 10, end gap extend penalty = 0.5.

As defined herein, an amino acid sequence “at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical” to a reference sequence may comprise mutations, such as deletions, insertions and/or substitutions compared to the reference sequence.

In case of substitutions, the substitution preferably corresponds to a conservative substitution as indicated in the Table 1 below. In a preferred embodiment, the sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference sequence only differs from the reference sequence by conservative substitutions.

Table 1

Conservative substitutions	Type of Amino Acid
Ala, Val, Leu, Ile, Met, Pro, Phe, Trp	Amino acids with aliphatic hydrophobic side chains
Ser, Tyr, Asn, Gln, Cys	Amino acids with uncharged but polar side chains
Asp, Glu	Amino acids with acidic side chains
Lys, Arg, His	Amino acids with basic side chains
Gly	Neutral side chain

In another preferred embodiment, the amino acid sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference sequence corresponds to a naturally-occurring allelic variant of the reference sequence.

Glycoprotein derived from an envelope glycoprotein F

The pseudotyped retrovirus-like particle or retroviral vector according to the invention also comprises at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family, wherein said glycoprotein preferably lacks at least one part of the cytoplasmic region of said envelope glycoprotein F.

The envelope glycoprotein F is as defined above in the section “Envelope glycoproteins of the Paramyxoviridae family”.

The virus of the Paramyxoviridae family is as defined above in the section “Pseudotyped retrovirus-like particle or retroviral vector”.

By the expression “protein derived from an envelope glycoprotein F”, it is herein meant that the protein comprises at least one modification by comparison to the sequence of the envelope glycoprotein F.

A preferred envelope glycoprotein F is a Nipah virus envelope glycoprotein F, also referred to as NiV envelope glycoprotein F, NiV F glycoprotein, or NiV-F.

Another preferred envelope glycoprotein F is a measles virus envelope glycoprotein F, also referred to as MeV glycoprotein F or MeV-F.

The envelope glycoprotein F is a wild-type virus or vaccine strain envelope glycoprotein or a variant thereof, provided that said variant retains the capacity of the wild-type or vaccine strain glycoprotein F to mediate the fusion of viral membranes with the cellular membrane of the targeted or host cell.

A reference sequence for a NiV envelope glycoprotein F is sequence SEQ ID NO: 11. The NiV envelope glycoprotein F may be of a sequence at least at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 11. For example, the NiV envelope glycoprotein F may be of sequence SEQ ID NO: 11.

A reference sequence for MeV envelope glycoprotein F is sequence SEQ ID NO: 12.

The MeV envelope glycoprotein F may be of a sequence at least at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to SEQ ID NO: 12. For example, the MeV envelope glycoprotein F may be of sequence SEQ ID NO: 12.

In a preferred embodiment, the protein derived from the envelope glycoprotein F lacks at least one part of the cytoplasmic region of said envelope glycoprotein F.

The protein lacking at least one part of the cytoplasmic region of said envelope glycoprotein F is also referred to as a protein truncated in its cytoplasmic region.

The expressions “protein lacking x amino acids of the cytoplasmic region”, “protein truncated by x amino acids in its cytoplasmic region” and “protein Δcx” are herein synonymous and may be used interchangeably.

Using an envelope glycoprotein F truncated in its cytoplasmic region greatly improves its incorporation into retrovirus-like particles and retroviral vectors, thereby allowing generating pseudotyped retrovirus-like particles or retroviral vectors with higher titers and production yield.

The cytoplasmic region of the envelope glycoprotein F is located at the C-terminus.

Thus, when ascertaining the location of the truncated part of a glycoprotein Δcx by reference to the non-truncated glycoprotein G, one begins counting from the C-terminal end of the glycoprotein F.

As an example, a glycoprotein that lacks X amino acids in its cytoplasmic region by comparison to the glycoprotein F of sequence SEQ ID NO: Z consisting of n amino acids differs from said glycoprotein F in that it lacks amino acids n-x+1 to n of sequence SEQ ID NO: Z.

The localisation of the cytoplasmic region in the envelope glycoprotein F sequence can be easily determined by the skilled person.

The cytoplasmic region of the MeV glycoprotein F for example consists of the amino acids 518 to 550 of sequence SEQ ID NO: 12 (33 amino acids).

The cytoplasmic region of the NiV glycoprotein F for example consists of the amino acids 519 to 546 of sequence SEQ ID NO: 11 (28 amino acids).

For example, the protein derived from the envelope glycoprotein F may lack at least 10 amino acids, at least 15 amino acids, at least 20 amino acids, at least 25 or at least 30 amino acids in the cytoplasmic region.

For example, the protein derived from the measles virus envelope glycoprotein F may lack 30 amino acids in the cytoplasmic region by comparison to said measles virus envelope glycoprotein F. For example, said protein derived from the measles virus envelope glycoprotein F and lacking 30 amino acids in the cytoplasmic region comprises or consists of sequence SEQ ID NO: 15.

For example, the protein derived from the Nipah virus envelope glycoprotein F may lack 22 amino acids in the cytoplasmic region by comparison to said Nipah virus envelope glycoprotein F. For example, said protein derived from the Nipah virus envelope glycoprotein F and lacking 22 amino acids in the cytoplasmic region comprises or consists of sequence SEQ ID NO: 16.

Target cells

The target cells are cells of interest, whose activity is to be modulated and/or that are to be transduced with at least one gene of interest.

The target cells express at their surface a specific cell surface receptor, thereby allowing to specifically target these cells and not the cells that do not express said cell surface receptor.

The expressions “target cells” and “target subset of cells” are herein synonymous and may be used interchangeably.

Target cells may be selected in the group consisting of hematopoietic cells, stroma cells, endothelial cells, liver cells, muscle cells (e.g. heart cells), cells of the nervous system and/or diseased cells.

Cells of the nervous system are for example neurons and/or glial cells.

By “hematopoietic cells”, it is herein meant cells of the hematopoietic system.

Preferred target cells are hematopoietic cells.

Hematopoietic cells may be selected in the group consisting of T cells, B cells, monocytes, Th1 cells, Th2 cells, Treg cells, mast cells, dendritic cells (DCs), natural killer (NK) cells, natural killer T (NKT) cells, macrophages, hematopoietic stem cells, progenitor T and/or B cells, erythroblasts, platelets, neutrophils and their combinations.

More preferred target hematopoietic cells are T cells, more preferably CD8+ T cells or CD4+ T cells.

Diseased cells may be tumor cells, tumor stem cells, cells lacking a specific functional gene, overexpressing a specific gene and/or expressing a truncated or mutated form of a specific gene, infected cells and/or functionally impaired cells.

Cell-specific targeting domain

The cell-specific targeting domain (also called “cell targeting domain”) allows the specific binding of the pseudotyped retrovirus-like particle or retroviral vector according to the invention to a surface receptor selectively expressed by target cells.

Target cells may be as defined above in the section of the same name.

The cell targeting domain of the targeting fusion protein is preferably selected in the group consisting of a DARPin, a scFv, targeting peptide and their combinations.

The term “DARPin” refers to designed ankytin repeat proteins.

The term scFv refers to single-chain variable fragment of an antibody.

For example, the cell targeting domain is specific for CD3, CD8, CD4, a cancer cell marker, CD11b, CD19, CD62L, CD56, Glut-1(glucose transporter), CD19, CD22, CD20, CCR5 or CXCR4.

A preferred cell targeting domain is a DARPin specific for CD4 or a scFv specific for CD8.

Functional domain

The functional domain allows modulating the activity of the target cells.

By “modulating the activity of a target cell”, it is herein meant to activate or inhibit, induce a phenotype change (for example maturation and/or differentiation), induce proliferation, and/or induce apoptosis of said target cell. Modulating the activity of a target cell for example comprises enhancing or suppressing immunity, promoting cell type-specific immune response(s).

The functional domain of the modulating fusion protein is preferably a receptor ligand.

By “receptor ligand”, it is herein meant a molecule, preferably a protein, preferably normally released by cells, and that alters the physiological state of cells expressing a cognate receptor at their cell surface. The receptor ligand is for example selected in the group consisting of a cytokine, growth factor, hormone, neuromediator, apoptosis ligand, a chemokine, glucose transporter and their combinations.

For example, the cytokine may be selected in the group consisting of interleukin (IL) TNF (*Tumor Necrosis Factor*) or interferon.

The interleukin may for example be IL-2, IL-3, IL-6, IL-7, IL15, IL21, IL-17 or IL-12.

For example, the apoptosis ligand may be FAS Ligand, CD40 Ligand or TNFalpha.

For example, the chemokine may be CXCL4, CCL5 or CXCL10.

For example, the growth factor may be GM-SCF (*Granulocyte-Macrophage Colony-Stimulating Factor*), Stem cells factor (SCF), thrombopoietin (TPO) or Flt-3 Ligand.

For example, the hormone may be insulin, growth hormone or a hormone peptide, for example vasopressin. For example, the neuromediator may be a neuropeptide, for example selected among insulin, glucagon, calcitonin, neurotensin or bradykinin.

A preferred functional domain is a cytokine, preferably an interleukin, more preferably IL-7.

<u>Cell targeting and modulating fusion proteins</u>

The retrovirus-like particle and retroviral vector according to the invention comprises two types of fusion proteins: one cell targeting fusion protein and one modulating fusion protein.

The cell targeting fusion protein comprises (i) a protein derived from an envelope glycoprotein G of H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain.

The modulating fusion protein comprises (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain.

It will be clearly understood by the skilled person that, since the cell targeting fusion protein and the modulating fusion protein are two different types of fusion proteins, the cell targeting domain and the functional domain are different.

The transmembrane domain may be any naturally-occurring or non-naturally occurring transmembrane domain.

The transmembrane domain may be a transmembrane domain of a receptor, a transmembrane protein, preferably a viral transmembrane protein, a fragment of a transmembrane protein, a transmembrane peptide or a variant thereof, such as a genetically modified transmembrane domain of a receptor, a genetically modified transmembrane protein, a genetically modified fragment of a transmembrane protein or a genetically modified transmembrane peptide.

Examples of transmembrane domain are the transmembrane domain (TMD) of the platelet-derived growth factor receptor (PDGFR), the transmembrane domain of CD34 or the VSVG glycoprotein transmembrane domain.

The C-terminus of the protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family is preferably fused, directly or indirectly (for example via a linker), to the N-terminus of the cell targeting or functional domain.

The C-terminus of the transmembrane domain is preferably fused, directly or indirectly (for example via a linker), to the N-terminus of the cell targeting or functional domain.

The protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, the glycoprotein derived from an envelope glycoprotein F, the cell targeting domain and the functional domain are as defined above.

When both present in the pseudotyped retrovirus-like particle or retroviral vector, the protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family in the cell targeting fusion protein and those in the modulating fusion protein may be derived from the same virus of the Paramyxoviridae family or from different viruses of the Paramyxoviridae family.

When the protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family in the cell targeting fusion protein and those in the modulating fusion protein are derived from different viruses of the Paramyxoviridae family, they preferably derive from a virus of the same genus, more preferably from a virus of the same species.

The protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family in the cell targeting fusion protein and those in the modulating fusion protein are preferably identical. In a preferred embodiment, the cell targeting fusion protein thus differs from the modulating fusion protein by its targeting domain instead of a functional domain and, possibly, when present, of at least one linker and/or at least one tag.

The two proteins of the fusion protein may be linked together with a linker. Any suitable linker well-known by the skilled person may be used.

For example, the linker may be $(G_4S)_3$, G_4S, Factor Xa cutting site or helical linker (for example HL3, HL7, …).

The fusion proteins may be tagged, for example to facilitate their purification and/or detection.

When present, the tag is preferably located at the C-terminus of the fusion protein, i.e. fused to the N-terminus of the cell targeting domain and/or of the functional domain. Any suitable tag well-known by the skilled person may be used.

For example, the tag may be a His-Tag, RGS-His tag, such as $RGSH_6$, HA tag or c-myc tag.

Specific pseudotyped retrovirus-like particle or retroviral vector

The present invention particularly relates to a pseudotyped retrovirus-like particle or retroviral vector comprising:

a) at least one cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain,
b) at least one modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain, and
c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family,

wherein said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

The pseudotyped retrovirus-like particle or retroviral vector thus comprises at least one fusion protein comprising a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, said fusion protein being a cell targeting fusion protein or a modulating fusion protein. In one embodiment, the pseudotyped retrovirus-like particle or retroviral vector comprises two fusion proteins comprising each a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, wherein one fusion protein is a cell targeting fusion protein and the second fusion protein is a modulating fusion protein.

In one embodiment, the pseudotyped retrovirus-like particle or retroviral vector comprises:

a) at least one cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family and (ii) at least one cell targeting domain,
b) at least one modulating fusion protein comprising (i) a transmembrane domain and (ii) at least one functional domain, and
c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family.

In one embodiment, the pseudotyped retrovirus-like particle or retroviral vector comprises:

a) at least one cell targeting fusion protein comprising (i) a transmembrane domain and (ii) at least one cell targeting domain,
b) at least one modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family and (ii) at least one functional domain, and
c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family.

In one embodiment, the pseudotyped retrovirus-like particle or retroviral vector comprises:

a) at least one cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family and (ii) at least one cell targeting domain,
b) at least one modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family and (ii) at least one functional domain, and
c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family.

The protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family in the cell targeting fusion protein, those in the modulating fusion protein and the glycoprotein derived from an envelope glycoprotein F may derived from the same virus of the Paramyxoviridae family or from different viruses of the Paramyxoviridae family.

When the protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family in the cell targeting fusion protein, those in the modulating fusion protein and/or the glycoprotein derived from an envelope glycoprotein F are derived from different viruses of the Paramyxoviridae family, they preferably derive from a virus of the same genus, more preferably from a virus of the same species.

In one embodiment, the protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family in the cell targeting fusion protein, those in the modulating fusion protein and the glycoprotein derived from an envelope glycoprotein F are derived from the same virus of the Paramyxoviridae family.

In another embodiment, the protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family in the cell targeting fusion protein and the glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family are derived from the same virus of the Paramyxoviridae family, whereas the modulating fusion protein comprises (i) a transmembrane domain, for example the transmembrane domain (TMD) of the platelet-derived growth factor receptor (PDGFR), and (ii) at least one functional domain.

In another embodiment, the protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family in the modulating fusion protein and the glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family are derived from the same virus of the Paramyxoviridae family, whereas the cell targeting fusion protein comprises (i) a transmembrane domain, for example the transmembrane domain (TMD) of the platelet-derived growth factor receptor (PDGFR), and (ii) at least one cell targeting domain.

The different components of the pseudotyped retrovirus-like particle or retroviral vector, in particular the cell targeting fusion protein, the modulating fusion protein, the glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family and the transmembrane domain are as defined above.

A preferred pseudotyped retrovirus-like particle or retroviral vector comprises:

a) at least one cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family, said protein lacking at least one part of the cytoplasmic region of said envelope glycoprotein G and being at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or a transmembrane domain and (ii) at least one cell targeting domain,

b) at least one modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family, said protein lacking at least one part of the cytoplasmic region of said envelope glycoprotein Gand being at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or a transmembrane domain and (ii) at least one functional domain, and

c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family and lacking at least one part of the cytoplasmic region of said envelope glycoprotein F,

wherein said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

Examples of pseudotyped retrovirus-like particle or retroviral vector are given below in Table 2.

Table 2

Name	**Cell targeting fusion protein**		**Modulating fusion protein**		**Glycoprotein derived from an envelope glycoprotein F**
	protein derived from envelope glycoprotein G or H	cell targeting domain	protein derived from envelope glycoprotein G or H	functional domain	
MeV 4^{H}/IL7^{H}	- HcΔ15, HcΔ18 or HcΔ20 - 4 mutations Y481A, R533A, S548L, F549S	DARpin specific of human CD4	- HΔ15, HΔ18 or HΔ20 - 4 mutations Y481A, R533A, S548L, F549S	IL-7	FΔ30
	For example SEQ ID NO: 2 comprising HcΔ15 and a His Tag		For example SEQ ID NO: 14 comprising HcΔ15 and a His Tag		For example SEQ ID NO: 15
NiV 8^{G}/IL7^{G}	- GcΔ34 - 4 mutations: E501A, W504A, Q530A, E533A	scFv specific of human CD8	- GcΔ34 - 4 mutations: E501A, W504A, Q530A, E533A	IL-7	FΔ22
	For example SEQ ID NO: 4 comprising a His Tag		For example SEQ ID NO: 6 comprising a His		For example SEQ ID NO: 16

	and a linker $(G_4S)_3$	Tag	

In a preferred embodiment, the pseudotyped retrovirus-like particle or retroviral vector is obtainable or obtained by the method of production described below in the section "*Method for producing a pseudotyped retrovirus-like particle or retroviral vector* " herein below.

Nucleic acid and vector

The present invention also relates to a nucleic acid encoding the cell targeting fusion protein and/or the modulating fusion protein used for pseudotyping the retrovirus-like particle or retroviral vector according to the invention.

The present invention thus particularly relates to a nucleic acid comprising a sequence encoding a cell targeting fusion protein and/or a sequence encoding a modulating fusion protein, wherein:

- said cell targeting fusion protein comprises (i) a protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or a transmembrane domain and (ii) at least one cell targeting domain,
- said modulating fusion protein comprises (i) a protein derived from an envelope glycoprotein G of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or a transmembrane domain and (ii) at least one functional domain.

When the nucleic acid comprises a sequence encoding a cell targeting fusion protein and a sequence encoding a modulating fusion protein, said cell targeting fusion protein and/or said modulating fusion protein comprise(s) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

The nucleic acid preferably comprises or consists of:

(i) a sequence encoding a protein derived from an envelope glycoprotein of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G,

(ii) a sequence encoding one cell targeting domain or one functional domain, and

(iii) optionally, a linker sequence between sequences (i) and (ii),

(iv) optionally, a sequence encoding a tag, preferably at the 3' end of sequence (ii),

wherein sequences (i) and (ii) are fused in frame.

The linker sequence encodes a linker as defined above, for example encodes $(G_4S)_3$.

The sequence (i) is located in 5' of the nucleic acid by comparison to the sequence (ii) located in 3' of the nucleic acid.

In one embodiment, the nucleic acid comprises or consists of a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a sequence selected in the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 13 and/or comprises or consists of a sequence encoding a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a sequence selected in the group consisting of sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 14.

In a preferred embodiment, the nucleic acid comprises or consists of a sequence selected in the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 13 and/or comprises or consists of a sequence encoding a sequence selected in the group consisting of sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 14.

A definition of the percentage of sequence identity is provided above.

A nucleic sequence "at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical" to a reference sequence may comprise mutations, such as deletions, insertions and/or substitutions compared to the reference sequence.

In case of substitutions, the substitution preferably corresponds to a silent substitution or a substitution leading to a conservative substitution in the translated amino acid sequence, by comparison to the reference sequence, for example as indicated in the Table 1 above.

In a preferred embodiment, the nucleic sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference sequence only differs from the reference sequence by silent substitution(s) and/or substitution(s) leading to a conservative amino-acid substitution.

The present invention also relates to a nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family and preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein F.

The glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family is as defined above.

The nucleic acid is preferably an isolated nucleic acid.

By the expression "nucleic acid", it is herein meant the phosphate ester polymeric form of ribonucleosides (also called "RNA molecule"), deoxyribonucleosides (also called "DNA molecule") or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form or a double-stranded form.

The term “isolated” in reference to a biological component (such as a nucleic acid, a vector or a protein) refers to a biological component that has been substantially separated or purified away from other biological components in the cell of the organism, or the organism itself, in which the component naturally occurs, such as other chromosomal and extra-chromosomal DNA and RNA, proteins, cells, and organelles. “Isolated nucleic acids” or “isolated vectors” include nucleic acid molecules purified by standard purification methods. These terms also encompass nucleic acids and vectors prepared by amplification and/or cloning, as well as chemically synthesized nucleic acids and vectors.

The nucleic acid according to the invention is preferably cloned into a vector.

The term “vector” has therefore a meaning different from a “retroviral vector”.

By the term “vector”, it is herein meant a nucleic acid vector.

A vector generally comprises an origin of replication, a multicloning site and a selectable marker.

A vector preferably comprises an expression cassette, i.e. a nucleic acid according to the invention placed under the control of at least one expression signal allowing its expression.

The expression signal is particularly selected among a promoter, a terminator, an enhancer and their combinations.

Suitable promoters, terminators and enhancers are well-known by the skilled person.

An example of vector is for example a plasmid.

By “plasmid”, it is herein meant a double-stranded circular DNA. The plasmid may include a marker gene enabling to select the cells comprising said plasmid, an origin of replication to allow the cell to replicate the plasmid and/or a multiple cloning site allowing the insertion of a nucleic acid according to the invention.

The present invention thus also relates to a vector comprising a nucleic acid as defined above.

The vector is preferably an isolated vector.

The vector may comprise or consist of:

- a first nucleic acid comprising or consisting of:
  - (i) a sequence encoding a) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G or H and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H or b) a transmembrane domain,
  - (ii) a sequence encoding one cell targeting domain,
  - (iii) optionally, a linker sequence between sequences (i) and (ii),
  - (iv) optionally a sequence encoding a tag, preferably at the 3' end of sequence (ii),

  wherein sequences (i) and (ii) are fused in frame,
- a second nucleic acid comprising or consisting of:
  - (i) a sequence encoding a) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family, said protein preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein G or H and being preferably at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H or b) a transmembrane domain,
  - (ii) a sequence encoding one functional domain,
  - (iii) optionally, a linker sequence between sequences (i) and (ii),
  - (iv) optionally a sequence encoding a tag, preferably at the 3' end of sequence (ii),

  wherein sequences (i) and (ii) are fused in frame,

wherein the first nucleic acid and/or the second nucleic acid comprises a sequence encoding a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

Said first nucleic acid thus encodes the cell targeting fusion protein and said second nucleic acid encodes the modulating fusion protein.

Gene of interest

When a pseudotyped retroviral vector is used, at least one gene of interest may be present in the retrovirus-derived genome of said retroviral vector.

The gene of interest may encode a therapeutic protein, apoptotic protein, chimeric antigen receptor, cell surface receptor, antibody, antibody fragment, shRNA, antigen, cytokine, microRNA, CRISPR ((*clustered, regularly interspaced, short palindromic repeat*))/CAS element(s) (for example CAS9 and/or guide RNAs, in particular for specific gene disruption or correction), other nuclease system, such as Zink Finger Nucleases, S/MAR (Scaffold / Matrix Attachment Region) - episomes, ligand and/or receptor.

A S/MAR-episome is inserted in the cell nucleus by retroviral vectors that then replicate with the host cell DNA.

Examples of gene of interest include globin gene, hematopoietic growth factor gene (for example erythropoietin (EPO) gene), interleukin gene (especially Interleukin-1, Interleukin-2, Interleukin-3, Interleukin-6 or Interleukin-12 gene), colony-stimulating factor gene (such as granulocyte colony-stimulating factor gene, granulocyte/macrophage colony-stimulating factor gene or stem-cell colony-stimulating factor gene), the platelet-specific integrin αIIbβ gene, multidrug resistance gene, the gp91 or gp 47 genes, which are defective in patients with chronic granulomatous disease (CGD), antiviral gene rendering cells resistant to infections with pathogens (such as human immunodeficiency virus), gene coding for blood coagulation factors VIII or IX which are mutated in hemophilia's, gene encoding a ligand involved in T cell-mediated immune responses (such as T cell antigen receptors, chimeric antigen receptor (CARs), B cell antigen receptors (immunoglobulins, neutralizing antibodies against HIV, Hepatitis C, Hepatitis B and/or other infectious diseases), the interleukin receptor common γ chain gene, TNF gene, gamma interferon gene, CTLA4 gene, genes expressed in tumor cells such as Melana, MAGE genes (such as MAGE-1, MAGE-3), P198 gene, P1A gene, gp100 gene.

Method for producing a pseudotyped retrovirus-like particle or retroviral vector

The present invention also relates to a method for producing a pseudotyped retrovirus-like particle or retroviral vector as defined above, wherein said method comprises co-transfecting a packaging cell line with:

(i) at least one nucleic acid encoding a cell targeting fusion protein,

(ii) at least one nucleic acid encoding a modulating fusion protein,

(iii) at least one nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family and preferably lacking at least one part of the cytoplasmic region of said envelope glycoprotein F,

(iv) at least one vector comprising a nucleic acid encoding core proteins from said retrovirus and

(v) optionally, at least one vector comprising a packaging competent retroviral derived genome,

thereby obtaining co-transfected cells.

Said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

The at least one vector comprising a packaging competent retroviral derived genome is required only for producing a retroviral vector.

Any suitable packaging cell line well known by the skilled person may be used.

By “packaging cell line”, it is herein meant a cell line which is able to express the different components of the pseudotyped retrovirus-like particle or retroviral vector of the invention.

The nucleic acids and vectors encoding the different components of the pseudotyped retroviral-like particle or retroviral vector of the invention may be integrated in the genome of the packaging cell line, for example in the case of murine leukemia based vectors.

The packaging cell line is preferably compatible with the expression of lentiviral Gag and Pol genes.

For example, the packaging cell line may be selected in the group consisting of 293T cells, insect cells, TE 671 cells and HT1080 cells.

The term "transfection" means the introduction of at least one foreign nucleic acid or vector (for example DNA, cDNA or RNA) into a cell, so that the host cell will express the protein(s) encoded by said nucleic acid(s) or vector(s). The foreign nucleic acid(s) or vector(s) may include regulatory or control sequences, such as start, stop, promoter, signal, secretion or other sequences used by a cell's genetic machinery.

A vector comprising the psi sequence necessary for encapsidation is called a psi-positive vector, whereas a vector not comprising said psi sequence is called a psi-negative vector.

Only the vector comprising a packaging competent retroviral derived genome is a psi-positive vector. The other nucleic acids and vectors used for the co-transfection are psi-negative.

The nucleic acid encoding a cell targeting fusion protein, the nucleic acid encoding a modulating fusion protein and the nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family are as defined above. They may be provided in the form of two or three separate vectors: for example, two separate vectors, such as the first one comprising or consisting of the nucleic acid encoding a cell targeting fusion protein and the nucleic acid encoding a modulating fusion protein and the second one comprising or consisting of nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family; or three separate vectors, the first one comprising or consisting of the nucleic acid encoding a cell targeting fusion protein, the second one comprising or consisting of the nucleic acid encoding a modulating fusion protein and the third one comprising or consisting of nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family. Alternatively, the nucleic acid encoding a cell targeting fusion protein, the nucleic acid encoding a modulating fusion protein and the nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family may be provided in a single vector comprising or consisting of these three nucleic acids.

A vector comprising a nucleic acid encoding core proteins from said retrovirus is also used.

By “core proteins from a retrovirus”, it is herein meant the proteins encoded by the gag and pol genes. The gag gene encodes a polyprotein which is further processed by the retroviral protease into structural proteins that comprise the core. The pol gene particularly encodes the retroviral protease, reverse-transcriptase and integrase.

A nucleic acid encoding core proteins from a retrovirus thus comprise the gag gene and the pol gene of said retrovirus.

At least one core protein from the retrovirus may be a modified, for example by comparison to the corresponding core protein from a wild-type retrovirus.

In one embodiment, at least one core protein from the retrovirus is modified by at least one amino acid mutation, such as an amino acid deletion, insertion or substitution.

In a preferred embodiment, said at least one modified core protein is a deficient integrase. An example of deficient integrase bears the D116A mutation.

The nucleic acid encoding core proteins may thus comprise at least one mutation in the pol gene and/or at least one mutation in the gag gene. Said at last one mutation may be a nucleotide deletion, insertion or substitution.

In one preferred embodiment, the nucleic acid encoding core proteins comprises at least one mutation in the pol gene, thereby encoding a deficient integrase.

A retroviral vector comprising a deficient integrase is called an integration-deficient retroviral vector. Such vector allows to transiently transfer an encapsidated RNA molecule encoding for a gene of interest.

A preferred integration-deficient retroviral vector is an IDLV (Integration-deficient lentiviral vector).

The origin of the gag and pol genes gives its name to the retrovirus-like particle or retroviral vector. For instance the expression “HIV-1-derived retrovirus-like particle or retroviral vector” usually indicates that the gag and pol genes are those of HIV-1 or are modified gag and pol genes from HIV-1.

A vector comprising a packaging competent retroviral derived genome may also be used for the co-transfection, in particular for the production of a retroviral vector.

By “vector comprising a packaging competent retrovirus derived genome”, it is herein meant a vector that comprises the retroviral nucleic acid sequences known as “cis-acting” sequences. These include the Long Terminal Repeats (LTRs) or modified (LTRs), for example lacking at least one part of the U3 region, for the control of transcription and integration, the psi sequence necessary for encapsidation and the Primer Binding site (PBS) and polypurine track (PPT) sequences necessary for reverse transcription of the retroviral genome.

A retroviral vector produced using a vector comprising LTRs lacking at least one part of the U3 region is for example a Self-inactivating (SIN-LTR) vector.

In one embodiment, said vector comprising a packaging competent retrovirus-derived genome further comprises gene(s) of interest, including CRISPR/CAS element(s) and/or a S/MAR-episome.

When using the CRISPR / CAS system, the vector comprising a packaging competent retrovirus-derived genome typically comprises a gene encoding an endonuclease CAS (for example CAS9), a DNA sequence corresponding to the guide RNA (gRNA) specific to the gene of interest to be targeted and, optionally, a sequence for gene correction.

Said retrovirus-derived genome is preferably replication-defective, in the absence of any trans-complementing function. A replication-competent genome would further comprise the gag, pol, and env retroviral genes. In a replication-defective genome, the viral genes gag, pol and env are deleted. Assembly of the retrovirus-like particles or retroviral vectors of the invention is achieved by providing in trans another vector that encodes gag and pol, but that is defective for the “cis” sequences (such as the vector of (iv)), and at least another vector or nucleic acid that encodes the pseudotyped envelope glycoproteins (such as the nucleic acids of (i), (ii) and (iii) or vector(s) comprising said nucleic acids). Their expression allows the encapsidation of the gene of interest, excluding the genes necessary for the multiplication of the viral genome and for the formation of complete viral particles.

The method for producing a pseudotyped retrovirus-like particle or retroviral vector as defined above may further comprise a step of:

b) culturing the co-transfected cells for a time sufficient to allow expression of the proteins encoded by said nucleic acid(s) and vector(s); and

c) allowing the encoded proteins to form retrovirus-like particles or retroviral vectors.

<u>Modulating the activity of and/or transducing specific cells or subtype of cells</u>

Another object of the invention is the use, preferably *in vitro* or *ex vivo* use, of a pseudotyped retrovirus-like particle or retroviral vector as defined above to selectively modulate the activity of target cells and, optionally, selectively transduce target cells.

Still another object of the invention is a method, preferably *in vitro* or *ex vivo* method, for selectively modulating the activity of target cells and, optionally, selectively transducing target cells, wherein said method comprises contacting a pseudotyped retrovirus-like particle or retroviral vector as defined above with cells comprising said target cells.

The term “selectively” in the expression “selectively modulating the activity of target cells” means that essentially only said target cells have their activity modulated by the pseudotyped retrovirus-like particle or retroviral vector. For example, the cells whose activity is modulated comprise less than 10%, for example less than 5% or less than 1% of non-targeted cells.

The term “selectively” in the expression “selectively transducing target cells” herein means that essentially only the target cells are transduced. For example, the transduced cells comprise less than 10%, for example less than 5% or less than 1% of non-targeted cells are transduced.

The expression “modulating the activity of a target cell” is as defined above in the section “Functional domain”.

As used herein, the term “transferring” or "transducing" relates to the capacity of the retrovirus-like particle or retroviral vector to deliver a biological material to the membrane or the cytoplasm of target cells, upon being bound to the target cells. After delivery, the biological material can be translocated to other compartment(s) of the cell.

As used 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 comprising a gene of interest, which may be comprised within the genome of the retroviral vector, as explained above.

Conditions to perform the transduction of cells are well-known from the skilled person and typically include incubating the cells to be transduced, preferably cultured in flasks, plates or dishes coated for example with retronectin, and optionally pre-stimulated with cytokine cocktails, with the pseudotyped retroviral vector, preferably at an MOI (Multiplicity of Infection) between 0.5 and 100.

The target cells are particularly as defined above in the section of the same name.

The pseudotyped retrovirus-like particle or retroviral vector may be contacted with cells comprising the target cells in a culture medium.

The skilled person knows the medium culture appropriate for given cells.

The pseudotyped retrovirus-like particle or retroviral vector may thus be used *in vitro* or *in vivo* for changing a defined cell function, thereby providing new tools for basic research.

Pharmaceutical composition

The present invention also relates to a pharmaceutical composition comprising at least one pseudotyped retrovirus-like particle or retroviral vector as defined above.

The amount of pseudotyped retrovirus-like particle or retroviral vector to be used in a pharmaceutical composition depends, for example, on the immunogenicity, the condition of the subject intended for administration (e.g. weight, age, sex, health, concurrent treatment, if any, and frequency of treatment), the mode of administration, the type of formulation and/or the number of the target cells.

The pharmaceutical composition preferably comprises at least one pseudotyped retrovirus-like particle or retroviral vector and at least one pharmaceutically acceptable carrier.

As used herein, the term "pharmaceutically acceptable carrier" is meant to encompass any carrier, which does not interfere with the effectiveness of the biological activity of the active ingredient and that is preferably not toxic to the host to which is administered.

Pharmaceutically acceptable carriers that may be used in a pharmaceutical composition of the invention are well-known by the skilled person. For example, they include ion exchangers, alumina, aluminium stearate, lecithin, self-emulsifying drug delivery systems (SEDDS), such as d-α-tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α-, β-, and γ-cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2-and 3-hydroxypropyl-β-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery of compositions according to the invention.

The pharmaceutical composition may comprise from 10^6 IU to 10^{12} IU of pseudotyped retroviral vector, preferably from 10^7 IU to 10^9 IU, more preferably from 10^7 IU to 10^8IU.

The terms “IU” or “infection unit” herein mean the quantity of infectious vector particles determined by titration on a cell line and expressed as IU/ml.

The administration may be achieved in a single dose or several doses of the pharmaceutical composition according to the invention, said several doses being injected at the same time or separately over time.

In one embodiment, the pharmaceutical compositions are presented in unit dosage forms to facilitate accurate dosing. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a pre-determined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient. Typical unit dosage forms include pre-filled, pre-measured ampoules or syringes of the liquid compositions. In such compositions, the pseudotyped retrovirus-like particle or retroviral vector is usually a minor component, with the remainder being various vehicles or carriers and processing aids helpful for forming the desired dosing form.

The invention further provides kits comprising a pharmaceutical composition comprising a pseudotyped retrovirus-like particle or retroviral vector as defined above and instructions regarding the mode of administration. These instructions may e.g. indicate the medical indication, the route of administration, the dosage and/or the group of patients to be treated.

Subject to be treated

The subject to be treated may be a mammal, for example a human being or a non-human mammal.

A human being is also referred to as an “individual” or a “patient”.

Said human being may be of any age, for example an infant, child, adolescent, adult, elderly people, and of any sex.

A non-human mammal is preferably a rodent (for example a mouse, rat or rabbit), a feline (for example a cat), a canine (for example a dog) or a primate (for example a chimpanzee).

The subject to be treated is preferably a human being.

Prevention and/or treatment of a disease

The present invention is particularly useful for the prevention and/or treatment of a disease that may be prevented and/or cured by selectively modulating the activity of specific cells or subset of cells and, optionally, by selectively transducing said specific cells or subset of cells.

Said disease preferably involves specific cells or subset of cells.

The subject to be treated may thus suffer from or may be likely to be affected by a disease that may be prevented and/or cured by selectively modulating the activity of specific cells or subset of cells and, optionally, by selectively transducing said specific cells or subset of cells.

Said disease may be selected in the group consisting of an immune disease (for example an auto-immune disease), cancer, genetic disease, allergic disease, inflammatory disease, infectious disease (in particular bacteria and/or virus infection), metabolic disease, neurodegenerative disease (for example neural dystrophy, Parkinson disease, Huntington disease, Alzheimer disease), muscular disease and their combinations.

As used herein, the expression "autoimmune disease" refers to a disease due to an overactive immune response of the body against substances and/or tissues normally present in the body. Accordingly, by specifically targeting immune cells involved in this overactive immune response, the pseudotyped retrovirus-like particle and retroviral vector of the invention are useful tools for the prevention and/or treatment of autoimmune disease.

Autoimmune diseases include in particular acute disseminated encephalomyelitis, acute hemorrhagic leukoencephalitis, Addison's disease, Agammaglobulinemia, Alopecia areata, amyotrophic lateral sclerosis, ankylosing spondylitis, antiphospholipid syndrome, antisynthetase syndrome, atopic allergy, autoimmune aplastic anemia, autoimmune cardiomyopathy, autoimmune enteropathy, autoimmune hemolytic anemia, autoimmune hepatitis, autoimmune inner ear disease, autoimmune lymphoproliferative syndrome, autoimmune peripheral neuropathy, autoimmune pancreatitis, autoimmune polyendocrine syndrome, autoimmune progesterone dermatitis, autoimmune thrombocytopenic purpura, autoimmune urticaria, autoimmune uveitis, Balo disease, Balo concentric sclerosis, Bechets syndrome, Berger's disease, Bickerstaff's encephalitis, Blau syndrome, bullous pemphigoid, Castleman's disease, celiac disease, chronic inflammatory demyelinating polyneuropathy, chronic recurrent multifocal osteomyelitis, Churg-Strauss syndrome, cicatricial pemphigoid, Cogan syndrome, cold agglutinin disease, complement component 2 deficiency, cranial arteritis, CREST syndrome, Crohn's disease, Cushing's syndrome, cutaneous leukocytoclastic angiitis, Dego's disease, Dercum's disease, dermatitis herpetiformis, dermatomyositis, diabetes mellitus type 1, diffuse cutaneous systemic sclerosis, Dressler's syndrome, discoid lupus erythematosus, eczema, enthesitis-related arthritis, eosinophilic fasciitis, eosinophilic gastroenteritis, epidermolysis bullosa acquisita, erythema nodosum, essential mixed cryoglobulinemia, Evan's syndrome, firodysplasia ossificans progressiva, fibrosing aveolitis, gastritis, gastrointestinal pemphigoid, giant cell arteritis, glomerulonephritis, goodpasture's syndrome, Grave's disease, Guillain-Barré syndrome (GBS), Hashimoto's encephalitis, Hashimoto's thyroiditis, hemolytic anaemia, Henoch-Schonlein purpura, herpes gestationis, hypogammaglobulinemia, idiopathic inflammatory demyelinating disease, idiopathic pulmonary fibrosis, idiopathic thrombocytopenic purpura, IgA nephropathy, inclusion body myositis, inflammatory demyelinating polyneuropathy, interstitial cystitis, juvenile idiopathic arthritis, juvenile rheumatoid arthritis, Kawasaki's disease, Lambert-Eaton myasthenic syndrome, leukocytoclastic vasculitis, lichen planus, lichen sclerosus, linear IgA disease (LAD), Lou Gehrig's disease, lupoid hepatitis, lupus erythematosus, Majeed syndrome, Ménière's disease, microscopic polyangiitis, Miller-Fisher syndrome, mixed connective tissue disease, morphea, Mucha-Habermann disease, multiple sclerosis, myasthenia gravis, myositis, neuropyelitis optica, neuromyotonia, ocular cicatricial pemphigoid, opsoclonus myoclonus syndrome, ord thyroiditis, palindromic rheumatism, paraneoplastic cerebellar degeneration, paroxysmal nocturnal hemoglobinuria (PNH), Parry Romberg syndrome, Parsonnage-Turner syndrome, pars planitis, pemphigus, pemphigus vulgaris, permicious anemia, perivenous encephalomyelitis, POEMS syndrome, polyarteritis nodosa, polymyalgia rheumatica, polymyositis, primary biliary cirrhosis, primary sclerosing cholangitis, progressive inflammatory neuropathy, psoriasis, psoriatic arthritis, pyoderma gangrenosum, pure red cell aplasia, Rasmussen's encephalitis, Raynaud phenomenon, relapsing polychondritis, Reiter's syndrome, restless leg syndrome, retroperitoneal fibrosis, rheumatoid arthritis, rheumatoid fever, sarcoidosis, Schmidt syndrome, Schnitzler syndrome, scleritis, scleroderma, Sjögren's syndrome, spondylarthropathy, Still's disease, stiff person syndrome, subacute bacterial endocarditis, Susac's syndrome, Sweet's syndrome, Sydenham chorea, sympathetic ophthalmia, Takayasu's arteritis, temporal arteritis, Tolosa-Hunt syndrome, transverse myelitis, ulcerative colitis, undifferentiated connective tissue disease, undifferentiated spondylarthropathy, vasculitis, vitiligo and Wegener's granulomatosis.

As used herein, the term "cancer" encompasses any type of cancer, such as glioblastoma, neuroblastoma, B cell lymphoma, T cell lymphoma, breast cancer, hepatocellular carcinoma, cancer arising from hematopoietic cells, including leukemia, in particular B-CLL (B-cell Chronic lymphocytic leukaemia), CML (Chronic Myelogenous Leukemia) or T cell based leukemia such as ATL (acute T cell leukemia), ALL (Acute Lymphoblastic Leukemia), AML (Acute Myeloid Leukemia) and/or melanoma.

Therapeutic applications

The present invention also relates to a pseudotyped retrovirus-like particle or retroviral vector as defined above for use as a medicament, preferably in immune therapy gene therapy and/or vaccination.

Gene therapy is a therapy using gene(s) as a medicament, which may for example be obtained by delivering in a cell a gene of interest and/or correcting gene(s) of interest at the endogenous site. In gene therapy, a nucleic acid comprising the gene(s) of interest is delivered into the cells of a patient, the expression of the protein(s) encoded by gene(s) of interest and/or the correction of the gene(s) of interest thereby allowing preventing and/or treating a disease.

Said gene(s) of interest may be present in the RNA molecule comprised in the retroviral vector according to the invention.

The correction of gene(s) may be carried out by the CRISPR/CAS system.

Immune therapy, also called immunotherapy, is a therapy based on modulating the activity of the immune system (for example, stimulation or inhibition) to prevent and/or treat a disease.

In the context of the present invention, immunotherapy consists in modulating the activity of only specific targeted immune cells, by using the retrovirus-like particle or retroviral vector according to the invention, and optionally selectively transducing said target immune cells. For example, the retroviral vector may be used to activate B cells, such as make them differentiate in plasma cells, and optionally transduced them with a nucleic acid encoding an ectopic antibody against an infectious agent (for example against HIV, HCV or HBC).

Immune therapy also comprises T cell therapy. In T cell therapy, T cells might in addition to being modulated for their function be more permissive to gene transfer, for example for the T cell receptor (CAR) gene transfer.

Adoptive T cell therapy is a therapy wherein T cells are transfused to a subject in need therefore. In the context of the present invention, the activity of said T cells may have been modulating prior to the transfusion, by using the retrovirus-like particle or retroviral vector according to the invention.

In the context of the present invention, vaccination consists in displaying at the surface of the retrovirus-like particle or retroviral vector specific viral epitopes that will be targeted to and activate at the same time the Antigen presenting cells (for example macrophages) that will subsequently present the epitopes to the immune system (T and B cells).

The present invention also relates to a pseudotyped retrovirus-like particle or retroviral vector as defined above for use as a medicament, wherein said pseudotyped retrovirus-like particle or retroviral vector selectively modulates the activity of target cells and optionally selectively transduces target cells.

The present invention particularly relates to a pseudotyped retrovirus-like particle or retroviral vector for use as defined above, in the prevention and/or treatment of a disease as defined above in the section “Prevention and/or treatment of a disease”.

Said disease is for example an immune disease (for example an auto-immune disease), cancer, genetic disease, allergic disease, inflammatory disease, infectious disease (in particular bacteria and/or virus infection), metabolic disease, neurological disease (such as neural dystrophy, Alzheimer disease, Parkinson disease, Huntington disease), muscular disease and their combinations.

In one embodiment, the pseudotyped retrovirus-like particle or retroviral vector is used for improving DC (*Dendritic Cell*) vaccine, for example, by co-displaying DC cell specific ligand (such as CD11b) and GM-SCF (*Granulocyte-Macrophage Colony-Stimulating Factor*) on the surface of the vector, for protein or gene transfer.

In an advantageous embodiment, T cell activation is coupled with T cell specific gene transfer, which significantly enhanced the gene transfer efficiency in resting T cells, for example in T cell-based gene therapy . Efficient *in vivo* gene transfer into T cell subset is a revolution in the gene and cell therapy field, since it allows omitting *ex vivo* culture and transduction processes which induce high costs for the clinical application. Moreover, leaving the cells into their normal micro-environment *in vivo* allows them to conserve their phenotype and to persist long-term into a patient (eg. tumor specific CD8 cytotoxic cells).

In another embodiment, the invention may be used to enhance selective gene delivery into resting B lymphocytes, for example in B cell-based gene therapy, such as immunotherapy that allows the B cells to produce neutralizing antibodies against infectious agent(s) or allows B cell secretion of recombinant proteins that are tolerated by the immune system since B cell can act as tolerogenic cells.

Moreover, displaying one or more cytokines on the surface of the pseudotyped retrovirus-like particle or retroviral vector may induce the T cell subset differentiating into phenotypes like TSCM or TCM, which might persist long term *in vivo*.

In another embodiment, the invention may be used for *in vivo* expansion of (autologous) anti-cancer natural killer cells, for cancer therapy.

In one embodiment, the pseudotyped retrovirus-like particle or retroviral vector is used to induce apoptosis of a defined cell subset by co-displaying an apoptosis ligand and a tumor or immune cell specific targeting domain.

The pseudotyped retrovirus-like particle or retroviral vector may be provided in the form of a pharmaceutical composition.

The pharmaceutical composition is preferably as defined above in the section of the same name.

The present invention also concerns a method for treating a subject in need thereof comprising administering a therapeutically effective amount of a pseudotyped retrovirus-like particle or retroviral vector as defined above, preferably in the frame of an immune therapy, gene therapy and/or vaccination.

The present invention also relates to a method for treating a subject in need thereof comprising administering a therapeutically effective amount of a pseudotyped retrovirus-like particle or retroviral vector as defined above, wherein said pseudotyped retrovirus-like particle or retroviral vector selectively modulates the activity of target cells and optionally selectively transduces target cells.

The present invention also concerns a method for preventing and/or treating a disease, comprising administering to a subject in need thereof a therapeutically effective amount of a pseudotyped retrovirus-like particle or retroviral vector as defined above, wherein said disease is preferably as defined above.

Any suitable method of administration known from one skilled in the art may be used. In particular, the pseudotyped retrovirus-like particle or retroviral vector according to the invention may be administered by the oral route, the parenteral route (preferably by intravenous injection), the medullar route, in particular the intra-femur (such as in the bone marrow cavity) or humerus medullar route injection and/or local intra-tumour injection.

When the parenteral route is selected, the pseudotyped retrovirus-like particle or retroviral vector may be in the form of injectable solution or suspension, for example conditioned in an ampoule or flask.

The pseudotyped retrovirus-like particle or retroviral vector is preferably used or administered in a therapeutically effective amount.

A "therapeutically effective amount" refers to a quantity of pseudotyped retrovirus-like particle or retroviral vector that confers a therapeutic effect on the treated subject. The therapeutic effect may be objective (*i.e.*, measurable by some test or marker) or subjective (*i.e.* subject gives an indication of or feels an effect). As known from the skilled person, effective doses will vary depending on route of administration, the size and/or weight of the subject, as well as the possibility of co-usage with other agents.

The term “comprising” as used herein encompasses the term “consisting of”.

The present invention will be further illustrated in view of the following examples and figures.

All references cited herein, including journal articles or abstracts, published or unpublished patent application, issued patents or any other references, are entirely incorporated by reference herein, including all data, tables, figures and text presented in the cited references.

Brief description of the sequences

SEQ ID NO: 1 corresponds to a nucleic acid sequence encoding a truncated and mutated (Y481A, R533A, S548L, F549S) MV H protein (HcDelta18) fused to Designed Ankyrin Repeat Protein (DARPin)-29.2 specific for human CD4.

SEQ ID NO: 2 corresponds to the amino acid sequence encoded by sequence SEQ ID NO: 1.

SEQ ID NO: 3 corresponds to a nucleic acid sequence encoding a truncated and mutated (E501A, W504, Q530A, E533A) NiV G protein (GcΔ34) fused to a single chain antibody directed to human CD8 (scFvC8-Vh1) linked with an additional $(G_4S)_3$ linker.

SEQ ID NO: 4 corresponds to the amino acid sequence encoded by sequence SEQ ID NO: 3.

SEQ ID NO: 5 corresponds to a nucleic acid sequence encoding a truncated and mutated (E501A, W504, Q530A, E533A) NiV G protein (GcΔ34) fused to IL-7.

SEQ ID NO: 6 corresponds to the amino acid sequence encoded by sequence SEQ ID NO: 5.

SEQ ID NO: 7 corresponds to a nucleic acid sequence encoding a truncated and mutated (E501A, W504, Q530A, E533A) NiV G protein (GcΔ34) fused to a designed ankaryin repeat protein (DARPin) directed against human EpCAM.

SEQ ID NO: 8 corresponds to the amino acid sequence encoded by sequence SEQ ID NO: 7.

SEQ ID NO: 9 corresponds to the full length amino acid sequence of the Nipah virus envelope glycoprotein G.

SEQ ID NO: 10 corresponds to the full length amino acid sequence of the measles virus envelope glycoprotein H.

SEQ ID NO: 11 corresponds to the full length amino acid sequence of the Nipah virus envelope glycoprotein F.

SEQ ID NO: 12 corresponds to the full length amino acid sequence of the measles virus envelope glycoprotein F.

SEQ ID NO: 13 corresponds to a nucleic acid sequence encoding a truncated and mutated MeV H protein (HcΔ15) fused to IL-7.

SEQ ID NO: 14 corresponds to the amino acid sequence encoded by sequence SEQ ID NO: 13.

SEQ ID NO: 15 corresponds to the amino acid sequence of the measles virus F protein truncated by 30 amino acids in the cytoplasmic tail (FcΔ30).

SEQ ID NO: 16 corresponds to the amino acid sequence of the Nipah virus F protein truncated by 22 amino acids in the cytoplasmic tail (FcΔ22).

Brief description of the figures

Figure 1: Efficient and stable ErbB2-CAR gene transfer into resting $CD4^+$ T cells by $4^H/IL7^H$-LV. To assess if $4^H/IL7^H$-LV can efficiently transfer therapeutic genes (ErbB2 specific CAR, ErbB2-CAR) into resting T cells, freshly isolated $CD3^+$ T cells were transduced with 4^H-LV or $4^H/IL7^H$-LV. Cells transduced with VSV-LV or left untransduced (ut) were used as negative controls. Three days later, half of the transduced cells were analyzed by FACS to determine the percentage of $ErbB2\text{-}CAR^+$ cells in the CD4- or CD8-gated cells. To confirm stable ErbB2-CAR expression, the other half of the transduced cells was further cultivated in the presence of anti-CD3/anti-CD28/IL-2 stimulus, after a washing step, for additional three days. Then, the percentages of $CD4^+/ErbB2^+$ cells and $CD8^+/ErbB2^+$ cells were determined by FACS.

Figure 2: Mutation of the NiV envelope glycoprotein G to ablate natural receptor recognition. Binding of Ephrin-B2 (A) and B3 (B) to NiV-G mutants is shown. 293T cells were transfected either mock, plasmids encoding for $GcΔ34^{His}$, or with different $GcΔ34^{EpCAM}$ mutants: unmutated ($GcΔ34^{EpCAM}$), E533A ($GcΔ34^{EpCAM}$mut1), Q530A+E533A ($GcΔ34^{EpCAM}$mut2.1), E501A+W504A ($GcΔ34^{EpCAM}$mut2.2) or E501A+W504A+Q530A+E533A ($GcΔ34^{EpCAM}$mut4) and incubated with 1µg/ml recombinant Fc-EphrinB2 or B3. The amounts of binding of the receptor by the different mutants are shown as MFI values. Statistics are relating to unmutated GΔ34-DARPin-Ac1 (n=3; mean ± standard deviations (SD) are shown; *, P<0,1 **, P<0,01; ***, P<0,001 by unpaired t test).

Figure 3: Selective activation of $CD4^+$ T cells by $4^H/IL7^H$-VLP. Freshly isolated $CD3^+$ T cells were left untransduced (ut) or transduced with different types of VLPs pseudotyped with CD4-DARPin-Hmut (4^H -VLP), CD4-DARPin/IL7-Hmut ($4^H/IL7^H$-VLP) or IL7-Hnse ($Hnse/IL7^H$-VLP). Three days later the cells were stained by CD8, CD4, and CD71 antibodies. The CD71 expression in the CD4- or CD8- gated T cells is shown.

Figure 4: Functionally displayed IL7 on the targeted-VLPs promote T cell survival. The forward/side scatter profiles of adult resting $CD3^+$ T cells that were incubated for 6 days with virus-like particles (VLP) pseudotyped with CD4-targeted MV-H (4^H-VLP), CD8-targeted MV-H (8^H-VLP), IL7-displaying CD4-targeted MV-H ($4^H/IL7^H$-VLP), or IL7-displaying CD8-targeted MV-H ($8^H/IL7^H$ -VLP). Cells cultivated in the medium alone

(untransduced, ut) or in the presence of 15 ng/ml IL7 were used as controls. The percentage of viable cells is indicated in each dot blot.

Figure 5: Functionally displayed IL7 on the CD4-targeted LVs promote T cell survival. The forward/side scatter profiles of adult resting $CD3^+$ T cells that were incubated for 6 days with lentiviral vectors (LV) pseudotyped with VSVG (VSV-LV), MV-Hmut (Hnse-LV), CD4-targeted MV-H (4^H-LV), or IL7-displaying CD4-targeted MV-H (4^H/IL7^H-LV). Cells cultivated in the medium alone (untransduced, ut) or in the presence of 15 ng/ml IL7 were used as controls. The percentage of viable cells is indicated in each dot blot.

Figure 6: Selective activation of $CD4^+$ T cells by 4^H/IL7^H-LV. Freshly isolated $CD3^+$ T cells were left untransduced (ut) or transduced with different types of LVs pseudotyped with CD4-DARPin-Hmut ($4^{H\Delta 18}$ -LV), Hnse (Hnse-LV), or VSVG (VSVG-LV). Here IL7 was displayed on two different versions of Hmut, either with 20 amino acids C-truncated (HΔ20) or with 15 amino acids C-truncated (HΔ15). Accordingly, two types of 4^H/IL7^H-LV were produced and transduced the resting T cells. Three days later the cells were stained by CD3, CD8, CD69, and CD71 antibodies. The activation markers CD69 and CD71 expression in $CD8^+$ and $CD8^-$ ($CD4^+$) cells is shown.

Figure 7: Schematic representation of pseudotyped lentiviral vectors (LV). 4^H-LV is a CD4-targeted LV based on MeV glycoprotein pseudotypes. 4^H/IL7^H-LV is a IL7-displaying CD4-targeted LV based on MV glycoprotein pseudotypes. The envelope glycoprotein H (MV-H) is truncated at its cytoplasmic tail, for example by 15, 18, or 20 amino acids and at least two out of four point mutations (Y481A, R533A, S548L, and F549S) are introduced to blind its natural receptor recognition of SLAM and CD46, thereby resulting in the envelope glycoprotein Hmut. Afterwards, a targeting domain (for example CD4-DARPin) and/or a cytokine (for example IL7) are fused to the mutant H (Hmut), with or without a linker. The MV envelope glycoprotein F is truncated at its cytoplasmic tail by 30 amino acids (MV-$F_{\Delta 30}$).

Figure 8: Schematic representation of pseudotyped virus-like particles (VLP). 8^G-VLP is a CD8-targeted VLP based on NiV glycoprotein pseudotypes. 8^G/IL7^G-VLP is a IL7-displaying CD8-targeted VLP based on NiV glycoprotein pseudotypes. The NiV-G envelope glycoprotein is truncated at its cytoplasmic tail by 34 amino acids (Δ34) and four point mutations (E501A, W504A, Q530A, and E533A) are introduced to blind its natural receptor recognition of Ephrin-B2/B3, thereby resulting in the envelope glycoprotein NiV-$G_{\Delta 34}$. Afterwards, a targeting domain (CD8-scFv) and/or a cytokine (IL7) are fused to the mutant G protein (NiV-$G_{\Delta 34}$) with or without a linker. The NiV envelope glycoprotein F is truncated at its cytoplasmic tail by 22 amino acids (NiV-$F_{\Delta 22}$).

Figure 9: Efficient and selective transduction of resting CD4^{+} T cells by 4^{H}/IL7^{H}-LV. Freshly isolated CD3^{+} T cells were left untransduced (ut) or transduced with different types of LVs pseudotyped with CD4-DARPin-Hmut ($4^{H\Delta18}$ -LV), Hnse (Hnse-LV), or VSVG (VSVG-LV). Here IL7 was displayed on two different versions of Hmut, either with 20 amino acids C-truncated (HΔ20) or with 15 amino acids C-truncated (HΔ15). Accordingly, two types of 4^{H}/IL7^{H}-LV ($4^{H\Delta18}$/IL7$^{H\Delta15}$-LV and $4^{H\Delta18}$/IL7$^{H\Delta20}$-LV) were produced and transduced the resting T cells. Three days later the cells were stained by CD3, CD8, and CD4 antibodies. The GFP expression in CD4- or CD8-gated T cells is shown.

Figure 10: Neutralization of NiV glycoprotein pseudotyped LVs. CHO-EpCAM or CHO-Ephrin-B2 cells were transduced with NiVmutEpCAM-LV (circle), MVEpCAM-LV (square), NiVwt-LV (triangle), or VSVG-LV (diamond) at an MOI of 0.4 after incubation with serial dilutions of pooled human serum (IVIG) for 2h at 37°C. After 72h, GFP+ cells were determined by flow cytometry and the number of GFP+ cells relative to untreated control is shown (n=3).

Figure 11: Selective activation of CD8^{+} T cells by 8^{G}/IL7^{G}-LV. Freshly isolated CD3^{+} T cells were left untransduced (ut) or transduced with different types of LVs pseudotyped with CD8-scFv-Gmut (8^{G}-LV) or CD8-scFv-Gmut /IL7-Gmut (8^{G}/IL7^{G}-LV). Three days later the cells were stained by CD8, CD4, and CD71 antibodies. The CD71 expression in the CD4- or CD8- gated T cells is shown.

Figure 12: Efficient and selective transduction of resting CD8^{+} T cells by 8^{G}/IL7^{G}-LV. Freshly isolated CD3^{+} T cells were left untransduced (ut) or transduced with different types of LVs pseudotyped with CD8-scFv-Gmut (8^{G}-LV) or CD8-scFv-Gmut /IL7-Gmut (8^{G}/IL7^{G}-LV). Three days later the cells were stained by CD3, CD8, and CD4 antibodies. The GFP expression in CD4- or CD8-gated T cells is shown.

Figure 13: Efficient CAR19 gene transfer into resting CD8^{+} T cells by 8^{G}/IL7^{G}-LV. Freshly isolated CD3^{+} T cells were left untransduced (ut) or transduced with different types of LVs pseudotyped with CD8-scFv-Gmut (8^{G}-LV), CD8-scFv-Gmut /IL7-Gmut (8^{G}/IL7^{G}-LV) or VSVG (VSV-LV). The therapeutic gene encoding a CD19-specfic CAR (CAR19) was delivered by the LVs. Three days after transduction, the CAR19 expression was detected by anti-cmyc antibody. The percentage of CAR19 expressing cells in CD4^{+} or CD8^{+} T cells is shown.

Figure 14: 4^{H}/IL7^{H}-LVs allow targeted *in vivo* activation of CD4+ T cells in humanized mice.

NOD/SCID gc-/- mouse were injected with cord blood T cells and 2 months upon engraftment (20% T cell reconstitution), 100 microliters of 4^{H}/IL7^{H}-LV (1E6 IU) or 4^{H}/IL7^{H}-

LV were injected by IV. Two weeks after injection of the vectors, the mice were sacrificed and the splenocytes were evaluated for % of CD71 + CD4+ T cells (activation marker).

EXAMPLES

Material and methods

Generation of the constructs

The plasmid pHL3-Ac1 coding for truncated and mutated MV HcΔ18mut protein and for a $(G_4S)_3$ linker (L3) between H and the His-tagged DARPin Ac1 was generated by inserting the PCR amplified coding sequence of the EpCAM specific DARPin Ac1 (Stefan et al. 2011) from pQE30ss_Ac1_corr into the backbone of plasmid pHL3-HRS3opt2#2 (Friedel et al. 2015) via SfiI/NotI.

All plasmids encoding Nipah virus G protein variants were derived from plasmid pCAGGS-NiV-codonop-Gn. The coding sequence for the Ac1 targeting domain was fused to the C-terminus of the G protein reading frame by PCR amplification of each fragment and simultaneously introducing a common AgeI restriction site, which was used for ligation resulting in plasmid pCAGGS- NiV-G-DARPin-Ac1. All other targeting domains were exchanged via AgeI/NotI. Truncations of the G protein cytoplasmic tail were introduced by PCR amplification of the G protein reading frame and insertion of the PCR fragments into pCAGGS-NiV-G- DARPin-Ac1 resulting in plasmids pCAGGS-NiV-GcΔ33-DARPin-Ac1 and pCAGGS-NiV-GcΔ34-DARPin-Ac1.The His-tagged G and GcΔ34 proteins were generated by PCR amplification from pCAGGS-NiV-codonop-Gn. The fragments were cloned via PacI/NotI restriction into the plasmid backbone of pCAGGS-NiV-G-DARPin-Ac1 resulting in pCAGGS-NiV-G-His and pCAGGS-NiV-GcΔ34-His, respectively. Mutations interfering with natural receptor recognition were introduced into the NiV-GcΔ34-DARPin-Ac1 protein coding sequence by site-directed mutagenesis. Each mutation was generated by amplification of two fragments carrying the designated mutation with homologous regions at the mutation site. These fragments were fused and amplified by a flanking primer pair. Resulting fragments were cloned into pCAGGS-NiV-GcΔ34-DARPin-Ac1 via RsrII/AgeI, generating the plasmids pCAGGS-NiV-GcΔ34EpCAMmut.

For the generation of the NiV-F variants, the coding sequences for FcΔ22 and FcΔ25 were amplified from pCAGGS-NiV-F and cloned via PacI/SacI restriction into the plasmid backbone of pCAGGS-NiV-codonop-Gn resulting in the plasmids pCAGGS-NiV-FcΔ22 and pCAGGS-NiV-FcΔ25. AU1 tagged NiV-F variants used for Western blot analysis of vector particles were generated by amplifying the NiV-F variants from pCAGGS-NiV-F and simultaneously adding the AU1 tag N-terminally. The resulting PCR fragments were cloned via PacI/SacI restriction digest into the backbone of pCAGGS-NiV-codonop-Gn, resulting in the plasmids pCAGGS-AU1-NiV-F, pCAGGS-AU1-NiV-FcΔ22, and pCAGGS-AU1-NiV-FcΔ25.

Vector production

Vector particles were generated by transient transfection of HEK-293T cells using polyethylenimine (PEI). Twenty-four hours before transfection, 2.5x107 cells were seeded into a T175 flask. On the day of transfection, the cell culture medium was replaced by 10 ml DMEM with 15% FCS and 3 mM L-glutamine. The DNA mix was prepared by mixing 35 µg of total DNA with 2.3 ml of DMEM without additives.

For example, following optimization of G to F ratios, 0.9 µg of plasmid encoding for GcΔ34DARPin/scFv variants was mixed with 4.49 µg plasmid encoding for F variants. 14.4 µg of HIV-1 packaging plasmid pCMVΔR8.9 and 15.1 µg of transfer-LV plasmids were used. The transfection reagent mix was prepared by mixing 140 µl of 18 mM PEI solution in H2O with 2.2 ml DMEM without additives. This solution was mixed with the DNA mix, vortexed, incubated for 20 minutes at room temperature and added to the HEK-293T cells, resulting in DMEM with 10% FCS, 2 mM L-glutatmine in total. 24 h later, the medium was exchanged with DMEM with 10% FCS, 2 mM L-glutatmine in order to remove remaining PEI/DNA complexes. At day two post transfection, the cell supernatant containing the lentiviral vectors was filtered through a 0.45 µm filter. If needed, vector particles were purified by centrifugation at 450 x g for 24 h over a 20% sucrose cushion. The pellet was resuspended in phosphate-buffered saline (PBS). For transduction, 8x103 of CHO-EpCAM and SK-OV-3 cells or 2x104 Molt4.8 and Raji cells were seeded into a single well of a 96-well-plate and transduced on the next day. When needed, medium of cells was replaced with medium containing different concentrations of Bafilomycin A1 (Santa Cruz Biotechnology, Inc, Dallas, USA) and cells were pre- incubated 30 min at 37°C before vector was added. For titration, at least four serial dilutions of vector particles were used. After 72 h, the percentage of green fluorescentprotein (GFP)-positive cells was determined by flow cytometry. Transducing units/ml (t.u./ml) was calculated by selecting the dilutions showing linear correlation betweendilution factor and number of GFP-positive cells (number of transduced cells/volume of vector in µl/0.001).

Schematic presentations of the pseudotyped retrovirus-like particle or retroviral vector according to the invention are depicted in Figures 7 and 8.

Example 1: Selective activation of CD4+ T cells

In order to activate $CD4^+$ but not $CD8^+$ lymphocytes, VLPs were generated that display a CD4-specific DARPin as targeting ligand and IL-7 as activation domain on the MV H protein along with the fusion protein (F) resulting in the 4^H/IL7^H-VLP (comprising proteins of sequence SEQ ID NO: 2, 14 and 15). To generate the particles, a transfection protocol was established. Briefly, 0.45 µg of pCG-Hmut-CD4-DARPin (Zhou et al., J Immunol, 2015), 0.45 µg of pCG-HΔ15-IL7 (provided by Els Verhoeyen), 4.7 µg of pCG-FcΔ30 (Funke et al., Molecular therapy, 2008), 14.4 µg of HIV-1 packaging plasmid pCMVΔR8.9 (Funke et al., Molecular therapy, 2008), and 15.1 µg of pCG-1 were used for transfection of HEK293T cells in one T175 flask. After harvesting, the vector particles were further concentrated via ultracentrifugation. Titers of ~10^7 tu/ml were obtained for LVs and titers of ~1.5 ug p24/ml were obtained for VLPs.

The function of 4^H/IL7^H-VLP was demonstrated on freshly isolated resting T cells. Briefly, $CD3^+$ T cells were isolated by negative selection using Pan T cell isolation kit (Miltenyi Biotech) from adult peripheral blood. Subsequently, the cells were incubated with 4^H/IL7^H-VLP in the absence of any stimulation. Three days later, the $CD4^+$ T cells but not the $CD4^-$ T cells in the cell culture were activated, as confirmed by FACS analysis for the CD71 activation maker (*Figure 3*). Non-targeted particles displaying IL-7 activated both, $CD4^+$ T cell and $CD4^-$ T cells. IL-7 is known to be a T cell survival cytokine. Therefore, it was demonstrated that all IL7-displaying VLPs were as effective as recombinant human IL-7 at preventing the death of primary T cells, while most of the resting T cells placed in culture were dead after six days in the presence of conventional IL7-deficient VLP particles (*Figure 4*).

The capacity of promoting T cell survival and selectively activating $CD4^+$ T cells was also acquired by IL7-displaying CD4-targeted LVs (4^H/IL7^H-LV). Viral particles were produced as described above, except that pCG-1 was substituted by the transfer plasmid encoding GFP. In addition, in order to test the flexibility of the system and if the function of particles could be enhanced by using other version of Hmut, two different plasmids encoding Hmut-IL7 were used for vector production. pCG-HΔ15-IL7 is with 15 aa cytoplasmic truncation of Hmut-IL7 (for 4^H/IL7$^{H\Delta15}$-LV production) and pCG-HΔ20-IL7 is with 20 aa cytoplasmic truncation of Hmut-IL7 (for 4^H/IL7$^{H\Delta20}$-LV production). Without specific indication, IL7-displaying VLP and LV were produced with pCG-HΔ15-IL7. Similar to what was achieved by 4^H/IL7^H-VLP, when freshly isolated human $CD3^+$ T cells were incubated with the indicated LVs in the absence of stimulation for 6 days, 4^H/IL7^H-LV significantly promoted T cell survival compared to the parental 4^H-LV and non-targeted Hnse-LV (*Figure 5*). As expected, the highest percentage of viable cells was observed in rhIL-7 treated positive group and the least viable cells were in the VSV-LV transduce or untransduced (ut) groups (*Figure 5*). Moreover, 4^H/IL7^H-LV was as potent as 4^H/IL7^H-VLP in selectively stimulating its target cell population in the mixed cell culture. As shown in Figure 6, 4^H/IL7^H-LV selectively upregulate activation maker CD69 and CD71 expression $CD4^+$ cells but not $CD4^-$ cells. There were no differences between 4^H/IL7$^{H\Delta15}$-LV and 4^H/IL7$^{H\Delta20}$-LV in $CD4^+$ T cell stimulation. Therefore, functional IL7-displaying targeted LVs are successfully generated.

<u>Example 2</u>: Delivery of tumor-specific chimeric antigen receptors into resting T cells

Since IL7-displaying vectors triggered the resting T cells activation, it is expected that these cells are permissive for lentiviral transduction. In order to prove it, two versions of 4^H/IL7^H-LV delivering GFP transgene were generated. As shown in Figure 9, both 4^H/IL7$^{H\Delta15}$-LV and 4^H/IL7$^{H\Delta20}$-LV can efficiently and selectively transduce resting $CD4^+$ T cells in the cell mixture.

Chimeric antigen receptors (CARs) are a powerful tool for cancer therapy. So far, T cell subsets must be purified and activated for genetic delivery of CARs. Here, it is demonstrated that CARs can be delivered selectively into resting $CD4^+$ cells. The particles were generated as described in example 1 except that pCG-1 was substituted by the CAR encoding transfer plasmid. When resting T cells were transduced with 4^H/IL7^H-LV delivering ErbB2-specific chimeric antigen receptors (ErbB2-CAR), ErbB2-CAR expression was only observed in the $CD4^+$ T cell population. Compared to the IL7-deficienct CD4-targeted LV (4^H-LV), the 4^H/IL7^H-LV was more efficient in delivering the CAR gene while retaining the selectivity for the $CD4^+$ T cells (*Figure 1*).

<u>Example 3</u>: The MV glycoproteins can be replaced by those of NiV to selectively activate and transduce resting human $CD8^+$ T cells

In order to efficiently pseudotype VLPs or LVs with NiV glycoproteins, the NiV-G was truncated at its cytoplasmic tail by 34 amino acids (GcΔ34) and the NiV-F was truncated at its cytoplasmic tail by 22 amino acids (FΔ22). Next, the natural receptor recognition of GcΔ34 for Ephrin-B2/B3 was destroyed by introducing four point mutations (E501A, W504A, Q530A, E533A) into GcΔ34. LVs pseudotyped with this engineered G protein completely lost binding to the natural Ephrin-B2 and -B3 NiV receptors (*Figure 2*) and did not enter into cells expressing these receptors.

Moreover, NiV-pseudotyped LVs have some attractive features, like high production yield and resistance to intravenous immunoglobulins. Since there is no vaccination against NiV and the few outbreaks are limited to a few cases in Malaysia, Bangladesh and India (SEARO | WHO South-East Asia Region), there should be no neutralizing antibodies present in humans. To demonstrate this, intravenous immunoglobulin (IVIG; Intratect®) covered the widest range of human serum donors, was incubated with NiVwt-LV, NiVmut^{EpCAM}-LV, MV^{EpCAM}-LV and VSV-LV at increasing concentrations prior to the transduction of target cells. GFP expression was then determined by flow cytometry three days post transduction. As expected, transduction mediated by VSVG-LV and NiVwt-LV was unaffected by the treatment with IVIG. MV^{EpCAM}-LV, on the other hand, showed a dose-dependent decrease in transduction rates with a complete *neu*tralization at 100 µg/ml of IVIG. In contrast, NiVmut^{EpCAM}-LV was resistant against IVIG at all concentrations used and must thus be at least 10000-fold less sensitive against human immunoglobulin than the corresponding MV-based vector (*Figure 10*). These results show that receptor-targeted vectors based on NiV glycoproteins will not be neutralized when injected into humans.

Therefore, due to the above features of the NiV-G, the NiV-pseudotypes were further used to generate cytokine-displaying CD8-targeted particles. Here, a CD8 specific scFv derived from OKT8 was displayed on the NiV-GΔ34mut4 to generate envelope plasmid pCG-Gmut-CD8scFv and human IL7 was displayed on the NiV-GΔ34mut4 to generate envelope plasmid pCG-Gmut-IL7. In order to produce 8^{G}/IL7^{G}-LV, 0.45 µg of pCG-Gmut-CD8scFv, 0.45 µg of pCG-Gmut-IL7, 4.7 µg of pCG-FcΔ22, 14.4 µg of HIV-1 packaging plasmid pCMVΔR8.9, and 15.1 µg of transfer-LV plasmids were used for transfection of HEK293T cells in one T175 flask. Titers of ~10^7 tu/ml were obtained for LVs and titers of ~1.5 ug p24/ml were obtained for VLPs.

Similar to what was achieved by 4^{H}/IL7^{H}-LV, 8^{G}/IL7^{G}-LV (comprising proteins of sequence SEQ ID NO: 4, 6 and 16) was able to selectively stimulate and transduce CD8^{+} T cells in the peripheral blood mixture. As shown in Figure 11, when freshly isolated human CD3^{+} T cells were incubated with 8^{G}/IL7^{G}-LV for 3 days, not only the CD8^{+} T cell subsets in the culture upregulated the CD71 expression but also only this cell population efficiently expressed the reporter transgene GFP (*Figure 12*) or therapeutic transgene CAR19 (*Figure 13*). In addition, compared to the parental IL7-deficient CD8-targeted LV (8^{G}-LV), the 8^{G}/IL7^{G}-LV was more efficient in activating the target cells and delivering transgenes without compromise on specificity for transduction of the CD8^{+} T cells.

Example 4: Identification of an optimal ratio of CD8-targeting NiV-G to IL7-displaying NiV-G

In order to hit more target cells, improve the titer and enhance the specificity of the particles for selective activation and transduction of a distinct cell population, while maximally reduce the off-target effect, the transfection protocol is further optimized for vector production and carefully determine the amounts of NiV-G$^{\text{targeting domain}}$ (or MV-H$^{\text{targeting domain}}$) and NiV-G$^{\text{functional domain}}$ (MV-H$^{\text{functional domain}}$) encoding plasmids maintained in the packaging cells.

For the production of G^{8}/G^{IL7}-LV, as described in Example 3, total amounts of 0.9 µg of pCG-Gmut plasmids together with 4.7 µg of pCG-FcΔ22, 14.4 µg of HIV-1 packaging plasmid pCMVΔR8.9, and 15.1 µg of pSEW were used for transfection of HEK293T cells in one T175 flask. Meanwhile, pCG-Gmut-CD8scFv and pCG-Gmut-IL7 plasmids are mixed at different ratios at 20:1, 10:1, 5:1, 1:1, or 1:5. The respective cell supernatants containing 8^{G}/IL7^{G}-LVs are used for transduction of CD8^{+} Molt or A301 cells and, 48h after transduction, the percentage of GFP^{+} cells are measured by FACS to calculate the titers. In addition, 8^{G}/IL7^{G} -LVs are used for transduction of freshly isolated CD3^{+} T cells and then the CD71 and GFP expression in CD8^{+} and CD8^{-} cells are determined. The best ratio is identified as giving the highest titer, highest expression of CD71 and GFP in target population and lowest expression in non-target population.

Example 5: Characterization of the transduced resting T cells by IL7-displaying targeted VLPs and LVs

T cell compartments are highly heterogeneous and T cell subsets are phenotypically and functionally distinct. As far as T cell therapy for cancer is concerned, less differentiated cells are usually correlated with better antitumor effect *in vivo*. Therefore, it's of importance to identify the T cell subsets that activated or modified by the vector particles according to the invention. Take 8^{G}/IL7^{G}-LV-CAR for example, the phenotypes of the transduced resting T cells should be characterized in comparison to transduced pre-stimulated T cells (with most widely used protocol of CD3/CD28 stimulation). Briefly, 3 days after transduction of resting or pre-stimulated CD3^{+} T cells, the expression of multiple T cell markers is monitored, including CD11a, CD11b, CD25, CD27, CD28, CD45RA, CD45RO, CD62L, CD69, CD71, CD95, CD127, and CCR7. These molecules are chosen as they have been used in the past to differentiate CD45RA^{+}CD45RO^{-}CD62L^{high}CD95^{-}CD27^{high}CCR7^{high} naïve (T_N), CD45RA^{+}CD45RO^{-}CD62L^{high}CD95^{+}CD27^{high}CCR7^{high} stem cell memory (T_{SCM}), CD45RA^{-}CD45RO^{high}CD62L^{+}CD95^{+}CD27^{+}central memory (T_{CM}), CD45RA$^{-/+}$CD45RO^{high}CD62L^{-}

$CD95^+CD27^{-/+}CCR7^-$ effector memory (T_{EM}), and $CD45RA^{-/+}CD45RO^+CD62L^-CD95^{high}CD27^-CD28^-CCR7^-$ effector (T_E) T cells. The phenotypes of CAR^+ T cells are analyzed by flow cytometry.

In addition, pre-sorted $CD4^+$ T cell subsets (T_{SCM}, T_{CM}, T_{EM}, and T_E) are incubated with 4^H/$IL7^H$-VLP for example, to demonstrate the advantages of current invention for T cell activation over the conversional TCR stimulation. Briefly, 3 days after incubation with 4^H/$IL7^H$-VLP or stimulation by CD3/28 antibodies, above indicated T cell subsets are analyzed for phenotype skewness and activation levels. It is expected that IL7-displaying T cell targeted vector particles activate and transduce less differentiated T cells.

<u>Example 6</u>: Enhance the function of *ex vivo* produced CART cells and simplify the CART cell production procedure

Adoptive T cell immunotherapy has been demonstrated to be an effective regime in clinic for treatment of various types of diseases. However, its broader application has been limited by several issues. On one hand, the production of CAR-engineered or TCR-engineered T cells is very expansive and labor-intensive due to the *in vitro* stimulation, long-term expansion, and cell isolation and purification. On the other hand, the effector T cells that have been expanded *in vitro* often persist poorly *in vivo*, and fail to exhibit a sustained antitumor effect. The current invention provides the potential solutions for these challenges by combining the cell stimulation and gene transfer in a single step and enabling resting T cell transduction. Using this invention, the T cell production process can be significantly simplified therefore reducing the cost of T cell manufacture. Moreover, compared to the conventionally produced cells, CAR/TCR-engineered T cells produced by current invention can be more potent *in vivo*, due to the potential of efficiently engineering less differentiated cells as demonstrated in Example 5.

To demonstrate these features, freshly isolated human PBMC or $CD8^+$ T cells are transduced with for example 8^G/$IL7^G$-LV-CAR or VSV-LV-CAR as control. Meanwhile, conventional CAR T cells are prepared in parallel according to the most widely uses protocol. Briefly, isolated human PBMC or $CD8^+$ T cells from the same donor are simulated with CD3/CD28 antibodies and IL2. Then cells are transduced with 8^G/$IL7^G$-LV-CAR or VSV-LV-CAR followed by 10 days expansion in the presence of IL2. 2-3 days after vector transduction of resting T cells or 12 days after transduction of stimulated T cells, these cells are co-cultivated with target tumor cells *in vitro* or infused in the tumor bearing humanized mouse. Afterwards, *in vitro* tumor cells lysis and *in vivo* tumor remission, animal survival, tumor-reactive T cell persistence and proliferation are assessed.

<u>Example 7</u>: Selective activation and transduction by IL7-displaying targeted particles in *in vivo* like conditions

In a next step, the feasibility of using the viral particles to target and functionally modify the distinct cells in *in vivo* settings is investigated. Thus, transduction of fresh human peripheral blood with the viral particles is performed, for example with G^{8}/G^{IL7}-LV. This allows evaluation of targeted activation and gene transfer in $CD8^{+}$ T cell population in the presence of an active human complement system, an obstacle encountered by viral particles *in vivo*.

Briefly, fresh peripheral blood is incubated with $8^{G}/IL7^{G}$-LV-GFP or other control vectors (VSV-LV, $8^{H}/IL7^{H}$ -LV, 8^{G}-LV, or VSV/$IL7^{G}$-LV) for 6-8 h. Afterwards, total PBMC are isolated from the blood and further cultured in the T cell culture medium without adding any stimulatory reagents. After 3-4 days, $CD8^{+}$ and $CD8^{-}$ cells in the culture are evaluated for CD71 and GFP expression. To confirm the stable gene transfer, a small fraction of cells are transferred into the culture medium supplemented with CD3/CD28 antibodies and IL2 for additional 3 days before the percentages of GFP^{+} cells are analyzed.

<u>Example 8</u>: IL7-displaying targeted particles selectively activate and transduce circulating resting T cells in humanized mouse models

In order to demonstrate the particles according to the invention are able to be locally or systemically applied *in vivo* to allow the cell type specific activation or modification, they are applied in mice engrafted with human $CD34^{+}$ hematopoietic stem cells (HSC) from healthy donors. In this mouse model, multilineage human hematopoietic cells can be generated and these cells are tolerated by the mouse host.

Here, the application of G^{8}/G^{IL7}-pseudotpyed particles for human $CD8^{+}$ T cell activation and modification is taken for example. Briefly, after having confirmed that the human immune system was successfully established by detecting around 10% of human $CD3^{+}$ T cells, G^{8}/G^{IL7}-pseudotpyed particles are applied systemically via intravenous injection (iv) or locally via intrasplenic or intrathymic injection. G^{8}/G^{IL7}-VLP is injected to assess the feasibility of selective activation of human $CD8^{+}$ T cells *in vivo*. In this experiment setting, the activation level/duration and $CD8^{+}$ T cells proliferation are analyzed at the different time points after VLP injection. G^{8}/G^{IL7}-LV-GFP is injected to assess the function of *in vivo* gene delivery. In this experiment setting, the GFP expression, the phenotypes of transduced cells, and their proliferation and persistence are analyzed at the different time points after LV injection. G^{8}/G^{IL7}-LV-CAR is injected to assess the feasibility of therapeutic gene delivery and the *in vivo* generation of CAR T cells. In this experiment setting, the CAR expression, the phenotypes of transduced cells, the proliferation and persistence of transduced cells in the presence/absence of target tumor cells/antigens, the elimination of local/systemic tumor are analyzed at the different time points after LV injection.

Example 9: Selective activation of Rhesus Macaque CD4^{+} T cells

T cells of non-human primate (NHP) are phenotypically similar to human T cells, making it the best animal model for human immunity and immunotherapies. As far as T cell therapy is concerned, the NHP model could be more reliable in predicting the effect, dosage, application routes, and safety of potential medicaments. Therefore, a tool to specifically modify or inducing a functional change in a distinct cell type in NHP is highly desirable.

Here, the 4^{H}/IL7^{H}-SIV vector and its application for rhesus macaque are described in detail as an example. First, a rhesus macaque CD4-specific DARPin57.2 (CD4$^{D57.2}$) is used as targeting domain displayed on the Hmut protein (H-CD4$^{D57.2}$) and the rhesus macaque reactive IL7 is used as functional domain displayed on the Hmut (H-rmIL7). Then the above two H proteins, for example with the ratios determined in Example 4, are used to pseudotype simian immunodeficiency virus mac 251 (SIVmac251)-derived LV. After determining the vector yield and titers by P27 ELISA/Nano-sight and transduction of CD4^{+} simian cells, freshly isolated simian CD3^{+} T cells are transduced with 4^{H}/IL7^{H}-SIV delivering GFP or CARs. Then, the activation makers and transgene expression in CD4^{+} vs CD4^{-} T cells was determined as described above. Next, the possibility of *in vivo* application is assessed in NHP models. For this purpose, a single dose of vectors is injected slowly into two axillary lymph nodes of each of the enrolled animals (eg. rhesus macaque). One week post-injection, blood samples are collected and one of the two injected lymph nodes is removed by surgery and dissociated into single cell suspensions. The detailed analysis is performed to analyse the phenotypes, specific activation, transgene expression levels and/or function (when therapeutic transgene is delivered) of *in vivo* transduced T cells. Moreover, the second lymph node receives a second dose of the same vector and the enrolled animals are monitored for at six months to assess the proliferation, persistence, and/or function (when therapeutic transgene is delivered) of *in vivo* transduced T cells.

Example 10: Selectively active and expand antigen-specific T cells in immunocompetent mouse model

The potential of targeting cytokine to function at the disease site while avoiding the systemic toxicity can be well evaluated in an immunocompetent mouse melanoma tumor model. IL12 displaying murine CD8-targeted VLP ($m8^G$/$mIL12^G$-VLP) is taken as an example. Previous studies have shown that IL12 application can enhance the transferred tumor-reactive T cells tolerance and antitumor efficacy, but it is very toxic systemically. To overcome this drawback, in our study, $m8^G$/$mIL12^G$-VLP is used to control the function of IL12 to take place at the tumor sites. Wide type C57/Bl6 mouse is transplanted with B16/OVA melanoma cells, after the establishment of tumor, the mouse is treated by irradiation and transferred with T cells isolated from C57/Bl6 OT-I transgenic mouse. After OT-I T cell transplantation, the mice are treated with $m8^G$/$mIL12^G$-VLP, or with murine IL12, or with PBS as controls via i.v. injection. The tumor volume, *in vivo* persistence and function of OT-I T cells, and mouse survival are analyzed.

Example 11: *In vivo* targeted targeted activation of human CD4+ T cells.

In order to evaluate the *in vivo* performance of the MV-4^H/$IL7^H$-LVs for activating human $CD4^+$ T cells, NOD/SCID gc-/- (NSG) mice were engrafted with $CD3^+$ cord blood T cells. Human T cell reconstitution was weekly determined in the blood. When 20% of hT cells was detected (% $hCD3^+$ cells / total $hCD45^+$+$mCD45^+$ cells), the mice were injected IV with 1E6 IU MV-4^H/$IL7^H$-LVs or MV-4^H- LVs. Two weeks post-injection of the vectors, the mice were sacrificed and the splenocytes and peripheral blood mononuclear cells were isolated. Remarkably, it was found a stronger specific IL-7 activation revealed by a CD71 late activation marker of the human CD4+ T cells in case of the MV-4^H/$IL7^H$-LVs (25% CD4+ CD71+ cells) as compared to MV-4^H- LVs (8 % CD4+ CD71+ cells) (*Figure 14*). Moreover, the CD4- T cells, which represent the CD8+ T cells, show identical expression of CD71 for both targeting vectors. The latter emphasizes that the IL-7 survival signaling was only activating the targeted CD4+ T cells and not the CD8+ cells.

In conclusion, next to the *in vitro* specific activation of the target cells achieved with MV-4^H/$IL7^H$-LVs, the specific activation of CD4+ T cells by MV-4^H/$IL7^H$-LVs was confirmed *in vivo* in human blood system mouse model.

CLAIMS

1. A pseudotyped retrovirus-like particle or retroviral vector comprising:
    a) at least one cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain,
    b) at least one modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain, and
    c) at least one glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family,

    wherein said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family,

    wherein said protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family lacks at least one part of the cytoplasmic region of said envelope glycoprotein G or H.

2. The pseudotyped retrovirus-like particle or retroviral vector according to claim 1, wherein said virus of the Paramyxoviridae family is a virus of the *Morbillivirus* genus or of the *Henipavirus* genus.

3. The pseudotyped retrovirus-like particle or retroviral vector according to claim 1 or 2, wherein said protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family is at least partially unable to bind at least one natural receptor of said envelope glycoprotein G or H.

4. The pseudotyped retrovirus-like particle or retroviral vector according to any one of claims 1 to 3, wherein said glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family lacks at least one part of the cytoplasmic region of said envelope glycoprotein F.

5. The pseudotyped retrovirus-like particle or retroviral vector according to any one of claims 1 to 4, wherein said cell targeting domain is selected in the group consisting of a DARPin, ScFv, targeting peptide and their combinations.

6. The pseudotyped retrovirus-like particle or retroviral vector according to any one of claims 1 to 5, wherein said functional domain is selected in the group consisting of a cytokine, growth factor, hormone, neurotransmitter, apoptosis ligand and their combinations.

7. Use of a pseudotyped retrovirus-like particle or retroviral vector according to any one of claims 1 to 6 to selectively modulate the activity of target cells and optionally selectively transduce said target cells.

8. A method for selectively modulating the activity of target cells and/or selectively transducing target cells, wherein said method comprises contacting a pseudotyped retrovirus-like particle or retroviral vector according to any one of claims 1 to 6 with cells comprising said target cells.

9. A pseudotyped retrovirus-like particle or retroviral vector according to any one of claim 1 to 6 for use as a medicament.

10. The pseudotyped retrovirus-like particle or retroviral vector for use according to claim 9 in immune therapy, gene therapy and/or vaccination.

11. The pseudotyped retrovirus-like particle or retroviral vector for use according to claim 9 or 10, in the prevention and/or treatment of an immune disease, cancer, genetic disease, allergic disease, inflammatory disease, infectious disease, metabolic disease, neurological disease, muscular disease and their combinations.

12. A nucleic acid comprising a sequence encoding a cell targeting fusion protein and/or a sequence encoding a modulating fusion protein, wherein:

- said cell targeting fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain, and
- said modulating fusion protein comprises (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain.

13. The nucleic acid according to claim 12, wherein said nucleic acid comprises a sequence selected in the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 and SEQ ID NO: 13 and/or comprises a sequence encoding a sequence selected in the group consisting of sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8 and SEQ ID NO: 14.

14. A vector comprising a nucleic acid according to claim 12 or 13.

15. A method for producing a pseudotyped retrovirus-like particle or retroviral vector according to any one of claims 1 to 6, wherein said method comprises co-transfecting a packaging cell line with:

(i) at least one nucleic acid encoding a cell targeting fusion protein, said cell targeting fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one cell targeting domain,

(ii) at least one nucleic acid encoding a modulating fusion protein, said modulating fusion protein comprising (i) a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family or a transmembrane domain and (ii) at least one functional domain,

(iii) at least one nucleic acid encoding a glycoprotein derived from an envelope glycoprotein F of a virus of the Paramyxoviridae family, and

(iv) at least one vector comprising a nucleic acid encoding core proteins from said retrovirus,

wherein said cell targeting fusion protein and/or said modulating fusion protein comprises a protein derived from an envelope glycoprotein G or H of a virus of the Paramyxoviridae family.

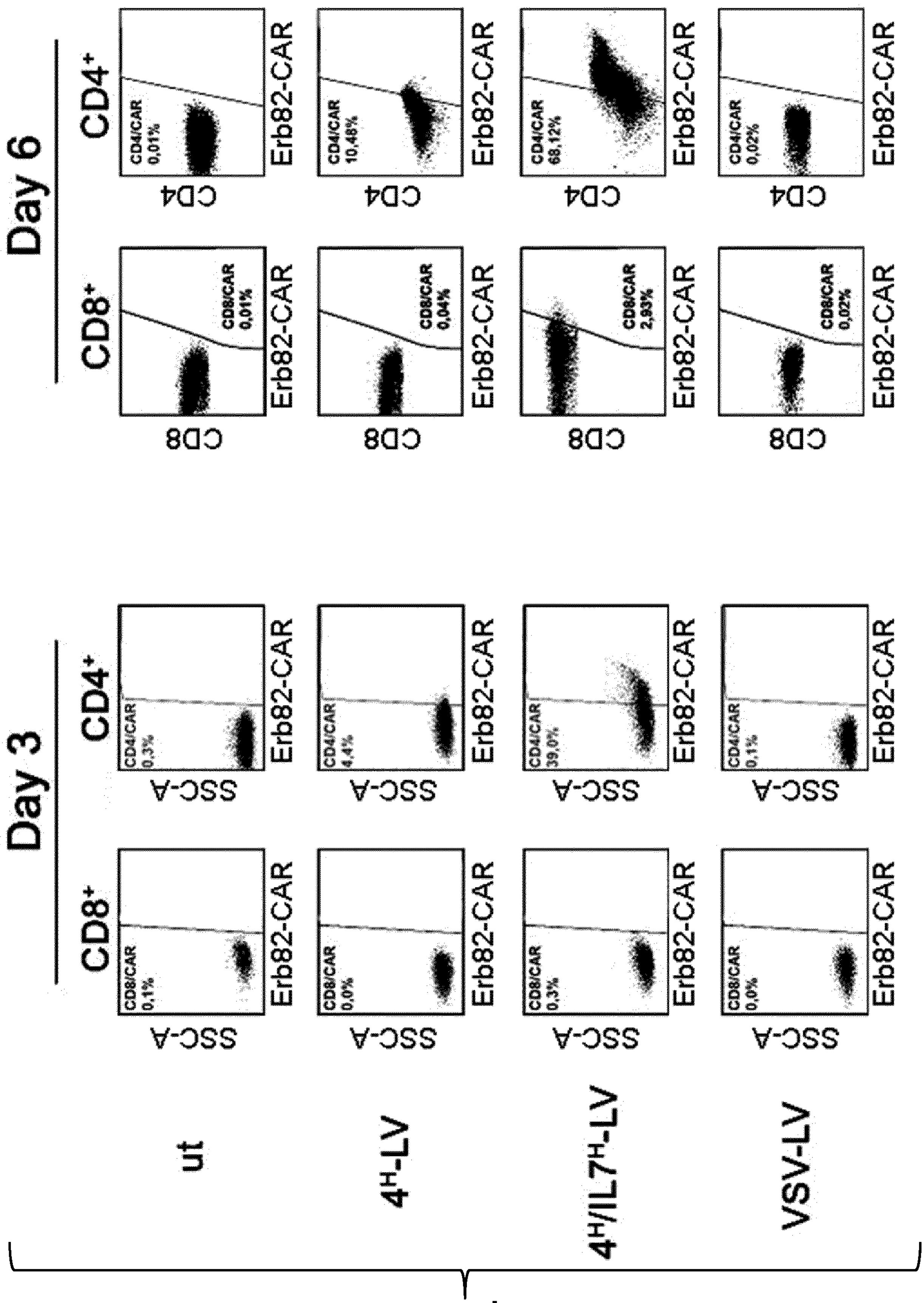

Day 3
Day 6
CD8⁺
CD4⁺
ut
4H-LV
4H/IL7H-LV
VSV-LV
SSC-A
CD8
CD4
Erb82-CAR
CD8/CAR 0,1%
CD4/CAR 0,3%
CD8/CAR 0,01%
CD4/CAR 0,01%
CD8/CAR 0,0%
CD4/CAR 4,4%
CD8/CAR 0,04%
CD4/CAR 10,48%
CD8/CAR 0,3%
CD4/CAR 39,0%
CD8/CAR 2,93%
CD4/CAR 68,12%
CD8/CAR 0,0%
CD4/CAR 0,1%
CD8/CAR 0,02%
CD4/CAR 0,02%

FIG.1

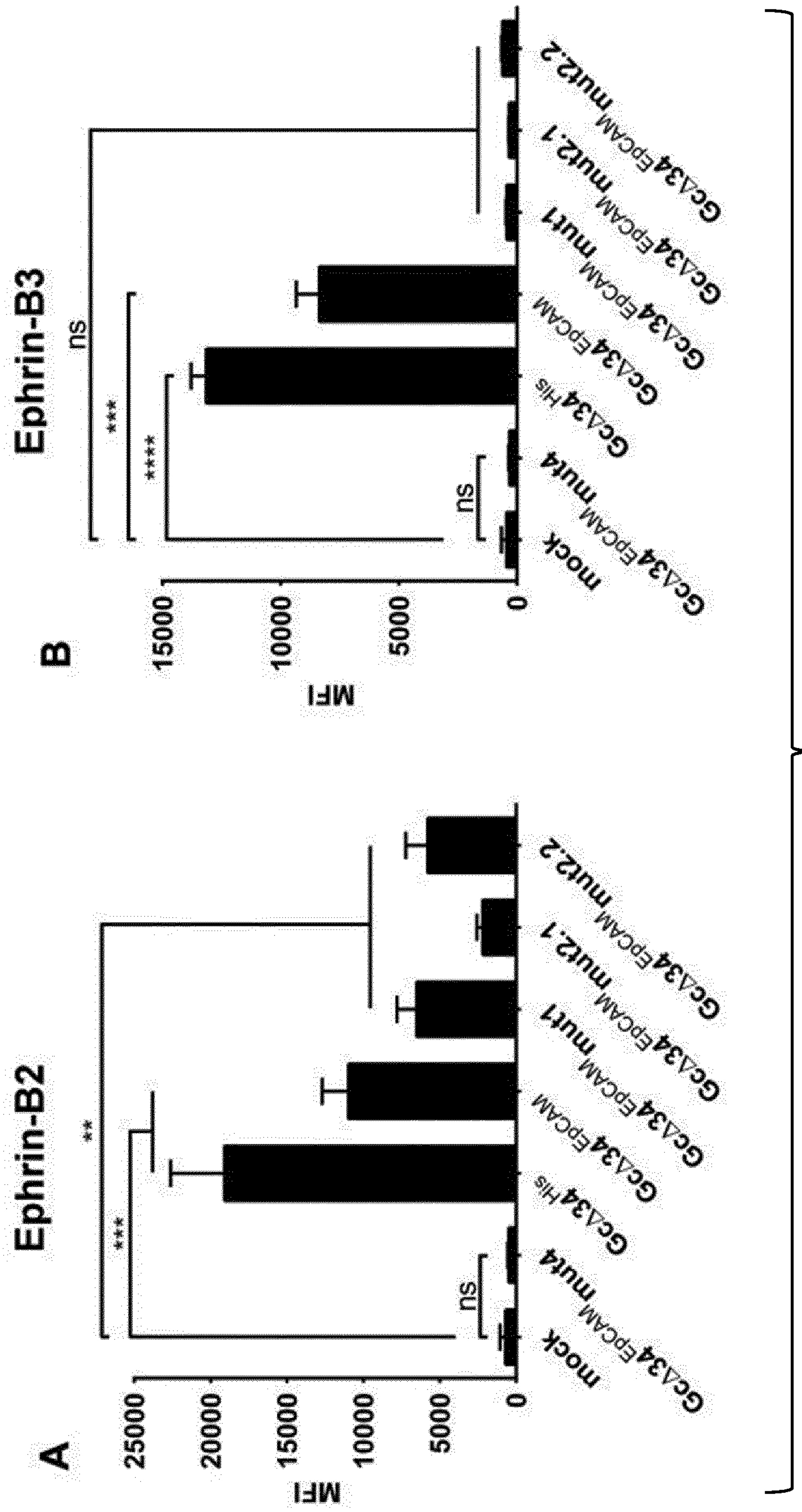

A
Ephrin-B2
MFI
25000
20000
15000
10000
5000
0
***
**
ns
mock
GcΔ34EpCAM mut4
GcΔ34His
GcΔ34EpCAM
GcΔ34EpCAM mut1
GcΔ34EpCAM mut2.1
GcΔ34EpCAM mut2.2
B
Ephrin-B3
MFI
15000
10000
5000
0
****
***
ns
ns
mock
GcΔ34EpCAM mut4
GcΔ34His
GcΔ34EpCAM
GcΔ34EpCAM mut1
GcΔ34EpCAM mut2.1
GcΔ34EpCAM mut2.2

FIG.2

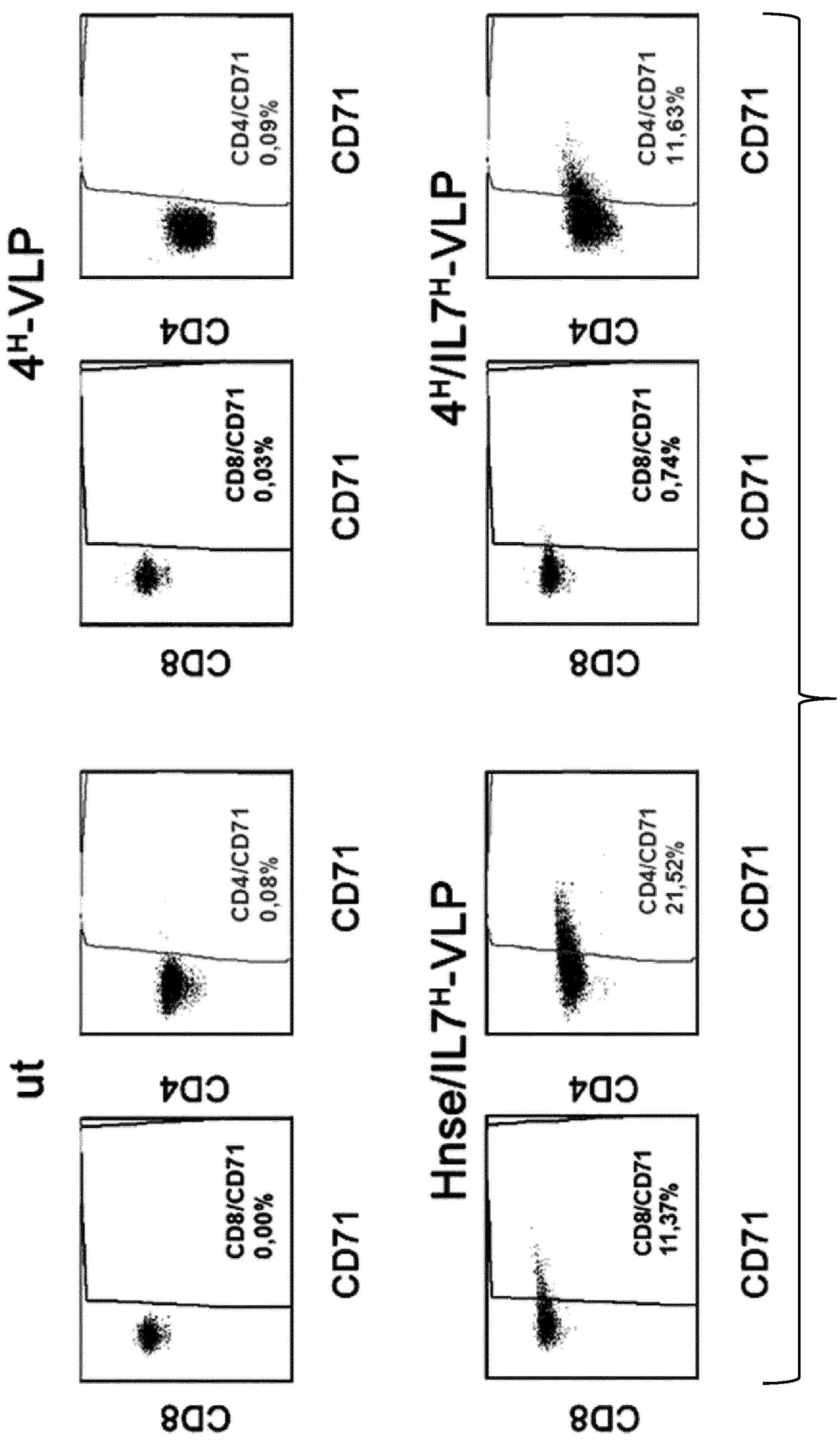

ut
CD8
CD71
CD8/CD71
0,00%
CD4
CD71
CD4/CD71
0,08%
4H-VLP
CD8
CD71
CD8/CD71
0,03%
CD4
CD71
CD4/CD71
0,09%
Hnse/IL7H-VLP
CD8
CD71
CD8/CD71
11,37%
CD4
CD71
CD4/CD71
21,52%
4H/IL7H-VLP
CD8
CD71
CD8/CD71
0,74%
CD4
CD71
CD4/CD71
11,63%

FIG.3

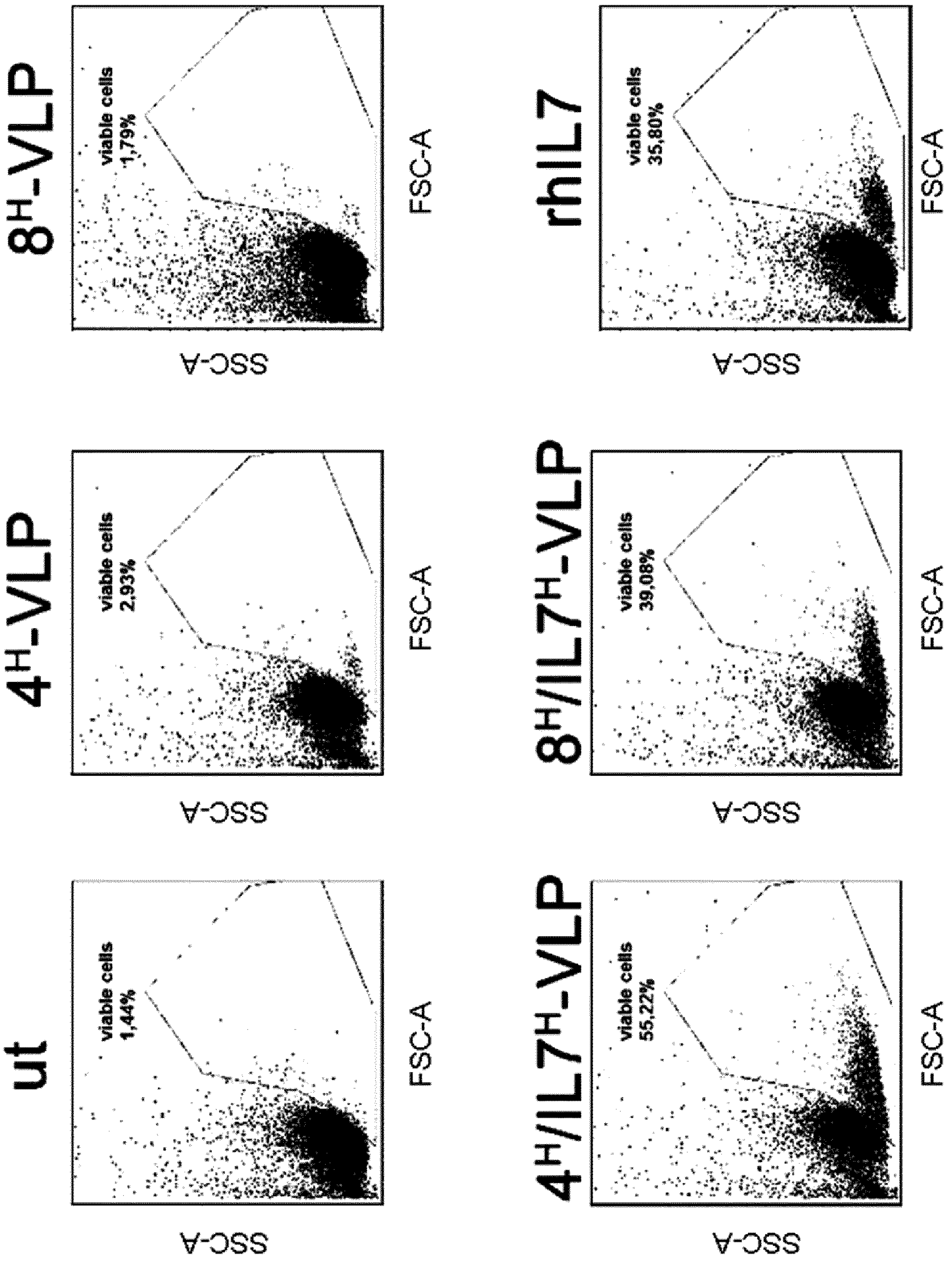
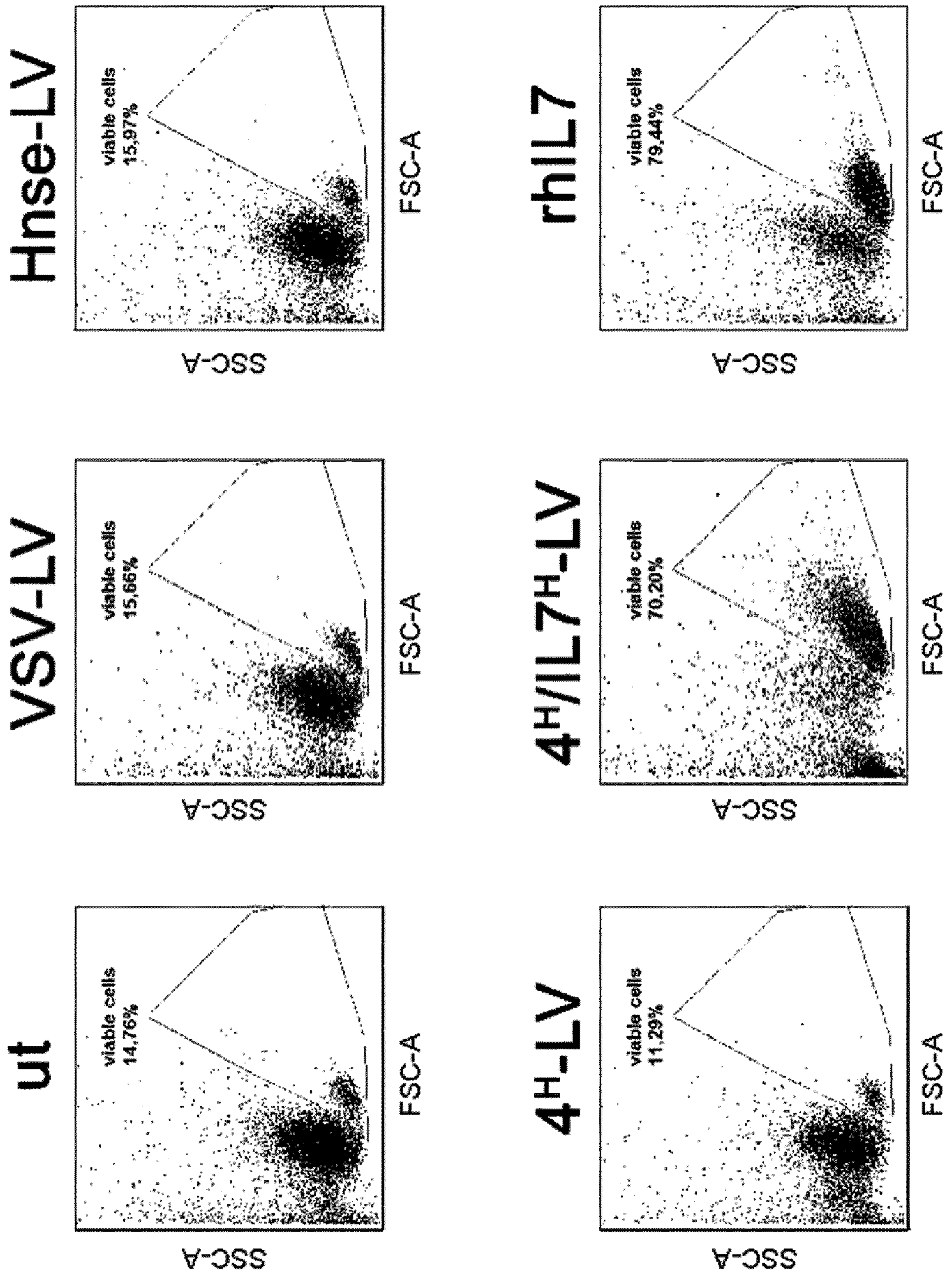

8H-VLP
viable cells 1,79%
SSC-A
FSC-A
rhIL7
viable cells 35,80%
4H-VLP
viable cells 2,93%
8H/IL7H-VLP
viable cells 39,08%
ut
viable cells 1,44%
4H/IL7H-VLP
viable cells 55,22%

FIG.4



Hnse-LV
viable cells 15,97%
rhIL7
viable cells 79,44%
VSV-LV
viable cells 15,66%
4H/IL7H-LV
viable cells 70,20%
ut
viable cells 14,76%
4H-LV
viable cells 11,29%
SSC-A
FSC-A

FIG.5

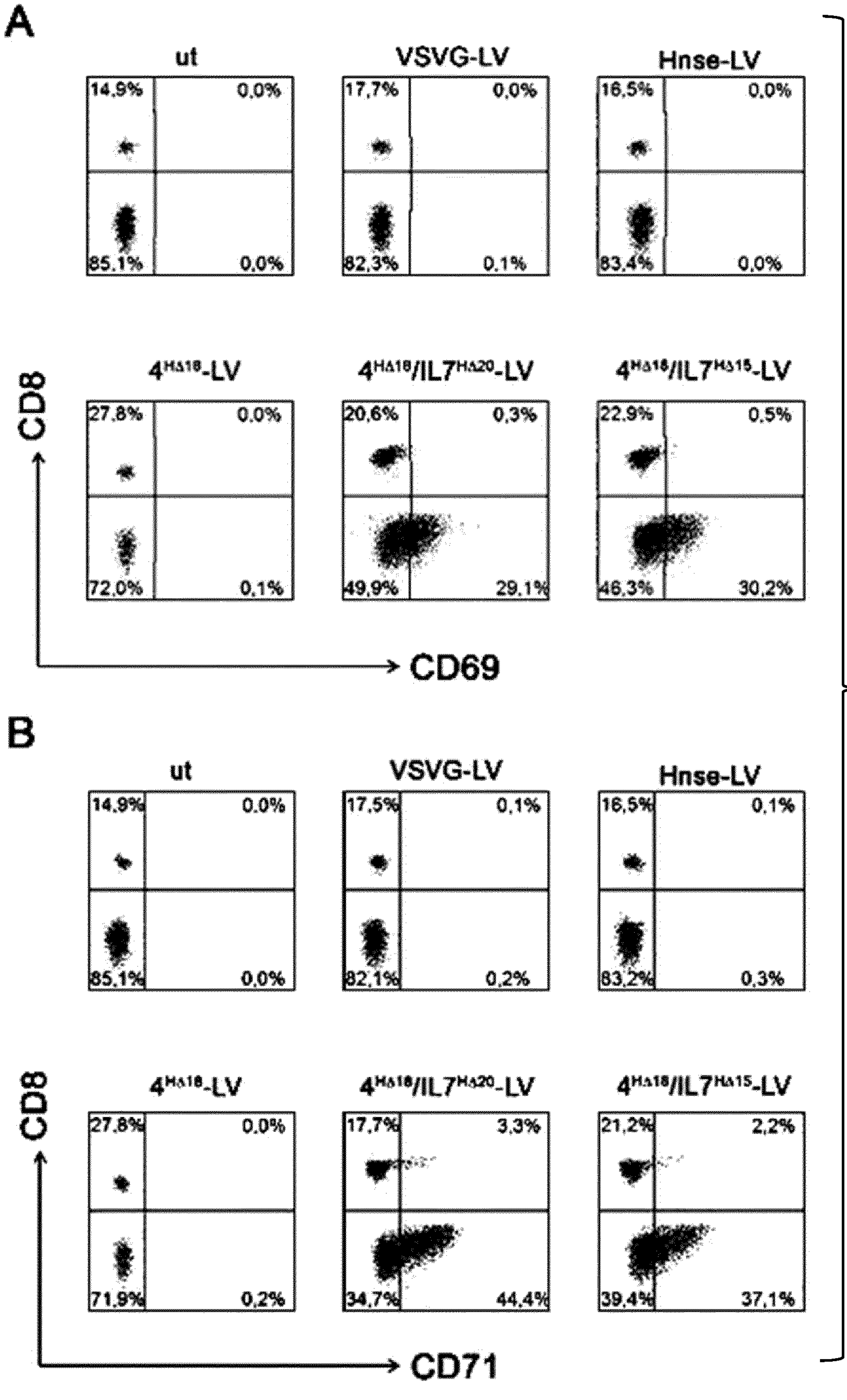

A
ut
VSVG-LV
Hnse-LV
14,9%
0,0%
85,1%
0,0%
17,7%
0,0%
82,3%
0,1%
16,5%
0,0%
83,4%
0,0%
4HΔ18-LV
4HΔ18/IL7HΔ20-LV
4HΔ18/IL7HΔ15-LV
27,8%
0,0%
72,0%
0,1%
20,6%
0,3%
49,9%
29,1%
22,9%
0,5%
46,3%
30,2%
CD8
CD69
B
ut
VSVG-LV
Hnse-LV
14,9%
0,0%
85,1%
0,0%
17,5%
0,1%
82,1%
0,2%
16,5%
0,1%
83,2%
0,3%
4HΔ18-LV
4HΔ18/IL7HΔ20-LV
4HΔ18/IL7HΔ15-LV
27,8%
0,0%
71,9%
0,2%
17,7%
3,3%
34,7%
44,4%
21,2%
2,2%
39,4%
37,1%
CD8
CD71

FIG.6

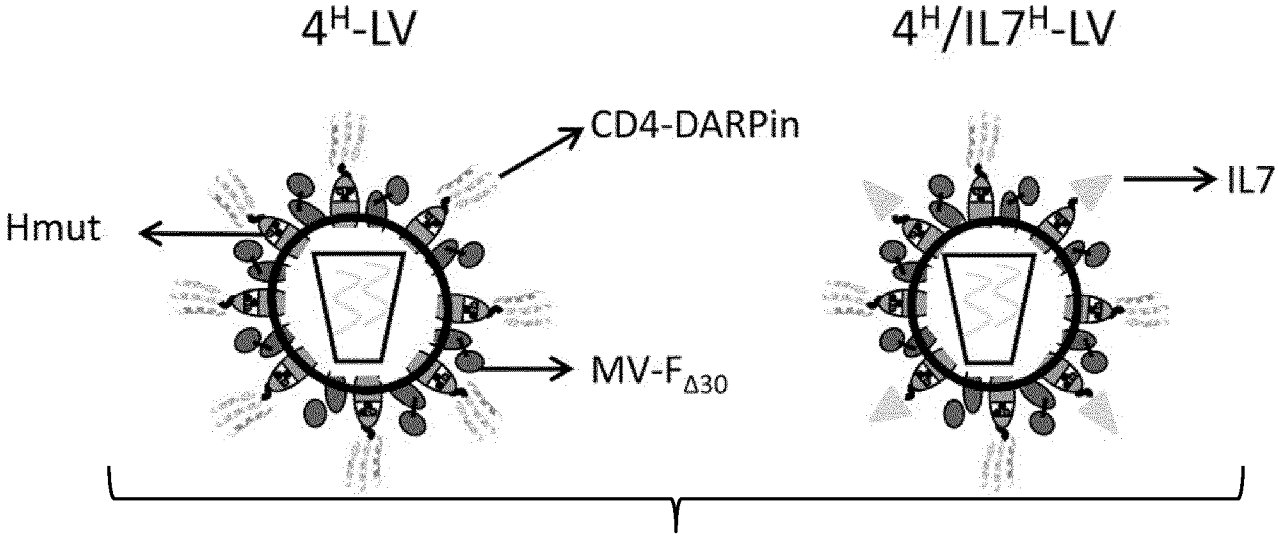

4H-LV
4H/IL7H-LV
CD4-DARPin
Hmut
MV-FΔ30
IL7

FIG.7

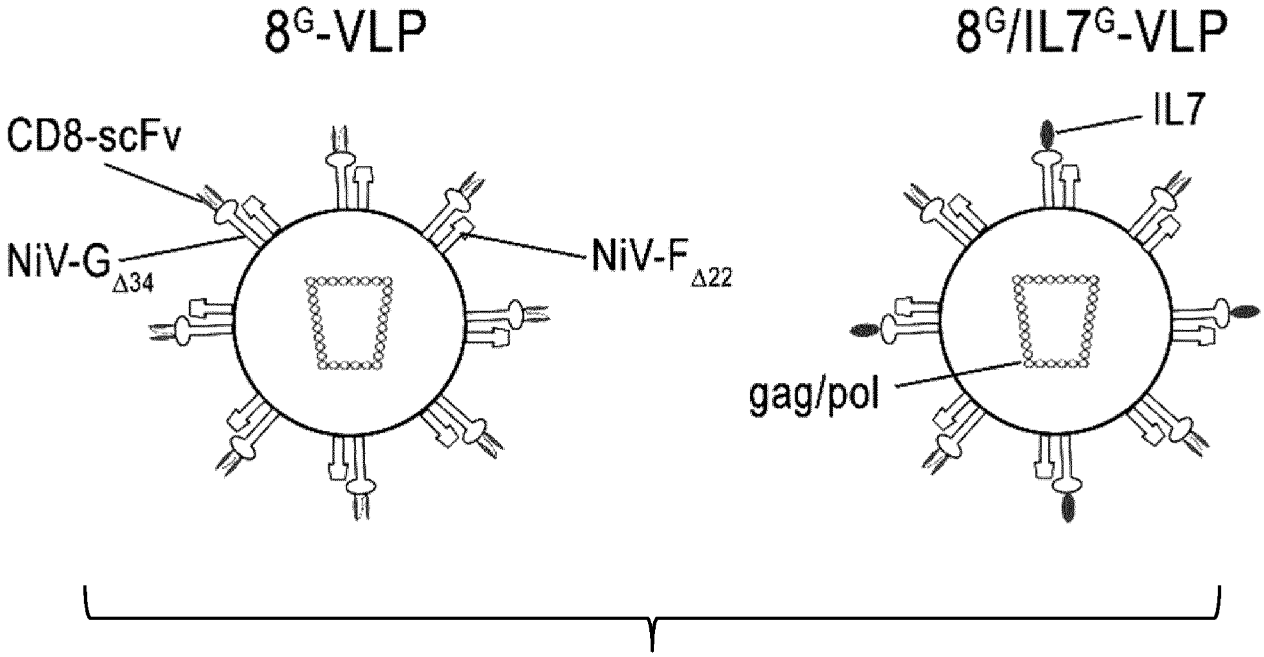

8G-VLP
8G/IL7G-VLP
CD8-scFv
NiV-GΔ34
NiV-FΔ22
IL7
gag/pol

FIG.8

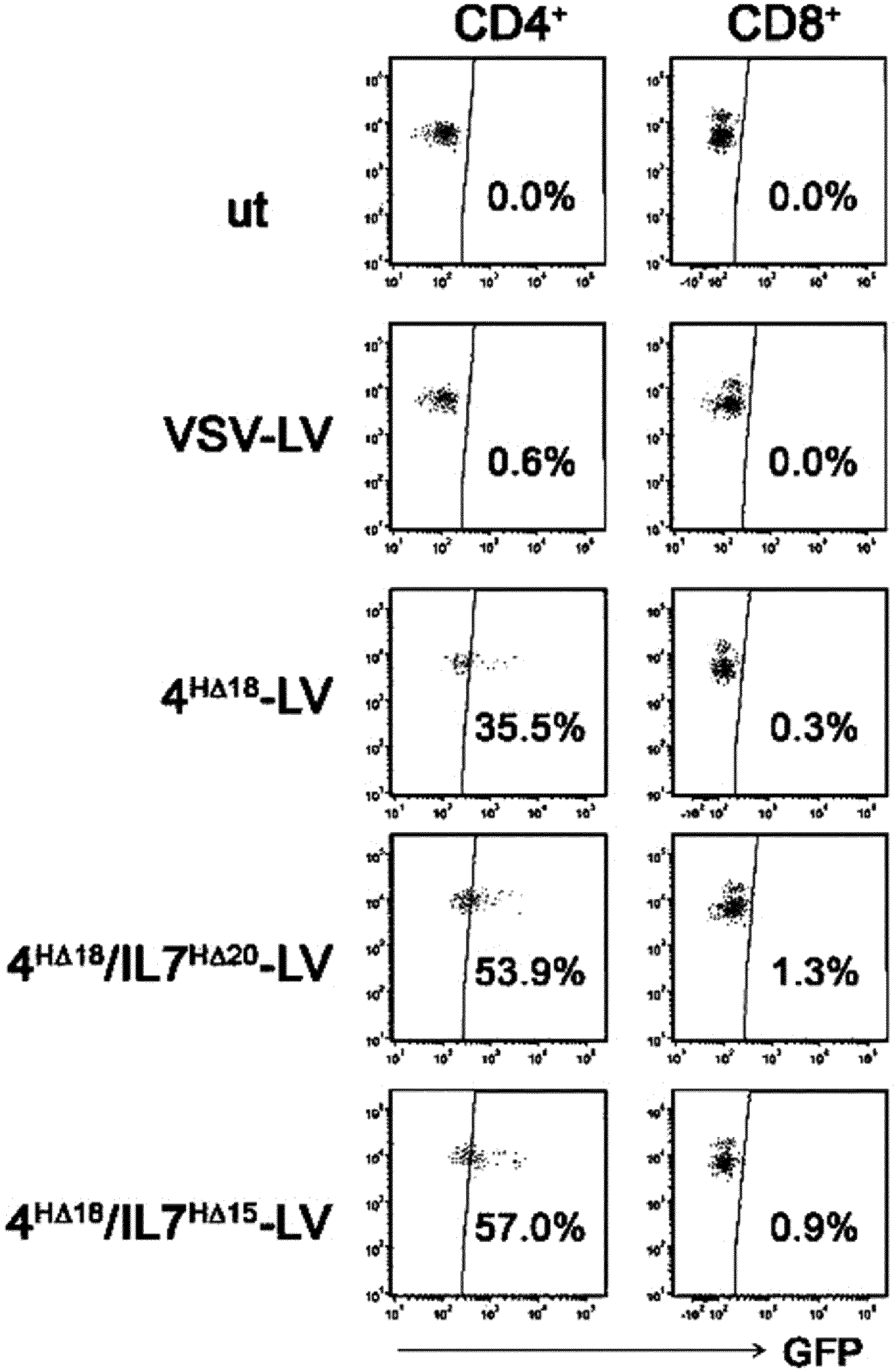

CD4+
CD8+
ut
0.0%
0.0%
VSV-LV
0.6%
0.0%
4HΔ18-LV
35.5%
0.3%
4HΔ18/IL7HΔ20-LV
53.9%
1.3%
4HΔ18/IL7HΔ15-LV
57.0%
0.9%
GFP

FIG.9

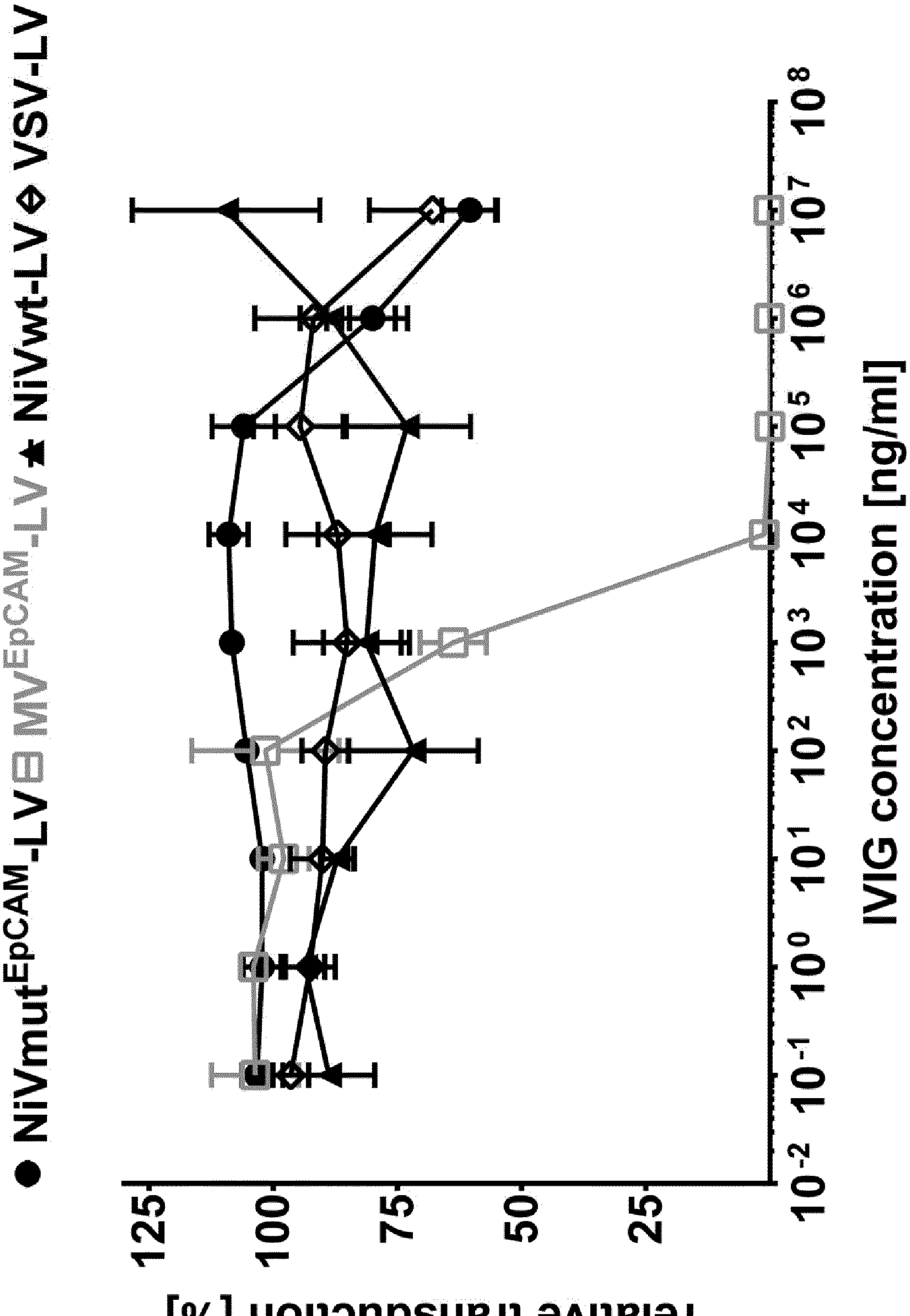

NiVmut^EpCAM-LV
MV^EpCAM-LV
NiVwt-LV
VSV-LV
relative transduction [%]
125
100
75
50
25
IVIG concentration [ng/ml]
10^-2
10^-1
10^0
10^1
10^2
10^3
10^4
10^5
10^6
10^7
10^8

FIG.10

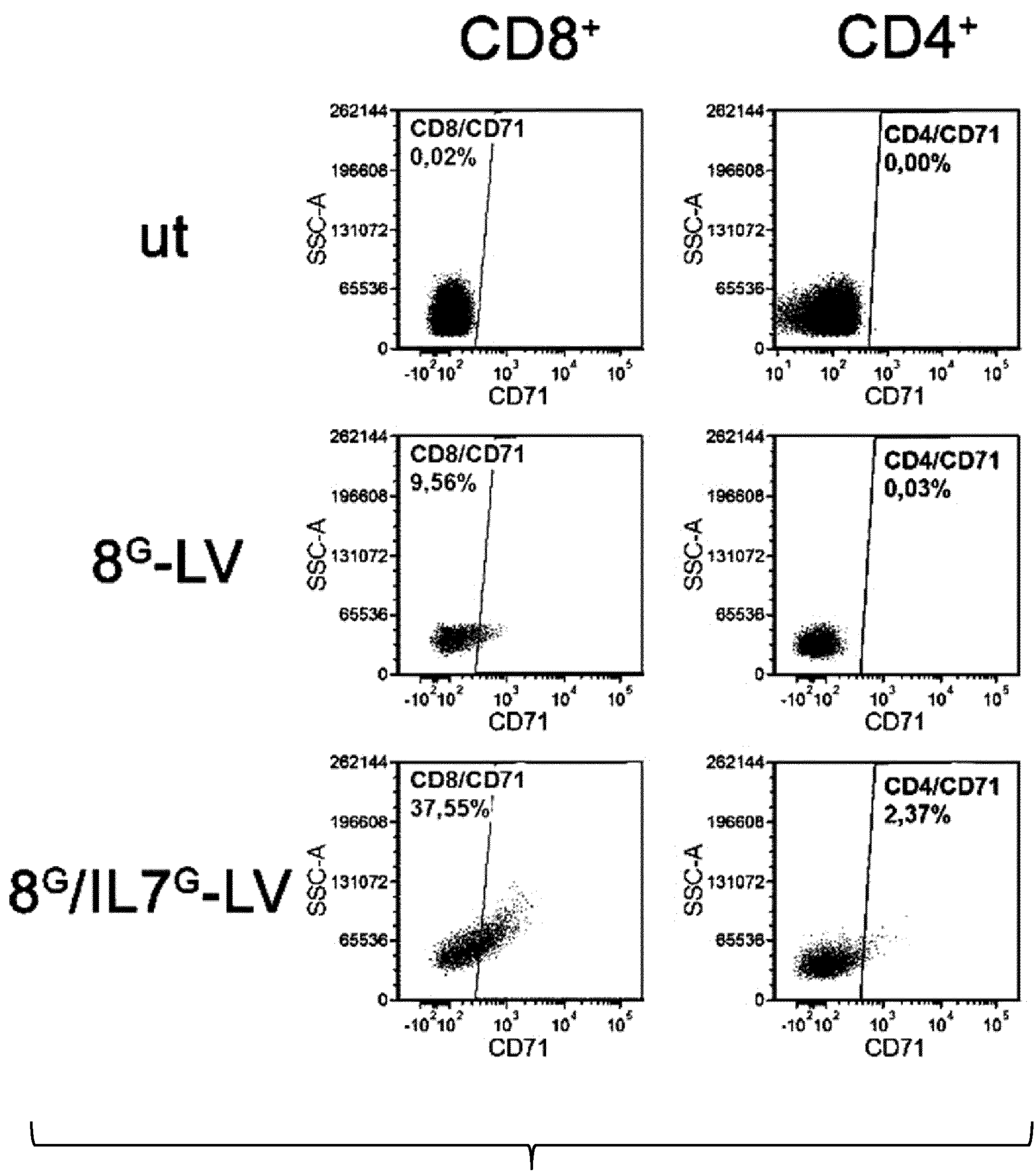

CD8+
CD4+
ut
8G-LV
8G/IL7G-LV
CD8/CD71
0,02%
CD4/CD71
0,00%
CD8/CD71
9,56%
CD4/CD71
0,03%
CD8/CD71
37,55%
CD4/CD71
2,37%
SSC-A
CD71
262144
196608
131072
65536
0

FIG.11

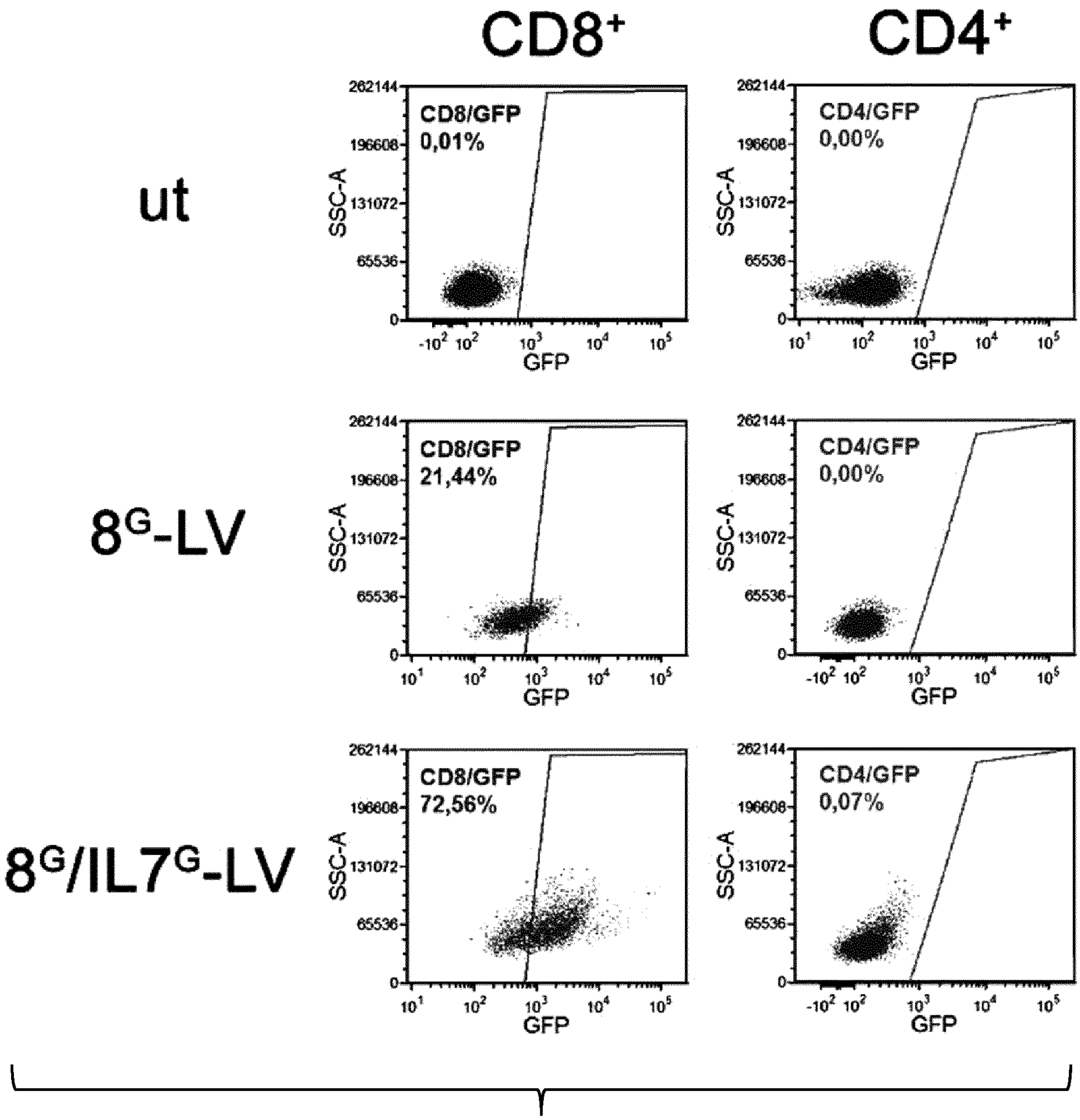

CD8+
CD4+
ut
8G-LV
8G/IL7G-LV
CD8/GFP
0,01%
CD4/GFP
0,00%
CD8/GFP
21,44%
CD4/GFP
0,00%
CD8/GFP
72,56%
CD4/GFP
0,07%
SSC-A
GFP
262144
196608
131072
65536
0

FIG.12

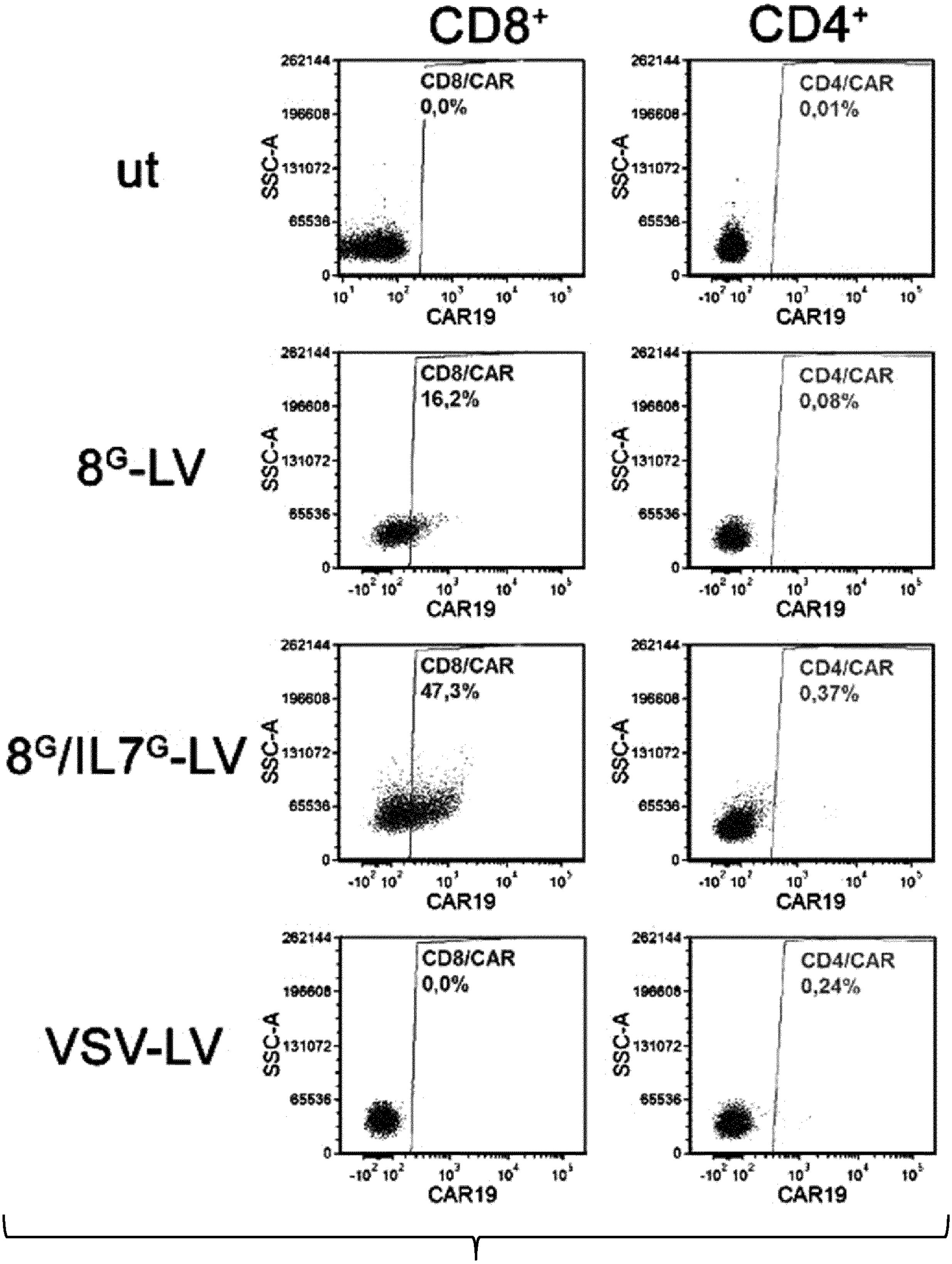

CD8⁺
CD4⁺
ut
8G-LV
8G/IL7G-LV
VSV-LV
SSC-A
CAR19
CD8/CAR 0,0%
CD4/CAR 0,01%
CD8/CAR 16,2%
CD4/CAR 0,08%
CD8/CAR 47,3%
CD4/CAR 0,37%
CD8/CAR 0,0%
CD4/CAR 0,24%

FIG.13

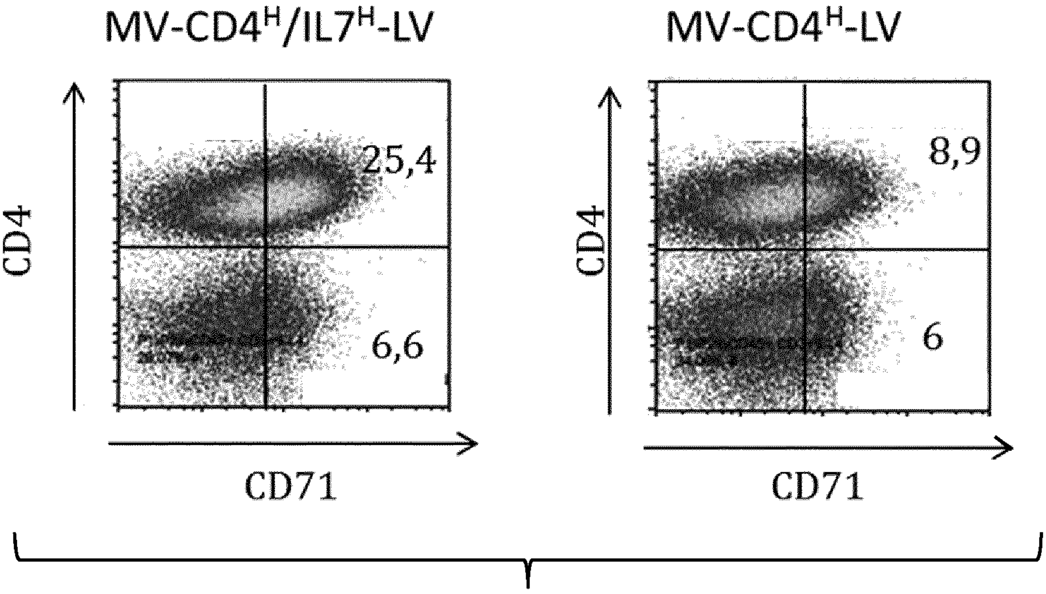


FIG.14

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2017/059435

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/86
ADD.

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

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 practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SABRINA FUNKE ET AL: "Targeted cell entry of lentiviral vectors", MOLECULAR THERAPY, NATURE PUBLISHING GROUP, GB , vol. 16, no. 8 1 August 2008 (2008-08-01), pages 1427-1436, XP008155467, ISSN: 1525-0016, DOI: DOI:10.1038/MT.2008.128 Retrieved from the Internet: URL:http://www.nature.com/mt/journal/v16/n8/index.html [retrieved on 2008-06-24] page 1428, right-hand column, last paragraph - page 1429, left-hand column, paragraph 1; figure 3a -----	12,14

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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 application or patent 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
30 May 2017	13/06/2017

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	Brenz Verca, Stefano

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRIGITTE ANLIKER ET AL: "Specific gene transfer to neurons, endothelial cells and hematopoietic progenitors with lentiviral vectors", NATURE METHODS, vol. 7, no. 11, 1 November 2010 (2010-11-01), pages 929-935, XP055033622, ISSN: 1548-7091, DOI: 10.1038/nmeth.1514 page 930, left-hand column, last paragraph - right-hand column, line 7; figure 1a -----	12,14
X	SABRINA KNEISSL ET AL: "Measles Virus Glycoprotein-Based Lentiviral Targeting Vectors That Avoid Neutralizing Antibodies", PLOS ONE, vol. 7, no. 10, 10 October 2012 (2012-10-10), page e46667, XP055309089, DOI: 10.1371/journal.pone.0046667 see the first 3 fusion protein constructs of Figure 1 -----	12,14
X	ROBERT C MÜNCH ET AL: "DARPins: An Efficient Targeting Domain for Lentiviral Vectors", MOLECULAR THERAPY, vol. 19, no. 4, 1 April 2011 (2011-04-01), pages 686-693, XP055092878, ISSN: 1525-0016, DOI: 10.1038/mt.2010.298 figure 1a -----	12,14
X	T ENKIRCH ET AL: "Targeted lentiviral vectors pseudotyped with the Tupaia paramyxovirus glycoproteins", GENE THERAPY, vol. 20, no. 1, 1 January 2013 (2013-01-01), pages 16-23, XP055309550, GB ISSN: 0969-7128, DOI: 10.1038/gt.2011.209 figure 1a ----- -/--	12,14

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. IMAMURA ET AL: "Autonomous growth and increased cytotoxicity of natural killer cells expressing membrane-bound interleukin-15", BLOOD, vol. 124, no. 7, 14 August 2014 (2014-08-14), pages 1081-1088, XP055309476, US ISSN: 0006-4971, DOI: 10.1182/blood-2014-02-556837 see the abstract, construct mbIL15 and the section "Plasmids, gene transduction, and functional analysis of NK cells"; figure 1A -----	12,14
X	E. VERHOEYEN ET AL: "IL-7 surface-engineered lentiviral vectors promote survival and efficient gene transfer in resting primary T lymphocytes", BLOOD, vol. 101, no. 6, 15 March 2003 (2003-03-15), pages 2167-2174, XP055272100, US ISSN: 0006-4971, DOI: 10.1182/blood-2002-07-2224 cited in the application see section "Envelope construction"; page 2169, left-hand column, last paragraph - right-hand column, paragraph 1 -----	12,14
A	C. FRECHA ET AL: "Stable transduction of quiescent T cells without induction of cycle progression by a novel lentiviral vector pseudotyped with measles virus glycoproteins", BLOOD, vol. 112, no. 13, 15 December 2008 (2008-12-15), pages 4843-4852, XP055309112, US ISSN: 0006-4971, DOI: 10.1182/blood-2008-05-155945 page 4848, left-hand column, paragraph 2 - page 4850, right-hand column, paragraph 2 -----	1-15
X	WO 2008/037458 A2 (BUNDESREPUBLIK DEUTSCHLAND LET [DE]; BUCHHOTZ CHRISTIAN [DE]; FUNKE SA) 3 April 2008 (2008-04-03) page 26, line 26 - page 33, line 30; claims 9-11,32,33,34; figure 5C; examples 5-8,10 ----- -/--	12,14

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 2 615 176 A1 (PAUL EHRLICH INST BUNDESAMT FUER SERA UND IMPFSTOFFE [DE]) 17 July 2013 (2013-07-17) paragraph [0008]; example 1 -----	12,14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2017/059435

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008037458 A2	03-04-2008	DK 2066795 T3	26-01-2015
		EP 1975239 A1	01-10-2008
		EP 2066795 A2	10-06-2009
		ES 2520024 T3	11-11-2014
		US 2010189690 A1	29-07-2010
		WO 2008037458 A2	03-04-2008
EP 2615176 A1	17-07-2013	EP 2615176 A1	17-07-2013
		EP 2802662 A1	19-11-2014
		US 2015291979 A1	15-10-2015
		WO 2013104728 A1	18-07-2013