- The transfer of one or more non-coding RNAs such as RNAs capable of inducing an effect on gene expression, for example via shRNA, miRNA, sgRNA, LncRNA or circRNA.
- The transfer of cell RNAs, of messaenger RNA type or other type (miRNA, etc.), sub genome replicons of virus RNA (VHC, etc.) or complete virus RNA genomes.
- The simultaneous expression of coding or non-coding sequences that are endogenous or exogenous to the target cell,
- The participation in the genome modification of the target cell by genetic engineering systems, for example the CRISPR system.

Advantageously, the retroviral particle according to the invention comprises a nucleocapsid protein, an envelope protein, optionally an integrase and at least two encapsidated non-viral RNAs, the encapsidated non-viral RNAs each comprising an RNA sequence of interest linked to an encapsidation sequence, each encapsidation sequence being recognized by a binding domain introduced into the nucleocapsid protein and/or into the integrase.

By “nucleocapsid protein” is meant the structural protein NC coded by the *gag* gene. When the binding domain is introduced into the nucleocapsid protein, that protein is then a chimeric protein, coming from a chimeric *gag* gene.

When the binding domain is introduced into the integrase, the integrase is then a chimeric protein, coming from a chimeric *pol* gene.

By “chimeric protein” is designated a recombinant protein comprising several different protein sequences that are combined.

According to a first embodiment, in the retroviral particle according to the invention, the binding domain is introduced into the nucleocapsid protein and the at least two non-viral encapsidated RNAs are distinguished by their RNA sequence.

This first embodiment enables early transient expression of the RNAs of interest, without any associated integration event in the genome of the target cells.

As a matter of fact, on placing a particle according to this first embodiment in contact with a target cell, the membrane of the retroviral particle and that of the target cell will combine and enable the release of the content of the particle into the target cell. The RNAs are then released into the cytoplasm and the cell mechanisms enable those RNAs to be directly translated into protein(s), that is to say without additional steps such as reverse transcription, translocation into the nucleus or integration into the genome of the target cell.

- **Encapsidation plasmid:** The p8.74, encapsidation plasmid, which carries the genes coding for the structural and functional proteins (Gag, Pol), is used for the production of the integrating lentiviral vectors (Figure 6).
- **Envelope plasmid** (pENV) : This plasmid is identical to the envelope plasmid used for the production of MS2RLP lentiviral particles (Figure 6).

1.3 Plasmids for the production of integration-deficient lentiviral vectors IDLVs: mutant D64L

The 3 plasmids are the same as those described in point 1.2 except for the encapsidation plasmid which comprises a D64L mutation of the *pol* sequence coding for the Integrase (Figure 7). This mutation on a single amino acid induces inhibition of the integration (Nightingale et al., 2006 and Apolonia et al., 2007). The D64L mutation on the integrase (IN) was introduced in the p8.74 plasmid by site-directed mutagenesis. The mutation was verified by sequencing.

1.4 pLuc and pCre plasmids

These expression plasmids are similar to those used for the production of the ILV and IDLV vectors (Figure 5b for pLuc and Figure 5c for pCre), used directly in transfection for control.

2. Batch production

After the transfection of the plasmids into cells, the supernatants are harvested and used crude or concentrated/purified according to the method described in application WO 2013/014537.

2.1 Production of the lentiviral vectors and lentiviral particles

The productions are carried out in a 10-stack CellSTACK (6360 cm^2, Corning) with HEK293T cells (ATCC, CRL-11268), cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, Paisley, UK) supplemented with 1% penicillin/streptomycin and 1% ultraglutamine (PAA) at 37°C in moist atmosphere with 5% CO_2. For the serum impact study assay, the DMEM is supplemented with 10% SVF. In particular, no induction with sodium butyrate is carried out. For each batch (MS2RLP, ILV and IDLV), the transfection mixture is composed of the following three plasmids:

- One of the expression plasmids described above, depending on whether

TU/mL) by qPCR is normalized with the internal calibration reference of which the titer was determined in advance by FACS.

3.2 Quantization of the physical particles by P24 Elisa test

The p24 capsid protein is directly detected in the viral supernatant using and following the recommendations for the HIV-1 p24 ELISA kit (Perkin Elmer). The p24 protein captured is complexed with a biotinylated polyclonal antibody, then detected by a streptavidin-HRP peroxidase conjugate. The resulting complex is detected by spectrophotometry after incubation with the substrate ortho-phenylenediamine-HCl (OPD) which produces a yellow coloration that is directly proportional to the amount of p24 captured. The absorbance of each well is quantified using the microplate reader Synergy H1 Hybrid (Biotek) and calibrated against absorbance of a p24 protein calibration reference range. The viral titer expressed in physical particles per mL is calculated from the p24 protein concentration obtained knowing that 1pg of p24 protein corresponds to 10^4 physical particles.

4. Monotransduction

This example is directed to developing the conditions for monotransduction by the lentiviral vectors or the lentiviral particles produced above.

5. Expression kinetics for ZsGreenI

The HCT116 cells seeded the previous day with 25000 cells/cm2 in a 24-well plate (Corning) are transduced to 100000 PP/cell in the presence of 8 µg/mL of Polybrene®. At 8h and 24h post-transduction, the cells are trypsinized and analyzed by flow cytometry to measure the percentage of fluorescent cells. Each assay is carried out in triplicate.

6. Expression kinetics for luciferase

6.1 HCT116 cells

The HCT116 cells (ATCC, CCL-247) are seeded in 6 or 24-well plates and incubated for 24h at 37°C / 5% CO2. The transduction by ILV or IDLV vectors or MS2RLP particles is carried out in the presence of 4µg/mL Polybrene. The transduction supernatant is eliminated 4 hours after and replaced by fresh

To increase the effectiveness of RNA transfer, the results obtained by Sutlu *et al*. (2012) to increase the transduction effectiveness of Natural Killer NK cells were transposed. More particularly, the hypothesis was formulated that the antiviral responses based on the Toll-like receptor (TLR) and/or RIG-I-like receptor (RLR) could restrict the effectiveness of the RNA transfer in certain cells. In order to test this hypothesis, an inhibitor of small signaling molecules TLR and RLR was used during the placing in contact of the cells with the MS2RLP particles. The BX795 molecule is an inhibitor of the BK1/IKKε complex, which acts as a common mediator in the signaling paths of RIG-I, MDA-5, and TLR3.

The use of the BX795 molecule at a concentration of 6 µM considerably increases the transduction effectiveness of HCT116 cells (Figure 14) and of Foreskin fibroblasts (Figure 13). The observation time (8h for the MS2RLP particles, 24h for the ILVs) takes into account the functioning of each particle (Figures 8 and 9). A synergistic effect may be observed between the MS2RLP particles and the use of BX795 in the HCT116 cells as in the Foreskin fibroblasts, whereas the BX795 induces a negative effect with the integrating lentiviral vectors ILV in these two cell types. This effect may be linked to a differential in the number of RNA molecules delivered by these two types of particles.

5. Study of gene transfer by Luciferase MS2RLP particles *in vivo*.

The injection *in vivo* of MS2RLP-Luc particles is directed to revealing the expression kinetics of a transgene, in the present case, luciferase, in the various mouse organs injected systemically. A suspension of purified ILV-EF1-Luc integrating vectors is used as positive control. The measurements are made as of 5h and up until 7 days post-injection. The intravenous administration engenders a dilution of the injected suspension and therefore a dispersion of the bioluminescence signal which is to be considered. The analyses are therefore made on the whole animal in order to take into account the distribution of the signal throughout the body. A suspension of MS2RLP-Luc particles produced in accordance with the method P1 in the presence of serum and a suspension of MS2RLP-Luc particles purified in accordance with the P2 method are tested. The injection of the suspension of MS2RLP-Luc particles, in accordance with the p2 method in the presence of serum in which the purification is less, led to difficulties both for sampling and for injection. As a matter of fact, this suspension of MS2RLP-Luc particles, obtained by the P1 method in the presence of serum, is characterized by a brown suspension of high viscosity which is particularly

CLAIMS

1. A retroviral particle comprising:

- a protein coming from the Gag polyprotein,
- an envelope protein,
- optionally an integrase, and
- at least two encapsidated different non-viral RNAs,

the encapsidated different non-viral RNAs each comprising an RNA sequence of interest linked to an encapsidation sequence, each encapsidation sequence being recognized by a binding domain introduced into the protein coming from the Gag polyprotein or alternatively, when the retroviral particle comprises an integrase, each encapsidation sequence being recognized by a binding domain introduced into the protein coming from the Gag polyprotein and/or into the integrase.

2. A retroviral particle according to claim 1, comprising:

- a nucleocapsid protein,
- an envelope protein,
- optionally an integrase, and
- at least two encapsidated different non-viral RNAs,

the encapsidated different non-viral RNAs each comprising an RNA sequence of interest linked to an encapsidation sequence, each encapsidation sequence being recognized by a binding domain introduced into the nucleocapsid protein or alternatively, when the retroviral particle comprises an integrase, each encapsidation sequence being recognized by a binding domain introduced into the nucleocapsid protein and/or into the integrase.

3. A retroviral particle according to claim 2, wherein the binding domain is introduced into the nucleocapsid protein ~~and the at least two non-viral encapsidated RNAs are distinguished by their RNA sequence~~.

4. A particle according to claim 3, wherein a second binding domain is introduced into the nucleocapsid protein.

5. A particle according to claim 3 or 4, wherein a binding domain is also introduced into the integrase.

6. A retroviral particle according to any one of claims 2 to 5, which is a lentiviral particle.

7. A lentiviral particle according to claim 6, wherein
   - the binding domain is the sequence of the Coat protein of the bacteriophage MS2,
   - the encapsidation sequence of the non-viral RNAs is a stem-loop sequence of MS2,

- the nucleocapsid protein is the nucleocapsid protein (NC) of HIV belonging to the Gag polyprotein, which NC is mutated at the second zinc finger in order to insert the Coat protein sequence of the bacteriophage MS2.

8. A lentiviral particle according to claim 6, wherein
   - the binding domain is the sequence of the Coat protein of the bacteriophage PP7,
   - the encapsidation sequence of the non-viral RNAs is a stem-loop sequence of PP7,
   - the nucleocapsid protein is the nucleocapsid protein (NC) of HIV belonging to the Gag polyprotein, which NC is mutated at the second zinc finger in order to insert the Coat protein sequence of the bacteriophage PP7.

9. A retroviral particle according to claim 1 or 2, wherein the binding domain is introduced into the integrase.

10. A composition comprising particles according to any one of claims 1 to 9.

11. A kit for producing particles according to any one of claims 1 to 9, comprising:
   (i) an expression plasmid comprising at least two different non-viral RNA sequences, each RNA sequence comprising ~~one~~ a sequence of interest, for which upstream or downstream of that sequence of interest is inserted an encapsidation sequence,
   (ii) an encapsidation plasmid coding for a protein coming from the Gag polyprotein and~~/or~~ optionally an integrase, the protein coming from the Gag polyprotein, which ~~are~~ is chimeric, comprising a binding domain enabling an encapsidation sequence to be recognized or alternatively, when the encapsidation plasmid codes for an integrase, the protein coming from the Gag polyprotein and/or the integrase, which are chimeric, comprising a binding domain enabling an encapsidation sequence to be recognized, and
   (iii) an envelope plasmid coding for an envelope protein.

12. A kit for producing particles according to any one of claims 3 to 8, comprising
   (i) an expression plasmid comprising at least two different non-viral RNA sequences, each RNA sequence comprising a sequence of interest for which upstream or downstream of that sequence of interest is inserted an

encapsidation sequence, or alternatively a first and a second expression plasmid each comprising a sequence of interest upstream or downstream of which is inserted an encapsidation sequence,

(ii) an encapsidation plasmid coding for a chimeric nucleocapsid protein comprising a binding domain making it possible to recognize the encapsidation sequence, and,

(iii) an envelope plasmid coding for an envelope protein.

13. A kit for producing particles according to claim 12, comprising:

(i) an expression plasmid comprising at least two sequences of interest, for which upstream or downstream of each of these sequences is inserted an encapsidation sequence, or alternatively a first and a second expression plasmid each comprising a sequence of interest upstream or downstream of which is inserted an encapsidation sequence,

the sequences of interest being different and the encapsidation sequences being identical,

(ii) an encapsidation plasmid coding for a chimeric nucleocapsid protein comprising a binding domain making it possible to recognize the encapsidation sequence, and,

(iii) an envelope plasmid coding for an envelope protein.

14. A method of manufacturing a kit according to claim 11, comprising:

(i) preparing an expression plasmid comprising at least ~~one~~ two different non-viral RNA sequences, each RNA sequence comprising a sequence of interest, for which upstream or downstream of that sequence of interest is inserted an encapsidation sequence,

(ii) preparing an encapsidation plasmid coding for a protein coming from the Gag polyprotein and optionally ~~/or~~ an integrase, the protein coming from the Gag polyprotein, which ~~are~~ is chimeric, comprising a binding domain enabling an encapsidation sequence to be recognized or alternatively, when the encapsidation plasmid codes for an integrase, the protein coming from the Gag polyprotein and/or the integrase, which are chimeric, comprising a binding domain enabling an encapsidation sequence to be recognized, and

(iii) preparing an envelope plasmid coding for an envelope protein.

15. A method of manufacturing a kit according to claim 12, comprising:

(i) preparing an expression plasmid comprising at least two different non-viral RNA sequences, each RNA sequence comprising a sequence of interest for which upstream or downstream of that sequence of interest is inserted

an encapsidation sequence, or alternatively a first and a second expression plasmid each comprising a sequence of interest upstream or downstream of which is inserted an encapsidation

sequence,

(ii) preparing an encapsidation plasmid coding for a chimeric nucleocapsid protein comprising a binding domain making it possible to recognize the encapsidation sequence, and,

(iii) preparing an envelope plasmid coding for an envelope protein.

16. A method of manufacturing a kit according to claim 13, comprising:

(i) preparing an expression plasmid comprising at least two sequences of interest, for which upstream or downstream of each of these sequences is inserted an encapsidation sequence, or alternatively a first and a second expression plasmid each comprising a sequence of interest upstream or downstream of which is inserted an encapsidation sequence,

the sequences of interest being different and the encapsidation sequences being identical,

(ii) preparing an encapsidation plasmid coding for a chimeric nucleocapsid protein comprising a binding domain making it possible to recognize the encapsidation sequence, and

(iii) preparing an envelope plasmid coding for an envelope protein.

17. A method of manufacturing particles according to any one of claims 1 to 9, comprising a step of co-transfecting cells with:

(i) an expression plasmid comprising at least ~~one~~ two different non-viral RNA sequences, each RNA sequence comprising a sequence of interest, for which upstream or downstream of that sequence of interest is inserted an encapsidation sequence,

(ii) an encapsidation plasmid coding for a protein coming from the Gag polyprotein and optionally~~/or~~ an integrase, the protein coming from the Gag polyprotein, which ~~are~~ is chimeric, comprising a binding domain enabling an encapsidation sequence to be recognized or alternatively, when the encapsidation plasmid codes for an integrase, the protein coming from the Gag polyprotein and/or the integrase, which are chimeric, comprising a binding domain enabling an encapsidation sequence to be recognized, and

(iii) an envelope plasmid coding for an envelope protein~~.~~,

and harvesting the supernatant of the transfected cells comprising the particles.

18. A method of manufacturing particles according to any one of claims 3 to 8,

comprising a step of co-transfecting cells with:

(i) an expression plasmid comprising at least two different non-viral RNA

sequences, each RNA sequence comprising a sequence of interest for which upstream or downstream of that sequence of interest is inserted an encapsidation sequence, or alternatively a first and a second expression plasmid each comprising a sequence of interest upstream or downstream of which is inserted an encapsidation sequence,

(ii) an encapsidation plasmid coding for a chimeric nucleocapsid protein comprising a binding domain making it possible to recognize the encapsidation sequence, and,

(iii) an envelope plasmid coding for an envelope protein,

and harvesting the supernatant of the transfected cells comprising the particles.

19. A method of manufacturing particles according to claim 18, comprising a step of co-transfecting cells with:

(i) an expression plasmid comprising at least two sequences of interest, for which upstream or downstream of each of these sequences is inserted an encapsidation sequence, or alternatively a first and a second expression plasmid each comprising a sequence of interest upstream or downstream of which is inserted an encapsidation sequence,

the sequences of interest being different and the encapsidation sequences being identical,

(ii) an encapsidation plasmid coding for a chimeric nucleocapsid protein comprising a binding domain making it possible to recognize the encapsidation sequence, and,

(iii) an envelope plasmid coding for an envelope protein,

and harvesting the supernatant of the transfected cells comprising the particles.

20. A manufacturing method according to any one of claims 17 to 19, wherein the supernatant is clarified.

21. A manufacturing method according to claim 20, wherein the supernatant is also concentrated and/or purified.

~~22. A composition able to be obtained by the method according to any one of claims 17 to 21.~~

2~~3~~2. The composition according to claim 10 ~~use of a particle according any one of claims 1 to 9, or of a composition according to claim 10 or 22~~ for transducing cells.