T cells are the major immune players. In addition to their long lifespan and their own direct effector functions, $CD4^+$ T cells orchestrate the immune system by regulating innate immunity, tailoring B-cell responses and supporting $CD8^+$ T-cell effector functions [4]. Therefore, leveraging the potential of LV to induce $CD4^+$ T cells will maximize their success rate in vaccine strategies.

Summary

In one aspect, the present disclosure relates to a recombinant lentiviral vector genome comprising a polynucleotide encoding a fusion polypeptide, wherein said fusion polypeptide comprises arranged from N-terminal to C-terminal ends a first recombinant polypeptide and a second polypeptide, wherein:

(i) said first recombinant polypeptide comprises a multimerization scaffold which comprises at least one collectin or a fragment thereof suitable to enable self-assembly of multimers of the first polypeptide, fused with at least one antigenic polypeptide;

(ii) said second polypeptide comprises a CD40L ectodomain or a receptor binding fragment thereof, in particular the CD40L ectodomain of the human CD40L, preferably the CD40L ectodomain of SEQ ID No. 19.

In another aspect, the present disclosure provides a DNA plasmid comprising the recombinant vector genome of the disclosure, in particular wherein said genome is inserted within the pTRIP vector plasmid of nucleotide sequence SEQ ID No.21 or within the pFlap-SP1beta2m-GFP-WPREm deposited at the CNCM (Paris, France) on February 16, 2021 under number CNCM I-5657 or variants thereof.

The present disclosure further relates to a DNA plasmid comprising the recombinant vector genome according to the disclosure.

In another aspect, the present disclosure also relates to a recombinant lentiviral vector or a recombinant lentiviral vector particle which comprises the recombinant lentiviral vector genome according to the disclosure.

The present disclosure also relates to a fusion polypeptide comprising arranged from N-terminal to C-terminal ends a first recombinant polypeptide and a second polypeptide, wherein:

(i) said first recombinant polypeptide comprises a multimerization scaffold which comprises at least one collectin or a fragment thereof suitable to enable self-assembly of multimers of the first polypeptide, fused with at least one antigenic polypeptide;

(ii) said second polypeptide comprises a CD40L ectodomain or a receptor binding fragment thereof, in particular the CD40L ectodomain of the human CD40L.

In yet another aspect, the disclosure further relates to a host cell, preferably a mammalian host cell, in particular a human host, transfected with a DNA plasmid according to the disclosure, in particular wherein said host cell is a HEK-293T cell line or a K562 cell line.

In another aspect, the disclosure relates to a pharmaceutical composition, in particular a vaccine composition, suitable for administration to a mammalian host, in particular a human host, comprising a recombinant lentiviral vector of the disclosure, a recombinant lentiviral vector particle of the disclosure, or a host cell of the disclosure together with one or more pharmaceutically acceptable excipient(s) suitable for administration to a host in need thereof, in particular a human host.

In particular, the disclosure relates to the pharmaceutical composition for use in the elicitation of a protective, preferentially prophylactic, immune response by the elicitation of T-cell responses directed against epitopes contained in the antigenic polypeptide or immunogenic fragments thereof, and/or cellular and/or humoral response in a host in need thereof, in particular a human host.

In another aspect, the disclosure provides use of the pharmaceutical composition of the disclosure in the manufacture of a medicament for the elicitation of a protective, preferentially prophylactic, immune response by the elicitation of antibodies directed against the antigenic polypeptide or immunogenic fragments thereof, and/or cellular and/or humoral response in a host in need thereof, in particular a human host. In yet another aspect, the disclosure provides use of the pharmaceutical composition of the disclosure in the manufacture of a medicament for preventing and/or treating an infection by a pathogen in a mammalian host in need thereof, in particular a human host.

Another aspect of the disclosure relates to a method for the preparation of recombinant lentiviral vector particles suitable for the preparation of a pharmaceutical composition, in particular a vaccine, comprising the following steps:

a) transfecting the recombinant lentiviral transfer vector carrying the lentiviral vector genome according to the disclosure, or the DNA plasmid according to the disclosure in a host cell, for example a HEK-293T cell line or a K562 cell line;

b) co-transfecting the cell of step a) with: (i) a plasmid vector encoding envelope proteins and with a plasmid vector encoding the lentiviral GAG and POL or mutated POL protein as packaging construct; and (ii) a plasmid encoding VSV-G Indiana or New Jersey envelope ;

c) culturing the host cell under conditions suitable for the production of recombinant lentiviral vector particles expressing the antigenic polypeptide, or an immunogenic fragment thereof;

d) recovering the recombinant lentiviral particles expressing the antigenic polypeptide, or an immunogenic fragment thereof.

Claims

1. A recombinant lentiviral vector genome comprising a polynucleotide encoding a fusion polypeptide, wherein said fusion polypeptide comprises arranged from N-terminal to C-terminal ends a first recombinant polypeptide and a second polypeptide, wherein:

(i) said first recombinant polypeptide comprises a multimerization scaffold which comprises at least one collectin or a fragment thereof suitable to enable self-assembly of multimers of the first polypeptide, fused with at least one antigenic polypeptide ;

(ii) said second polypeptide comprises a CD40L ectodomain or a receptor binding fragment thereof, in particular the CD40L ectodomain of the human CD40L, preferably the CD40L ectodomain of SEQ ID No. 19.

2. The recombinant lentiviral vector genome according to claim 1, wherein said collectin or fragment thereof comprises arranged from N-terminal to C-terminal ends:

- at least one crosslinking region of a collectin;
- at least one collagen-like region of a collectin; and
- at least one neck region of a collectin.

3. The recombinant lentiviral vector genome according to claim 2, wherein the polynucleotide encoding the said at least one antigenic polypeptide is fused in-frame within the nucleotide sequence of the collagen-like region of said collectin.

4. The recombinant lentiviral vector genome according to any one of claims 1 to 3, wherein said collectin or fragment thereof is a carbohydrate recognition domain (CRD)-truncated form of collectin, wherein said at least one antigenic polypeptide is inserted within the collagen-like region of the collectin.

5. The recombinant lentiviral vector genome according to any one of claims 1 to 4, wherein said fusion polypeptide further comprises a polypeptide comprising a

chemokine, in particular selected from CCL20, a pro-inflammatory Th1-related chemokine such as CXCL9, CXCL10, CCL3, CCL4 and/or CCL5, and/or a Th17-promoting chemokine such as CXCL20, or a receptor binding domain thereof, preferably wherein said chemokine is the CCL20 domain of SEQ ID No. 20, in particular wherein said chemokine or fragment thereof is inserted within the collagen-like domain of said collectin.

6. The recombinant lentiviral vector genome according to any one of claims 1 to 5, wherein said collectin is selected from mannan-binding lectin (MBL), surfactant protein D (SP-D), surfactant protein A (SP-A), collectin liver 1 (CL-L1), collectin placenta 1 (CL-P1), conglutinin collectin of 43 kDa (CL-43), collectin of 46 kDa (CL-46),and collectin kidney 1 (CL-K1), preferably wherein said collectin is a human collectin selected from MBL (SEQ ID No. 17), SP-D (SEQ ID No. 18), CL-L1 (SEQ ID No. 34), SP-A1 (SEQ ID No. 35), SP-A2 (SEQ ID No. 36), CL-P1 (SEQ ID No. 37) and CL-K1 (SEQ ID no. 38).

7. The recombinant lentiviral vector genome according to any one of claims 1 to 6, wherein said antigenic polypeptide is a mono-antigenic polypeptide comprising one antigen or immunogenic fragment thereof, or is a poly-antigenic polypeptide comprising at least two antigens or immunogenic fragments thereof.

8. The recombinant lentiviral vector genome according to any one of claims 1 to 7, wherein the at least one antigen or immunogenic fragment thereof is selected from a bacterial, parasite or viral pathogen, in particular from *Mycobacterium tuberculosis*, an influenza virus or a coronavirus such as SARS-CoV-2 or is a tumoral antigen or immunogenic fragment thereof.

9. The recombinant lentiviral vector genome according to any one of claims 1 to 8, wherein said antigenic polypeptide comprises one or more *Mycobacterium tuberculosis* (Mtb) antigens selected from EsxA, EspC, EsxH, PE19, Hypoxic response

protein 1 (Hrp1) and Resuscitation promoting factor D (RpfD), or an immunogenic fragment thereof, in particular one of the following Mtb antigenic combinations:

(a) EsxH;
(b) EsxH and EsxA;
(c) EsxH, EsxA and PE19;
(d) EsxH, EsxA, PE19 and EspC;
(e) EsxA, PE19, EspC, HRp1 and RpfD;
or an immunogenic fragment thereof.

10. The recombinant lentiviral vector genome, wherein said genome is obtained from the pTRIP vector plasmid of nucleotide sequence SEQ ID No. 21, wherein the polynucleotide encoding the fusion polypeptide has been cloned under control of a promoter functional in mammalian cells, in particular the CMV promoter, the human beta-2 microglobulin promoter, the composite BCUAG promoter of SEQ ID No. 22 and wherein the vector optionally comprises post-transcriptional regulatory element of the woodchuck hepatitis virus (WPRE).

11. A DNA plasmid comprising the recombinant vector genome according to any one of claims 1 to 10, in particular wherein said genome is inserted within the pTRIP vector plasmid of nucleotide sequence SEQ ID No.21 or within the pFlap-SP1beta2m-GFP-WPREm deposited at the CNCM (Paris, France) on February 16, 2021 under number CNCM I-5657 or variants thereof.

12. A recombinant lentiviral vector particle which comprises the recombinant lentiviral vector genome according to any one of claims 1 to 10.

13. The recombinant lentiviral vector particle according to claim 12 which is a recombinant integration-deficient lentiviral vector particle, in particular the recombinant integration-deficient lentiviral vector is a HIV-1 based vector and is integrase deficient as a result of a mutation of the integrase gene encoded in the genome of the lentivirus in such a way that the integrase is not expressed or not functionally expressed, in

particular the mutation in the integrase gene leads to the expression of an integrase substituted on its amino acid residue 64, in particular the substitution is D64V in the catalytic domain of the HIV-1 integrase encoded by Pol.

14. The recombinant lentiviral vector particle according to any one of claim 12 or 13, wherein said recombinant lentiviral vector genome is the genome of a replication-incompetent pseudotyped lentiviral vector, in particular a replication-incompetent pseudotyped HIV-1 lentiviral vector, in particular wherein the vector is pseudotyped with the glycoprotein G from a Vesicular Stomatitis Virus (V-SVG) of Indiana or of New-Jersey serotype.

15. A host cell, preferably a mammalian host cell, transfected with a DNA plasmid according to claim 11, in particular wherein said host cell is a HEK-293T cell line or a K562 cell line.

16. A pharmaceutical composition, in particular a vaccine composition, suitable for administration to a mammalian host, comprising a recombinant lentiviral vector particle of any one of claims 12 to 14 together with one or more pharmaceutically acceptable excipient(s) suitable for administration to a host in need thereof, in particular a human host.

17. Use of the pharmaceutical composition of claim 16 in the manufacture of a medicament for the elicitation of a protective, preferentially prophylactic, immune response by the elicitation of antibodies directed against the antigenic polypeptide or immunogenic fragments thereof, and/or cellular and/or humoral response in a host in need thereof, in particular a human host.

18. The use of claim 17, wherein the immune response involves the induction of MHC-II restricted presentation of the antigenic polypeptide or immunogenic fragments thereof, by an antigen-presenting cell, in particular a dendritic cell, and the induction of a CD4-mediated cellular immune response.

19. Use of the pharmaceutical composition of claim 16 in the manufacture of a medicament for preventing and/or treating an infection by a pathogen in a mammalian host in need thereof, in particular a human host.

20. A method for the preparation of recombinant lentiviral vector particles suitable for the preparation of a pharmaceutical composition, in particular a vaccine, comprising the following steps:

a) transfecting the recombinant lentiviral transfer vector carrying the lentiviral vector genome according to any one of claims 1 to 10, or the DNA plasmid according to claim 11 in a host cell, for example a HEK-293T cell line or a K562 cell line;

b) co-transfecting the cell of step a) with: (i) a plasmid vector encoding envelope proteins and with a plasmid vector encoding the lentiviral GAG and POL or mutated POL protein as packaging construct; and (ii) a plasmid encoding VSV-G Indiana or New Jersey envelope,

c) culturing the host cell under conditions suitable for the production of recombinant lentiviral vector particles expressing the antigenic polypeptide, or an immunogenic fragment thereof;

d) recovering the recombinant lentiviral particles expressing the antigenic polypeptide, or an immunogenic fragment thereof.