

Boosting SARS-CoV-2 immunity with a lentiviral-based nasal vaccine

The invention relates to the field of immunity against coronaviruses. In this respect, the invention provides a lentiviral-based immunogenic agent that is suitable for use in boost or target immunization treatment in a subject, in particular a human subject, who had previously developed an immunity against Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) based on: (i) vaccination with a first generation of vaccines against SARS-CoV-2 infection or disease such as a protein, an mRNA, an adenovirus, an inactivated virus or a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an mRNA-based vaccine, or (ii) SARS-CoV-2-induced or correlated disease. The invention accordingly concerns a lentiviral-based immunogenic agent that in particular may help overcome the deficiencies of available vaccines against SARS-CoV-2, especially may be efficient in overcoming the waning immune response or insufficient cellular memory response observed after immunization with available first generation of vaccines such as a protein, an mRNA, an adenovirus, an inactivated virus or a protein subunit vaccine, in particular protein or mRNA vaccine, by triggering a mucosal humoral and cellular immune response against coronaviruses, including a long-lasting immune response.

Considering: (i) the sustained pandemicity of coronavirus disease 2019 (COVID-19), (ii) weakening protection potential of the first-generation vaccines against SARS-CoV-2, and (iii) ceaseless emergence of new viral Variants of Concerns (VOCs), new effective vaccine platforms can be critical for the future primary or booster vaccines (*Global COVID-19 Vaccination – Strategic Vision for 2022, World Health Organization, SAGE meeting October 2021*). The inventors recently demonstrated the strong performance of a lentiviral vaccination vector (LV) encoding the full-length sequence of Spike glycoprotein (S) from the ancestral SARS-CoV-2 (LV::S), when used in systemic prime followed by intranasal (i.n.) boost in multiple preclinical models (*Ku MW, et al. Cell Host Microbe, 29(2), 236-249 e236, 2021*). LV::S ensures complete (cross) protection of the respiratory tract against ancestral SARS-CoV-2 and VOCs (*Ku MW, et al. EMBO Mol Med, e14459, 2021*). In addition, in new transgenic mice, expressing human Angiotensin Converting Enzyme 2 (hACE2) and displaying unprecedented permissiveness of the brain to SARS-CoV-2 replication, an i.n. boost with LV::S is required for full protection of the central nervous system (*Ku MW, et al. EMBO Mol Med, e14459, 2021*). LV::S is intended to be used as a primary vaccine or a booster to reinforce and broaden protection against emerging VOCs with immune evasion potential (*Juno JA, Wheatley AK. Nat Med, 27(11), 1874-1875, 2021*).

The duration of the protection conferred by the first generation COVID-19 vaccines is not yet well established, hardly predictable with serological laboratory tests and variable in diverse individuals and against distinct VOCs. Despite high vaccination rates, the current exacerbation of the world-wide pandemic indicates that repeated booster immunizations will be needed to ensure individual and collective immunity against COVID-19. In this context, the safety and potential adverse effects of multiple additional homologous doses of the first generation COVID-19 vaccines, for instance related to allergic reaction to polyethylene glycol (PEG) contained in mRNA vaccines, have to be taken into account (*Castells MC, Phillips EJ. N Engl J Med, 384(7), 643-649, 2021*). Importantly, unmatched vaccine delivery methods, i.e., heterologous prime-boost format, has been proven to be a more successful strategy than homologous prime-boost approach in numerous preclinical models of various infectious diseases (*He Q, et al. Emerg Microbes Infect, 10(1), 629-637, 2021; Lu S. Curr Opin Immunol, 21(3), 346-351, 2009; Nordstrom P, et al. Lancet Reg Health Eur, 100249, 2021*). Therefore, new efficient vaccination platforms are of particular interest to develop heterologous boosters against COVID-19. The LV::S vaccine candidate has a serious potential for prophylactic use against COVID-19, mainly based on its strong capacity to induce, not only strong neutralizing humoral responses, but also robust protective T-cell responses which are not impacted by the escape mutations accumulated in the SARS-CoV-2 VOCs (*Ku MW, et al. EMBO Mol Med, e14459, 2021*).

Besides, it has been observed that with the decreasing prophylactic potential of the immunity initially induced by the first-generation vaccines, especially against new VOCs, administration of additional vaccine doses becomes essential (*Global COVID-19 Vaccination – Strategic Vision for 2022, World Health Organization, SAGE meeting October 2021*). As an alternative to additional doses of the same vaccines, combining vaccine platforms in a heterologous prime-boost regimen may hold promise for gaining protective efficacy (*Barros-Martins J, et al. Nat Med, 27(9), 1525-1529, 2021*). Compared to homologous vaccine dose administration, heterologous prime-boost strategies may reinforce better the specific adaptive immune responses and long-term protection, without triggering/reinforcing vector-specific immunity or the risk of aggravation of possible reactogenicity to the vaccines themselves or excipients. Furthermore, the sequence of the Spike antigen has to be adapted according to the dynamics of SARS-CoV-2 VOC emergence in order to induce the greatest neutralization breadth. In addition, whereas protection against symptomatic SARS-CoV-2 infection is mainly related to sero-neutralizing activity, protection against severe COVID-19 involves CD8⁺ T-cell immunity. These cells with their ability to cytolyze virus-infected cells, especially controls the virus replication and result in resolution of SARS-CoV-2 infection (*Sette A, Crotty S. Cell,*

184(4), 861-880, 2021). Therefore, an appropriate B- and T-cell vaccine platform, including an adapted Spike sequence, is of utmost interest at the current step of the pandemic.

The inventors have reasoned that LV::S could be remarkably suitable to be used as a heterologous i.n. booster vaccine, to reinforce and broaden protection against the SARS-CoV-2 in particular against its known and emerging VOCs (including but not limited to Alpha, Beta, Gamma, Delta and Omicron variants of SARS-CoV-2), while collective immunity in early vaccinated nations is waning only a few months after completion of the initial immunization, and while new waves of infections are on the rise (*Juno JA, Wheatley AK. Nat Med, 27(11), 1874-1875, 2021*).

LVs for use in the present invention are in particular non-integrating, non-replicative, non-cytopathic and negligibly inflammatory (*Hu B, Tai A, Wang P. Immunol Rev, 239(1), 45-61, 2011; Ku MW, Charneau P, Majlessi L. Expert Rev Vaccines, 1-16, 2021*). These vectors are pseudotyped with the heterologous glycoprotein from Vesicular Stomatitis Virus (VSV-G) which confers them a broad tropism for diverse cell types, notably including dendritic cells. The latter are mainly non-dividing cells and thus barely permissive to gene transfer. Hence, LVs possess the central property to efficiently transfer genes to the nuclei of non-dividing cells, which therefore renders possible efficient transduction of dendritic cells. The resulting endogenous antigen expression in these cells with unique ability to activate naïve T cells (*Guermonprez P, et al. J. Int Rev Cell Mol Biol, 349, 1-54, 2019*) correlates with outstanding ability of LV at inducing high-quality effector and memory T cells (*Ku MW, et al. Commun Biol, 4(1), 713, 2021*). Importantly, VSV-G pseudo-typing also avoids LVs to be targets of preexisting vector-specific immunity in humans which is key in vaccine development (*Hu B, Tai A, Wang P. Immunol Rev, 239(1), 45-61, 2011; Ku MW, Charneau P, Majlessi L. Expert Rev Vaccines, 1-16, 2021*). The safety of LV has been established in humans in a phase I/IIa Human Immunodeficiency Virus-1 therapeutic vaccine trial (*EU Clinical Trials Register, Clinical Trials for 2011-006260-52*). Because of their non-cytopathic and non-inflammatory properties (*Cousin C, et al. Cell Rep, 26(5), 1242-1257 e1247, 2019; Lopez J, et al. An optimized lentiviral vector induces CD4+ T-cell immunity and predicts a booster vaccine against tuberculosis. in revision*), LVs are well suitable for mucosal vaccination. The i.n. administration route presents well-recognized advantages of triggering mucosal IgA responses, as well as resident memory B and T lymphocytes in the respiratory tract (*Lund FE, Randall TD. Science, 373(6553), 397-399, 2021*). This route has also been shown to be the most effective at reducing SARS-CoV-2 transmission in both hamster and macaque preclinical models (*van Doremalen N, et al. Sci Transl Med, 13(607), 2021*). Induction of mucosal immunity by i.n. immunization allows SARS-CoV-2 neutralization, directly at the gateway to the host organism, before it gains access to

major infectable anatomical sites (*Ku MW, et al. Cell Host Microbe, 29(2), 236-249 e236, 2021*).

In the present invention, the inventors generated an LV encoding the down-selected S_{CoV-2} of the Beta variant, stabilized by K⁹⁸⁶P and V⁹⁸⁷P substitutions in the S2 domain of S_{CoV-2} (LV::S_{Beta-2P}). In mice, primed and boosted intramuscularly (i.m.) with mRNA-1273 (Moderna) vaccine, and in which the (cross) sero-neutralization potential was progressively turning down, the inventors compared the systemic and mucosal immune responses and the protective potential of an i.n. LV::S_{Beta-2P} heterologous boost vs an i.m. mRNA-1273 (Moderna) (*Jackson LA, et al. Preliminary Report. N Engl J Med, 383(20), 1920-1931, 2020; Wang F, et al. Med Sci Monit, 26, e924700, 2020*) homologous boost. The inventors observed multiple advantages with respect to the former regimen with an improved antigen design, new vaccine delivery LV platform and the alternative i.n. administration route.

According to a first aspect, the invention hence relates to a pseudotyped lentiviral vector particle encoding a Spike (S) protein of a Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) or a derivative thereof for use as a heterologous boost or target immunization agent in a vaccine regimen for administration to the upper respiratory tract of a subject, in particular a human subject, who received a prime immunization with a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein or an mRNA vaccine composition against SARS-CoV-2 infection or disease.

Spike (S) protein of SARS-CoV-2 virus is well identified in the art as an envelop-anchored glycoprotein (*Walls et al, 2020, Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell 181:281-292 e286*). More precisely, the SARS-CoV-2 S (S_{CoV-2}) is a (180 kDa)₃ homotrimeric class I viral fusion protein, which engages the carboxypeptidase Angiotensin-Converting Enzyme 2 (ACE2), expressed on host cells. The monomer of S_{CoV-2} protein possesses an ecto-domain, a transmembrane anchor domain, and a short internal tail. S_{CoV-2} is activated by a two-step sequential proteolytic cleavage to initiate fusion with the host cell membrane. Subsequent to S_{CoV-2}-ACE2 interaction, which leads to a conformational reorganization, the extracellular domain of S_{CoV-2} is first cleaved at the highly specific furin 682^{RRAR}685 (SEQ ID NO: 21) site (*Guo et al., 2020, The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak - an update on the status. Mil Med Res 7, 11; Walls et al., 2020*), a key factor determining the pathological features of the virus, linked to the ubiquitous furin expression (*Wang et al., 2020 A Unique Protease Cleavage Site Predicted in the Spike Protein of the Novel Pneumonia Coronavirus (2019-nCoV)*

Potentially Related to Viral Transmissibility. *Virol Sin* 2020 Jun;35(3):337-339. doi: 10.1007/s12250-020-00212-7. Epub 2020 Mar 20). The resulted subunits are constituted of: (i) S1, which harbors the ACE2 Receptor Binding Domain (RBD), with the atomic contacts restricted to the ACE2 protease domain and also harbors main B-cell epitopes, targeted of neutralizing antibodies (NAbs) (Walls et al., 2020), and (ii) S2, which bears the membrane-fusion elements. Like for S_{CoV-1}, the shedding of S1 renders accessible on S2 the second proteolytic cleavage site 797^R, namely S2' (Belouzard et al., 2009, Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc Natl Acad Sci U S A* 106:5871-5876). According to the cell or tissue types, one or several host proteases, including furin, trypsin, cathepsins or TransMembrane Protease Serine Protease (TMPRSS)-2 or -4, can be involved in this second cleavage step (Coutard et al., 2020, The spike glycoprotein of the new coronavirus 2019-nCoV contains a furin-like cleavage site absent in CoV of the same clade. *Antiviral Res* 176:104742). The consequent "fusogenic" conformational changes of S result in a highly stable post-fusion form of S_{CoV-2} that initiates the fusion reaction with the host cell membrane (Sternberg and Naujokat, 2020 Structural features of coronavirus SARS-CoV-2 spike protein: Targets for vaccination. *Life Sci* 257, 118056) and lead to the exposure of a Fusion Peptide (FP), adjacent to S2'. Insertion of FP to the host cell/vesicle membrane primes the fusion reaction, whereby the viral RNA release into the host cytosol (Lai et al., 2017 The SARS-CoV Fusion Peptide Forms an Extended Bipartite Fusion Platform that Perturbs Membrane Order in a Calcium-Dependent Manner. *J Mol Biol* 429:3875-3892). The facts that the S_{CoV-2}-ACE2 interaction is the only mechanism, thus far identified for the host cell infection by SARS-CoV-2, and that the RBD contains numerous conformational B-cell epitopes (Walls et al., 2020), designate this viral envelop glycoprotein as the main target for neutralization antibodies (NAbs).

25 The S protein for expression by the lentiviral particles of the invention may originate from a SARS-CoV-2 strain and accordingly maybe characterized by an amino acid sequence that is the native sequence of the viral protein. In a particular embodiment the invention is performed using the S protein of known SARS-CoV-2 strains such as the S protein of the Ancestral strain (wherein the amino acid sequence is SEQ ID NO: 1), or of variant strains discovered later such as the Alpha, Beta, Gamma, Delta or Omicron strain (all regarded as variant strains with respect to one another).

35 The invention may alternatively be performed with a derivative of the S protein, i.e., a derivative of a native S protein obtained by mutation in the amino acid sequence of the S protein, as will be disclosed herein. In order to express LV::S recombinant particles, the nucleic acid encoding the S protein may have the sequence of the gene present in the viral strain of

origin or may be a codon-optimized acid nucleic suitable for expression in mammalian cells, in particular in human cells. In order to express LV recombinant particles expressing a derivative of the S protein, the nucleic acid encoding the derivative of the S protein may have the sequence deduced from the sequence of the gene of the S protein present in a viral strain and
5 may be a codon-optimized acid nucleic suitable for expression in mammalian cells.

In a particular embodiment, the recombinant lentiviral particles (LV) used in the invention are HIV-1-based lentiviral particles. Accordingly, where the expressions "lentiviral particle" or "LV" are used herein it is in particular directed to the HIV-1 based lentiviral particles especially LV particles pseudotyped with VSV-G protein, in particular LV as illustrated in the
10 examples.

The expressions "boost" or "boost immunization" or "boost administration" or "target immunization" refer according to the invention to an administration of the immunogenic agent that comes after a first administration of a heterologous immunization agent, in particular a heterologous vaccine, or after a second or later administration of such heterologous
15 immunization agent or vaccine. Otherwise stated the immunization agent used according to the invention is administered to a subject who previously received a prime administration, or a prime and further one or multiple administration doses, of a heterologous immunization agent or vaccine against the same SARS-CoV-2 or against a variant strain thereof. The boost or target immunization is achieved through administration to the upper respiratory tract, in particular as an intranasal administration, that accordingly distinguish over administration route
20 of a first generation of vaccines against SARS-CoV-2 infection or disease such as a protein, an mRNA, an adenovirus, an inactivated virus or a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular protein or mRNA vaccines that most often make use of systemic, including intramuscular, intradermal or subcutaneous administration route.

25 The boost or target immunization is intended to enhance, improve or lengthen the immune response previously raised and possibly to broaden such response to elicit cross-neutralization against multiple SARS-CoV-2 viruses. Improvement of the response may arise from the capability of the immunization agent used in the invention to elicit a mucosal response and to accordingly protect, not only the systemic sites, but also the upper and lower respiratory tracts
30 and the central nervous system that may not have been successfully targeted or protected with heterologous vaccines against SARS-CoV-2 infection or disease such as a protein, an mRNA, an adenovirus, an inactivated virus or a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular protein or mRNA vaccines injected via systemic routes. In a particular embodiment the boost administration is intended to raise cross-
35 neutralizing immune response in the subject against emerging strains of the virus. In a particular

embodiment, the boost or target immunization may be administered to a subject who received a heterologous immunization agent as disclosed herein and who had and recovered from infection by SARS-CoV-2 or disease related to such infection such as COVID-19. Additional features relating to the use of the immunization agent and to the treatment course of the subject will be disclosed in the following description.

Administration "to the upper respiratory tract" includes any type of administration that results in delivery to the mucosa lining of the upper respiratory tract and includes in particular nasal administration. Administration to the upper respiratory tract includes without limitation, aerosol inhalation, nasal instillation, nasal insufflation, and all combinations thereof. In some embodiments the administration is by aerosol inhalation. In some embodiments the administration is by nasal instillation. In some embodiments the administration is by nasal insufflation.

According to a particular embodiment, the pseudotyped lentiviral vector particle encoding a SARS-CoV-2 S protein or a derivative thereof is for administration as intranasal mucosal boost or target immunization in a subject who received a prime administration with a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein or an mRNA vaccine composition against SARS-CoV-2 infection or disease.

According to a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use in accordance with the embodiments disclosed herein is further characterized by the following features:

- the S protein is from a SARS-CoV-2 virus which is pathogenic for a human host, in particular (i) a S protein from a SARS-CoV-2 virus selected from the group consisting of SARS-CoV-2 Ancestral strain, D614G strain, Alpha strain, Beta strain, Gamma strain, Delta strain and Omicron strain, preferably from Beta strain or Omicron strain, more preferably from Beta strain, or (ii) a S protein from a variant of said Ancestral, D614G strain, Alpha, Beta, Gamma, Delta or Omicron strains wherein such variants encode a S protein with an amino acid sequence at least 90% identical to SEQ ID NO: 1 or,
- the S protein is a derivative of the native S protein of one of the Ancestral, D614G, Alpha, Beta, Gamma, Delta or Omicron strains by mutation of 1 to 12, especially 1 to 6 amino acid residues, in particular by substitution and/or deletion of 1 to 12, especially 1 to 6 amino acid residues, in particular **(a)** is a stabilized form of the S protein characterized by substitution of two consecutive amino acid residues for Proline

residues in the S2 domain of the S protein at positions provided as residues 986 and 987 by reference to SEQ ID NO: 1 in the amino acid sequence of the S protein or **(b)** is a prefusion form of the S protein by deletion of the furin site located from residue 675 to residue 685 by reference to SEQ ID NO: 1 in the amino acid sequence of the S protein or **(c)** is a stabilized prefusion form of the S protein by deletion of the furin site and substitution of two consecutive amino acid residues for Proline residues in the S2 domain at positions 986 and 987 by reference to SEQ ID NO: 1.

5 In a particular embodiment, the S protein of the Ancestral strain of SARS-CoV-2 has an amino acid sequence of SEQ ID NO: 1 and the native sequence of the polynucleotide encoding the S protein of the Ancestral strain of SARS-CoV-2 is defined in SEQ ID NO: 2.

10 In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the D614G strain of SARS-CoV-2 comprising a mutation of the aspartic acid residue to the glycine residue at position 614 of the amino acid sequence of SEQ ID NO: 1 (D614G), a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 3 ($S_{D614G-2P}$). The S protein of the D614G strain of SARS-CoV-2 comprising said mutation 2P ($S_{D614G-2P}$) has an amino acid sequence of SEQ ID NO: 4.

15 In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Alpha strain of SARS-CoV-2 comprising a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 5 ($S_{Alpha-2P}$). The S protein of the Alpha strain of SARS-CoV-2 comprising said mutation 2P ($S_{Alpha-2P}$) has an amino acid sequence of SEQ ID NO: 6.

20 In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Beta strain of SARS-CoV-2 is defined in SEQ ID NO: 7 (S_{Beta}). The S protein of the Beta strain of SARS-CoV-2 (S_{Beta}) has an amino acid sequence of SEQ ID NO: 8.

30 In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Beta strain of SARS-CoV-2 comprising a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 9 ($S_{Beta-2P}$).

The S protein of the Beta strain of SARS-CoV-2 comprising said mutation 2P ($S_{\text{Beta-2P}}$) has an amino acid sequence of SEQ ID NO: 10.

In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Gamma strain of SARS-CoV-2 comprising a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 11 ($S_{\text{Gamma-2P}}$). The S protein of the Gamma strain of SARS-CoV-2 comprising said mutation 2P ($S_{\text{Gamma-2P}}$) has an amino acid sequence of SEQ ID NO: 12.

In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Delta strain of SARS-CoV-2 comprising a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 13 ($S_{\text{Delta-2P}}$). The S protein of the Delta strain of SARS-CoV-2 comprising said mutation 2P ($S_{\text{Delta-2P}}$) has an amino acid sequence of SEQ ID NO: 14.

In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Omicron strain of SARS-CoV-2 is defined in SEQ ID NO: 15 (S_{Omicron}). The S protein of the Omicron strain of SARS-CoV-2 (S_{Omicron}) has an amino acid sequence of SEQ ID NO: 16.

In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Omicron strain of SARS-CoV-2 comprising a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 17 ($S_{\text{Omicron-2P}}$). The S protein of the Omicron strain of SARS-CoV-2 comprising said mutation 2P ($S_{\text{Omicron-2P}}$) has an amino acid sequence of SEQ ID NO: 18.

In another particular embodiment, the native sequence of the polynucleotide encoding the S protein of the Ancestral strain of SARS-CoV-2 comprising a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), *i.e.* mutation 2P, is defined in SEQ ID NO: 19 (S_{2P}). The S protein of the Ancestral strain of SARS-CoV-2 comprising said mutation 2P (S_{2P}) has an amino acid sequence of SEQ ID NO: 20.

In a particular embodiment, the pseudotyped lentiviral vector particle encodes the S protein of the Beta strain of SARS-CoV-2 comprising the mutation 2P ($S_{\text{Beta-2P}}$) that is encoded by the vector pFlap-ieCMV-S-B351-2P-WPREm that has been deposited at the COLLECTION NATIONALE DE CULTURES DE MICROORGANISMES (CNCM) located at Institut Pasteur, 25-28 rue du Docteur Roux, 75724 Paris Cedex 15 FRANCE, on July 6, 2021 under N°CNCM I-5710.

Also provided is the vector pFlap-ieCMV-S-B351-2P-WPREm (CNCM I-5710). The nucleotide sequence of pFlap-ieCMV-S-B351-2P-WPREm is defined in SEQ ID NO: 22.

Also provided is a host cell comprising the vector pFlap-ieCMV-S-B351-2P-WPREm (CNCM I-5710 or SEQ ID NO: 22).

Also provided is a pseudotyped lentiviral vector particle encoding the S protein of the Beta strain of SARS-CoV-2 comprising the mutation 2P ($S_{\text{Beta-2P}}$), wherein the pseudotyped lentiviral vector particle is made by a method comprising co-transfection of a host cell with the vector pFlap-ieCMV-S-B351-2P-WPREm (CNCM I-5710 or SEQ ID NO: 22).

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use in accordance with the embodiments disclosed herein, is further characterized by the following features: the amino acid sequence of the S protein is SEQ ID NO: 1 or is a derivative thereof having an amino acid sequence at least 90% identical to SEQ ID NO: 1, and the derivative of the S protein of SARS-CoV-2 comprises at least five amino acid mutations including (i) a mutation of the lysine residue to the asparagine residue at position 417 of the amino acid sequence of SEQ ID NO: 1 (K417N), (ii) a mutation of the glutamic acid residue to the lysine residue at position 484 of the amino acid sequence of SEQ ID NO: 1 (E484K) or a mutation of the glutamic acid residue to the alanine residue at position 484 of the amino acid sequence of SEQ ID NO: 1 (E484A), (iii) a mutation of the asparagine residue to the tyrosine residue at position 501 of the amino acid sequence of SEQ ID NO: 1 (N501Y), (iv) a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and (v) a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P).

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention, is such that the S protein of SARS-CoV-2 further comprises amino acid mutations selected from the group consisting of (vi) a mutation of the glycine residue to the serine residue at position 446 of the amino acid sequence of SEQ ID NO: 1 (G446S), (vii) a mutation of the threonine residue to the lysine residue at position 478 of the amino acid sequence of SEQ ID NO: 1 (T478K), (viii)

a mutation of the glutamine residue to the arginine residue at position 493 of the amino acid sequence of SEQ ID NO: 1 (Q493R) and (ix) a mutation of the glutamine residue to the arginine residue at position 498 of the amino acid sequence of SEQ ID NO: 1 (Q498R).

5 In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention is such that the encoded mutated S protein of SARS-CoV-2 has the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 18, preferably of SEQ ID NO: 10.

10 In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention is pseudotyped with a vesicular stomatitis virus glycoprotein G (VSV-G) protein.

In particular the VSV-G protein is advantageously provided by a VS virus of the Indiana strain or the New-Jersey strain.

15 In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention is such that the pseudotyped lentiviral vector particle is non-integrative, non-cytopathic and non-replicative.

20 In accordance with the herein disclosed use of the immunogenic agent according to the invention, in some embodiments, the immunogenic agent or composition comprising the agent is for use in a method of prevention of infection of a human subject by SARS-CoV-2. In some embodiments, the immunogenic agent or composition is for use in a method of protection against SARS-CoV-2 replication in a human subject at risk of being exposed to SARS-CoV-2 or infected by SARS-CoV-2. In some embodiments, the immunogenic composition is for use in a method of preventing development of symptoms or development of a disease associated with infection by SARS-CoV-2, such as COVID-19 in a human subject at risk of being exposed to SARS-CoV-2 or infected by SARS-CoV-2. In some embodiments, the immunogenic composition is for use in a method of preventing the onset of neurological outcome associated with infection by SARS-CoV-2 in a human subject at risk of being exposed to SARS-CoV-2 or infected by SARS-CoV-2. In some embodiments, the immunogenic composition is for use in a method of protecting the Central Nervous System (CNS) of a human subject at risk of being exposed to SARS-CoV-2 or infected by SARS-CoV-2. In some embodiments the vaccine provides protection against the infection by SARS-CoV-2, especially sterilizing protection.

30 In any of these applications for use in a method disclosed, the immunogenic agent or composition is to be administered to the subject as a prophylactic agent in a boost or target administration step in an effective amount for administration to the upper respiratory tract in order to elicit an immune response against SARS-CoV-2.

In some embodiment the immunogenic composition is for use in a method of protection of a human subject against SARS-CoV-2 infection or against development of the symptoms or the COVID-19 disease associated with SARS-CoV-2 infection, wherein the subject is at risk of developing lung and/or CNS pathology. In particular the human subject is in need of immune protection of CNS from SARS-CoV-2 replication because he/she is affected with comorbid conditions, in particular comorbid conditions affecting the CNS.

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of the embodiments disclosed herein is administered in a subject selected from the group consisting of (a) a subject that has previously received a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease as a systemic prime and/or boost administration(s) such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular prime and/or boost administration(s), (b) a subject that has received a systemic prime administration such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular prime administration, of a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease and has then recovered from coronavirus disease such as coronavirus disease 2019 (COVID-19), (c) a subject that has first recovered from coronavirus disease such as COVID-19 and has then received a systemic prime administration such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular administration, of a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease, and (d) a subject that has received more than two, in particular more than three, systemic administrations such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular administration, of a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease.

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof is for use in a prime/boost or a target immunization regimen for elicitation of a long-lasting protective mucosal humoral immune response and/or a long-lasting mucosal cellular immune response against SARS-CoV-2

infection or disease, wherein said response protects the respiratory system and/or the CNS of the subject.

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof is for use in an immunization regimen wherein
5 the pseudotyped lentiviral vector particle elicits a CD8⁺ T-cell response against SARS-CoV-2.

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof is for use in an immunization regimen wherein the pseudotyped lentiviral vector particle elicits lung-resident memory CD8⁺ T cells (Trm) and/or effector CD8⁺ T cells (Tc1) specific to Spike and able to produce Interferon-gamma
10 (IFN-γ)/Tumor Necrosis Factor (TNF)/Interleukin-2 (IL-2) cytokines. In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention is used in an immunization regimen wherein the subject has a waning immunity from week 12 after the first injection of the initial vaccination with a vaccine composition against SARS-CoV-2 infection or disease selected
15 from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease or post SARS-CoV-2 disease recovery, in particular post-COVID-19 recovery.

In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention is formulated
20 as a liquid composition or a dry powder for an administration as intranasal aerosols, intranasal drops or intranasal insufflations. In a particular embodiment, the pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to the invention, is used in an immunization regimen wherein the administration regimen comprises
25 administration of one or more dosage form(s) of the pseudotyped lentiviral vector particle wherein the dose of each dosage form is from 10⁷ to 10⁹ Transduction Unit (TU).

According to another aspect the invention also concerns an immunogenic composition comprising a pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof and a pharmaceutically acceptable carrier, wherein the pseudotyped
30 derivative of the S protein of SARS-CoV-2 comprises at least nine amino acid mutations including (i) a mutation of the lysine residue to the asparagine residue at position 417 of the amino acid sequence of SEQ ID NO: 1 (K417N), (ii) a mutation of the glutamic acid residue to the alanine residue at position 484 of the amino acid sequence of SEQ ID NO: 1 (E484A), (iii) a mutation of the asparagine residue to the tyrosine residue at position 501 of the amino acid
35 sequence of SEQ ID NO: 1 (N501Y), (iv) a mutation of the lysine residue to the proline residue

at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P), (v) a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), (vi) a mutation of the glycine residue to the serine residue at position 446 of the amino acid sequence of SEQ ID NO: 1 (G446S), (vii) a mutation of the threonine residue to the lysine residue at position 478 of the amino acid sequence of SEQ ID NO: 1 (T478K), (viii) a mutation of the glutamine residue to the arginine residue at position 493 of the amino acid sequence of SEQ ID NO: 1 (Q493R) and (ix) a mutation of the glutamine residue to the arginine residue at position 498 of the amino acid sequence of SEQ ID NO: 1 (Q498R).

This immunogenic composition may be such that the pseudotyped lentiviral vector particle encodes mutated S protein of SARS-CoV-2 the amino acid sequence of which is SEQ ID NO: 18.

According to a further embodiment, the immunogenic composition is formulated for intranasal administration as disclosed in the embodiments herein.

The invention also concerns kits suitable for use in practicing a use or a method disclosed herein. In some embodiments the kit comprises a dosage form for administration to the upper respiratory tract of a subject of the pseudotyped lentiviral vector particle encoding a SARS-CoV-2 S protein or a derivative thereof according to this disclosure, and an applicator. In some embodiments the applicator is an applicator for aerosol inhalation. In some embodiments the applicator is an applicator for nasal instillation. In some embodiments the applicator is an applicator for nasal insufflation. Suitable examples of each are known in the art and may be used.

Preparation of recombinant LV particles is known in the art, including to obtain non-integrative, non-replicative recombinant LV particles. In particular reference is made to the disclosure in *Ku MW, et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021; *Ku MW et al, EMBO Mol Med*, e14459, 2021, *Ku MW, Charneau P, Majlessi L. Expert Rev Vaccines*, 1-16, 2021). Polynucleotide constructs may be adapted with the sequence encoding the selected Spike protein or derivative thereof.

In some embodiments, the lentiviral vector particle comprises HIV-1 Gag and Pol proteins. In some embodiments, the lentiviral vector particle comprises subtype D, especially HIV-1_{NDK}, Gag and Pol proteins.

According to some embodiments, the lentivector particles are obtained in a host cell transformed with a DNA plasmid.

Such a DNA plasmid can comprise:

- bacterial origin of replication (ex: pUC ori);
- antibiotic resistance gene (ex: Amp^r or Kan^r) for selection; and more particularly:

- a lentiviral vector comprising at least one nucleic acid encoding a SARS-CoV-2 S protein or a derivative thereof, transcriptionally linked to a promoter, for example a CMV promoter.

Such a method allows producing a recombinant vector particle for use according to the invention, comprising the following steps of:

- i) transfecting a suitable host cell with a lentiviral vector;
- ii) transfecting said host cell with a packaging plasmid vector, containing viral DNA sequences encoding at least structural and polymerase (+ integrase) activities of a retrovirus (preferably lentivirus); Such packaging plasmids are described in the art (Dull *et al.*, 1998, *J Virol*, 72(11):8463-71; Zufferey *et al.*, 1998, *J Virol* 72(12):9873-80);
- iii) culturing said transfected host cell in order to obtain expression and packaging of said lentiviral vector into lentiviral vector particles; and
- iv) harvesting the lentiviral vector particles resulting from the expression and packaging of step iii) in said cultured host cells.

An appropriate host cell is preferably a human cultured cell line as, for example, a HEK cell line, such as a HEK293T line.

Alternatively, the method for producing the vector particle is carried out in a host cell, which genome has been stably transformed with one or more of the following components: a lentiviral vector DNA sequence, the packaging genes, and the envelope gene. Such a DNA sequence may be regarded as being similar to a proviral vector according to the invention, comprising an additional promoter to allow the transcription of the vector sequence and improve the particle production rate.

In a preferred embodiment, the host cell is further modified to be able to produce viral particle in a culture medium in a continuous manner, without the entire cells swelling or dying. One may refer to Strang *et al.*, 2005, *J Virol* 79(3):1165-71; Relander *et al.*, 2005, *Mol Ther* 11(3):452-9; Stewart *et al.*, 2009, *Gene Ther*, 16(6):805-14; and Stuart *et al.*, 2011, *Hum gene Ther*, with respect to such techniques for producing viral particles.

The lentiviral particle vectors can comprise the following elements, as previously defined:

- cPPT/CTS polynucleotide sequence; and
- a nucleic acid encoding a CAR under control of a β 2m or Major Histocompatibility Complex classe I (MHC-I) promoter, and optionally one of the additional elements described above.

Other features and advantages of the invention will be apparent from the examples which follow and will also be illustrated in the figures.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Down-selection of a S_{CoV-2} variant with the highest potential to induce cross

sero-neutralizing antibodies. (A) Timeline of prime-boost vaccination with LV::S_{Alpha}, LV::S_{Beta} or LV::S_{Gamma} and (cross) sero-neutralization assays in C57BL/6 mice ($n = 4-5/\text{group}$). (B) Half maximal Effective Concentration (EC₅₀) of neutralizing activity of sera from vaccinated mice was evaluated before and after the boost, against pseudo-viruses carrying S_{CoV-2} from D614G, Alpha, Beta or Gamma variants. (C) EC₅₀ of sera from C57BL/6 mice, vaccinated following the regimen detailed in (A) with LV encoding for S_{D614G}, either WT or carrying the K^{986P} - V^{987P} substitutions in the S2 domain. EC₅₀ was evaluated before and after the boost, as indicated in (B).

Figure 2: Anti-S_{CoV-2} humoral responses in mRNA-1273-vaccinated mice which

were further intranasally boosted with LV::S_{Beta-2P}. (A) Timeline of mRNA-1273 intramuscular (i.m.)-i.m. prime-boost vaccination in C57BL/6 mice which were later immunized i.n. by escalating doses of LV::S_{Beta-2P} ($n = 4-5/\text{group}$) and the (cross) sero-neutralization follow-up. (B) Serum EC₅₀ determined at the indicated time points against pseudo-viruses carrying S_{CoV-2} from D614G, Alpha, Beta, Gamma, Delta or Delta+ variants. (C) Anti-S_{CoV-2} IgG or IgA in the sera two weeks after late LV::S_{Beta-2P} i.n. or mRNA-1273 (Moderna) i.m. boost. Statistical significance was determined by Mann-Whitney test (*= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$).

Figure 3: Lung resident memory B-cell subsets in mRNA-1273-vaccinated mice which were further intranasally boosted with LV::S_{Beta-2P}.

The mice are those detailed in Figure 2. Mucosal immune cells were studied two weeks after LV::S_{Beta-2P} i.n. boost. (A) Cytometric gating strategy to detect lung B resident memory cells, and (B) percentages of Brm among surface IgM/IgD⁺ B cells in various groups.

Figure 4: Systemic CD8⁺ T-cell responses to S_{CoV-2} in mRNA-1273-vaccinated

mice which were further intranasally boosted with LV::S_{Beta-2P}. The mice are those detailed in Figure 2. T-splenocyte responses were evaluated two weeks after LV::S_{Beta-2P} i.n. boost by IFN- γ ELISPOT after stimulation with S:256-275, S:536-550 or S:576-590 synthetic 15-mer peptides encompassing S_{CoV-2} MHC-I-restricted epitopes, pertinent in C57BL/6 (H-2^b) mice. Statistical significance was evaluated by Mann-Whitney test (*= $p < 0.05$).

Figure 5: Mucosal CD8⁺ T-cell responses to S_{CoV-2} in mRNA-1273-vaccinated mice which were further intranasally boosted with LV::S_{Beta-2P}. The mice are those detailed in Figure 2. (A) Representative IFN- γ response by lung CD8⁺ T cells detected by IntraCellular Cytokine Staining (ICS) after *in vitro* stimulation with a pool of S:256-275, S:536-550 and S:576-590 peptides. Cells are gated on alive CD45⁺ CD8⁺ T cells.

Figure 6. Lung resident memory T-cell subset in mRNA-1273-vaccinated mice which were further intranasally boosted with LV::S_{Beta-2P}. The mice are those detailed in Figures 2 and 3. Mucosal immune cells were studied two weeks after LV::S_{Beta-2P} i.n. boost. (A) Cytometric gating strategy to detect lung CD8⁺ T resident memory cells (Trm, CD44⁺CD62L⁻CD69⁺CD103⁺), and (B) percentages of Trm among CD8⁺ CD44⁺ T-cells in various groups.

Figure 7. Full protective potential of a late LV::S_{Beta-2P} i.n. boost in mRNA-1273-primed and-boosted mice. (A) Timeline of mRNA-1273 i.m.-i.m. prime-boost vaccination in C57BL/6 mice which were later immunized i.n. with the sub-optimal dose of 1×10^8 TU/mouse of LV::S_{Beta-2P} ($n = 4$ -5/group), pre-treated i.n. with Ad5::hACE-2 4 days before i.n. challenge with 0.3×10^5 TCID₅₀ (Tissue Culture Infectious Dose of 50 %) of SARS-CoV-2 Delta variant. (B) Comparative quantitation of hACE-2 mRNA in the lungs of different groups of mice pre-treated i.n. with Ad5::hACE-2. (C) Viral RNA contents, evaluated by conventional E_{CoV-2}-specific (top) or sub-genomic Esg-specific (bottom) qRT-PCR at 3 dpi. The bottom line indicates the detection limits. Statistical significance was evaluated by Mann-Whitney test (*= $p < 0.05$, **= $p < 0.01$).

Figure 8. Anti-S_{CoV-2} humoral responses in mRNA-1273-vaccinated mice which were further intranasally boosted with LV::S_{Beta-2P}. (A) Follow-up of anti-S_{CoV-2} (left) and anti-RBD (right) IgG in the sera of mice initially primed and boosted i.m. with mRNA-1273. (B) Anti-S_{CoV-2} IgG (top), anti-RBD IgG (middle), and anti-S_{CoV-2} IgA (bottom) in the total lung extracts of mice initially primed and boosted i.m. with mRNA-1273 and then boosted later with a third i.m. dose of mRNA-1273 or an i.n. boost of LV::S_{Beta-2P}.

30

Figure 9. Absence of mucosal CD8⁺ Tc2 responses to S_{CoV-2} in mRNA-1273-vaccinated mice which were further intranasally boosted with LV::S_{Beta-2P}. The mice are those detailed in Figure 2. Absence of IL-4, IL-5, IL-10 and IL-13 production by lung CD8⁺ T cells after *in vitro* stimulation with a pool of S:256-275, S:536-550 and S:576-590 peptides,

studied by ICS in parallel to the assay performed to detect IFN- γ /TNF/IL-2 (see **Figure 5**). Cells are gated on alive CD45⁺ CD8⁺ T cells.

Figure 10. Maps of plasmids used for production of LV encoding (A) S_{D614G-2P}, (B) S_{Alpha-2P}, (C) S_{Gamma-2P}, (D) S_{Delta-2P}, (E) S_{Beta-2P} and (F) S_{Omicron-2P} antigens.

The LV-based strategy, which is highly productive, not only in inducing humoral responses but also and particularly in establishing high quality and memory T-cell responses (*Ku MW, et al. Commun Biol, 4(1), 713, 2021*), is a favorable platform for a heterologous boost, even if it is also largely efficacious by its own as a primary COVID-19 vaccine candidate (*Ku MW, et al. Cell Host Microbe, 29(2), 236-249 e236, 2021; Ku MW, et al. EMBO Mol Med, e14459, 2021*). Furthermore, and importantly, LV is non-cytopathic, non-replicative and scarcely inflammatory and thus can be used to perform non-invasive i.n. boost, to efficaciously induce sterilizing mucosal immunity which protects the respiratory system, as well as the CNS (*Ku MW, et al. Cell Host Microbe, 29(2), 236-249 e236, 2021; Ku MW, et al. EMBO Mol Med, e14459, 2021*). The i.n. route of vaccination has been shown by several teams to be the most efficacious route at reducing viral contents in nasal swabs and nasal olfactory neuroepithelium (*Bricker TL, Cell Rep, 36(3), 109400, 2021; Hassan AO, et al. Cell Rep Med, 2(4), 100230, 2021*). The inventors thus hypothesized that i.n. vaccination could contribute efficaciously in blocking/reducing the respiratory chain of SARS-CoV-2 transmission.

Another major advantage of LV-based immunization is the induction of strong T-cell immune responses with high cross-reactivity of T-cell epitopes from Spike of diverse VOCs. Therefore, when the neutralizing antibody fails or wanes, the T-cell arm remains largely protective, as the inventors recently described in antibody-deficient, B-cell compromised μ MT KO mice (*Ku MW, et al. EMBO Mol Med, e14459, 2021*). This property is relative to a high-quality and long-lasting T-cell immunity induced against multiple preserved T-cell epitopes, despite the mutation accumulated in the Spike of the emerging VOCs (*Ku MW, et al. EMBO Mol Med, e14459, 2021*).

In the present invention, the inventors first down-selected S_{Beta} antigen which induced the greatest neutralization breadth against the VOCs and designed a non-integrating LV encoding a stabilized version of this antigen. In mRNA-1273-primed and -boosted mice with waning (cross) sero-neutralization capacity, the inventors used escalating doses of LV::S_{Beta-2P} in i.n. boost. The inventors demonstrated a dose-dependent increase in anti-S_{CoV-2} IgG and IgA titers, and a broadened sero-neutralization potential both in the sera and lung homogenates against VOCs. No anti-S_{CoV-2} IgA was detected in the lungs of mice injected i.m.

with the third dose of mRNA-1273. Increasing proportions of lung surface IgM/D⁺ B cells with CD38⁺ CD73⁺ CD62L⁺ CD69⁺ CD80⁺ phenotype, which have been associated with resident memory characteristic, were detected in a dose-dependent manner, in these mice. No such increases in this B-cell subset were detected in their counterparts which received an additional
5 mRNA-1273 dose via i.m.

Spike-specific, effector lung CD8⁺ Tc1 cells were largely detected in the initially mRNA-1273-primed and boosted mice which received a late i.n. LV::S_{Beta-2P} boost. These lung CD8⁺ T cells did not display Tc2 phenotype. Increasing proportions of lung CD8⁺ CD44⁺ CD69⁺ CD103⁺ Trm were also detected, in a dose-dependent manner, only in LV::S_{Beta-2P} i.n. boosted
10 mice, but not in their counterparts boosted i.m. with mRNA-1273. The systemic CD8⁺ T-cell responses against various immunogenic regions of S_{CoV-2} were also increased with 1×10^8 or 1×10^9 TU of LV::S_{Beta-2P} i.n. boost in initially mRNA-1273-primed and boosted mice. The highest i.n. dose of LV::S_{Beta-2P} was comparable to the additional i.m. dose of mRNA-1273. The fact that the i.n. administration of LV::S_{Beta-2P} has a boost effect on the systemic T-cell immunity
15 represents another advantage of this vaccination regimen.

Evaluation of the protective potential of the lungs of mice primed and boosted with mRNA-1273, without additional boosting, showed that 20 wks after the first injection of mRNA-1273, there was no detectable protective capacity left against the Delta variant of SARS-CoV-2. In these mice, an i.n. booster injection of suboptimal dose, i.e., 1×10^8 TU of LV::S_{Beta-2P}
20 completely inhibited SARS-CoV-2 replication in the lungs. A third late i.m. booster injection of mRNA-1273 reduced SARS-CoV-2 RNA content in the lungs in a similar manner, but did not completely inhibit viral replication in all mice.

The lack of protective potential against the Delta variant 4 months after prime-boost to mRNA-1273 could be explained by the hypothesis that adaptive immune memory is likely to
25 be located in secondary lymphoid organs, i.e., distant anatomical sites far from the respiratory tract. In this context, the extraordinarily rapid replication of new VOCs, such as Delta and Omicron variants, at the site of the infection would not allow sufficient time for reactivation of immune memory and early enough to prevent local mucosal infection, replication and viral transmission.

In the present invention, the inventors provided numerous evidences that LV::S_{Beta-2P} i.n. boost can be used to induce robust systemic and mucosal adaptive immunity, to broaden the specificity of the protective response. The LV::S_{Beta-2P} i.n. boost strengthen the intensity, broaden the VOC cross-recognition, and targets B-and T-cell immune responses to the principal entry point of SARS-CoV-2 to the mucosal respiratory of the host organism and avoid
35 the infection of main anatomical sites. A phase I/IIa clinical trial is currently in preparation for

the use of i.n. boost by LV::S_{Beta-2P} in previously vaccinated persons or in COVID-convalescents.

Materials and Method

5 Mice immunization and SARS-CoV-2 infection

Female C57BL/6JRj mice were purchased from Janvier (Le Genest Saint Isle, France), housed in individually-ventilated cages under specific pathogen-free conditions at the Institut Pasteur animal facilities and used at the age of 7 wks. Mice were immunized i.m. with 1 µg/mouse of mRNA-1273 (Moderna) vaccine. For i.n. injections with LV, mice were
 10 anesthetized by i.p. injection of Ketamine (Imalgene, 80255mg/kg) and Xylazine (Rompun, 5 mg/kg). For protection experiments against SARS-CoV-2, mice were transferred into filtered cages in isolator. Four days before SARS-CoV-2 inoculation, mice were pretreated with 3 x 10⁸ IGU of Ad5::hACE2 as previously described (*Ku MW, et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021). Mice were then transferred into a level 3 biosafety cabinet and inoculated
 15 i.n. with 0.3 × 10⁵ TCID₅₀ of the Delta SARS-CoV-2 clinical isolate (*Lescure FX, et al. Lancet Infect Dis*, 20(6), 697-706, 2020) contained in 20 µl. Mice were then housed in filtered cages in an isolator in BioSafety Level 3 animal facilities. The organs recovered from the infected animals were manipulated according to the approved standard procedures of these facilities.

20 Ethical approval of animal experimentation.

Experimentation on animals was performed in accordance with the European and French guidelines (Directive 86/609/CEE and Decree 87-848 of 19 October 1987) subsequent to approval by the Institut Pasteur Safety, Animal Care and Use Committee, protocol agreement delivered by local ethical committee (CETEA #DAP20007, CETEA
 25 #DAP200058, and Ministry of High Education and Research APAFIS#24627-2020031117362508 v1, APAFIS#28755-2020122110238379 v1).

Construction and production of vaccinal LVs

First, a codon-optimized sequence of Spike from the Ancestral, D614G, Alpha, Beta or
 30 Gamma VOCs were synthesized and inserted into the pMK-RQ_S-2019-nCoV_S501YV2 plasmid. The S sequence was then extracted by BamHI/XhoI digestion to be ligated into the pFlap lentiviral plasmid between the BamHI and XhoI restriction sites, located between the native human ieCMV promoter and the mutated *atg* starting codon of Woodchuck Posttranscriptional Regulatory Element (WPRE) sequence (See the plasmid maps, **Figure**
 35 **10**). To introduce the K⁹⁸⁶P-V⁹⁸⁷P “2P” double mutation in S_{D614G} or S_{Beta}, a directed

mutagenesis was performed by use of Takara In-Fusion kit on the corresponding pFlap plasmids. Various pFlap-*ieCMV-S-WPREm* or pFlap-*ieCMV-S_{2P}-WPREm* plasmids were amplified and used to produce non-integrative vaccinal LV, as described elsewhere, (Ku MW, *et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021; Ku MW, Charneau P, Majlessi L. *Expert Rev Vaccines*, 1-16, 2021).

Analysis of humoral and systemic T-cell immunity

Anti-S_{CoV-2} IgG and IgA antibody titers were determined by ELISA by use of recombinant stabilized S_{CoV-2} or RBD fragment for coating. Neutralization potential of clarified and decomplemented sera or lung homogenates was quantitated by use of lentiviral particles pseudo-typed with S_{CoV-2} from diverse variants, as previously described (Ku MW, *et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021; Sterlin D, *et al. Sci Transl Med*, 13(577), 2021).

T-splenocyte responses were quantitated by IFN- γ ELISPOT after *in vitro* stimulation with S:256-275, S:536-550 or S:576-590 synthetic 15-mer peptides which contain S_{CoV-2} MHC-I-restricted epitopes in H-2^d mice (Ku MW, *et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021). Spots were quantified in a CTL Immunospot S6 ultimate-V Analyser by use of CTL Immunocapture 7.0.8.1 program.

Phenotypic and Functional cytometric analysis of lung immune cells

Enrichment and staining of lung immune cells were performed as detailed elsewhere (Ku MW, *et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021; Ku MW, *et al. EMBO Mol Med*, e14459, 2021) after treatment with 400 U/ml type IV collagenase and DNase I (Roche) for a 30-minute incubation at 37°C and homogenization by use of GentleMacs (Miltenyi Biotech). Cell suspensions were then filtered through 100 μ m-pore filters, centrifuged at 1200 rpm and enriched on Ficoll gradient after 20 min centrifugation at 3000 rpm at RT, without brakes. The recovered cells were co-cultured with syngeneic bone-marrow derived dendritic cells loaded with a pool of A, B, C peptides, each at 1 μ g/ml or negative control peptide at x290 μ g/ml. The following mixture was used to detect lung Tc1 cells: PerCP-Cy5.5-anti-CD3 (45-0031-82, eBioScience), eF450-anti-CD4 (48-0042-82, eBioScience) and APC-anti-CD8 (17-0081-82, eBioScience) for surface staining and BV650-anti-IFN- γ (563854, BD), FITC-anti-TNF (554418, BD) and PE-anti-IL-2 (561061, BD) for intracellular staining. The following mixture was used to detect lung Tc2 cells: PerCP-Cy5.5-anti-CD3 (45-0031-82, eBioScience), eF450-anti-CD4 (48-0042-82, eBioScience), BV711-anti-CD8 (563046, BD Biosciences), for surface staining and BV605-anti-IL-4 (504125, BioLegend Europe BV), APC-anti-IL-5 (504306, BioLegend Europe BV), FITC-anti-IL-10 (505006, BioLegend Europe BV), PE-anti-

IL-13 (12-7133-81, eBioScience) for intracellular staining. The intracellular staining was performed by use of the Fix Perm kit (BD), following the manufacturer's protocol. Dead cells were excluded by use of Near IR Live/Dead (Invitrogen). Staining was performed in the presence of FcγII/III receptor blocking anti-CD16/CD32 (BD).

5 To identify lung resident memory CD8⁺ T-cell subsets, a mixture of PerCP-Vio700-anti-CD3 (130-119-656, Miltenyi Biotec), PECy7-CD4 (552775, BD Biosciences), BV510-anti-CD8 (100752, BioLegend), PE-anti-CD62L (553151, BD Biosciences), APC-anti-CD69 (560689, BD Biosciences), APC-Cy7-anti-CD44 (560568, BD Biosciences), FITC-anti-CD103 (11-1031-82, eBiosciences) and yellow Live/Dead (Invitrogen) was used. Lung B cells were studied
10 by surface staining with a mixture of PerCP Vio700-anti-IgM (130-106-012, Miltenyi), and PerCP Vio700-anti-IgD (130-103-797, Miltenyi), APC-H7-anti-CD19 (560143, BD Biosciences), PE-anti-CD38 (102708, BioLegend Europe BV), PE-Cy7-anti-CD62L (ab25569, AbCam), BV711-anti-CD69 (740664, BD Biosciences), BV421-anti-CD73 (127217, BioLegend Europe BV), FITC-anti-CD80 (104705, BioLegend Europe BV) and yellow Live/Dead
15 (Invitrogen).

Cells were incubated with appropriate mixtures for 25 minutes at 4°C, washed in PBS containing 3% FCS and fixed with Paraformaldehyde 4% after an overnight incubation at 4°C. Samples were acquired in an Attune NxT cytometer (Invitrogen) and data analyzed by FlowJo software (Treestar, OR, USA).

20

Determination of viral RNA content in the organs

Organs from mice were removed and immediately frozen at -80°C on dry ice. RNA from circulating SARS-CoV-2 was prepared from lungs as described elsewhere (*Ku MW, et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021). Lung homogenates were prepared by
25 thawing and homogenizing in lysing matrix M (MP Biomedical) with 500 µl of PBS using a MP Biomedical Fastprep 24 Tissue Homogenizer. RNA was extracted from the supernatants of organ homogenates centrifuged during 10 min at 2000g, using the Qiagen Rneasy kit, except that the neutralization step with AVL buffer/carrier RNA was omitted. The RNA samples were then used to determine viral RNA content by E-specific qRT-PCR. To determine viral RNA
30 content by Esg-specific qRT-PCR, total RNA was prepared using lysing matrix D (MP Biomedical) containing 1 mL of TRIzol reagent (ThermoFisher) and homogenization at 30 s at 6.0 m/s twice using MP Biomedical Fastprep 24 Tissue Homogenizer. The quality of RNA samples was assessed by use of a Bioanalyzer 2100 (Agilent Technologies). Viral RNA contents were quantitated using a NanoDrop Spectrophotometer (Thermo Scientific NanoDrop).
35 The RNA Integrity Number (RIN) was 7.5-10.0. SARS-CoV-2 E or E sub-genomic mRNA were

quantitated following reverse transcription and real-time quantitative TaqMan® PCR, using SuperScript™ III Platinum One-Step qRT-PCR System (Invitrogen) and specific primers and probe (Eurofins), as recently described (Ku MW, et al. *EMBO Mol Med*, e14459, 2021).

5 **Examples**

Antigen design and down-selection of a lead candidate

To select the most suitable S_{CoV-2} variant, which can consider the dynamics of virus spread of the known variants and able to induce the greatest neutralization breadth, the inventors generated LVs encoding the full length S_{CoV-2} from the Alpha, Beta or Gamma SARS-CoV-2 VOCs. C57BL/6 mice ($n = 5/\text{group}$) were primed i.m. (wk 0) and boosted i.m. (wk 3) with 1×10^8 TU/mouse of each individual LV and the (cross) neutralization potential of their sera was assessed before boost (wk 3) and after boost (wk 5) against pseudoviruses carrying various S_{CoV-2} (**Figure 1A**). Immunization with LV::S_{Alpha} generated appropriate neutralization capacity against S_{D614G} and S_{Alpha} but not against S_{Beta} and S_{Gamma} (**Figure 1B**). Between 15 LV::S_{Beta} and LV::S_{Gamma}, the former generated the highest cross sero-neutralization potential against S_{D614G}, S_{Alpha} and S_{Gamma} variants. In accordance with previous observations using other vaccination strategies in the context of immunization with LV, the K^{986P} - V^{987P} substitutions in the S2 domain of S_{CoV-2} improved the (cross) sero-neutralization potential (**Figure 1C**), which the most probably results from an extended half-life of S_{CoV-2-2P} (Walls AC, 20 et al. *Cell*, 181(2), 281-292 e286, 2020).

Taken together these data allowed to down select S_{Beta-2P} as the best cross-reactive antigen candidate to be used in the context of LV (LV::S_{Beta-2P}) to strengthen the waning immunity previously induced by the first generation COVID-19 vaccines, like mRNA-1273.

25 **Follow-up of humoral immunity in mRNA-1273-primed and -boosted mice and effect of LV::S_{Beta-2P} i.n. boost**

The inventors analyzed the potential of LV::S_{Beta-2P} i.n. boost vaccination to strengthen and broaden the immune responses in mice which have been initially primed and boosted with mRNA-1273 and in which the (cross) sero-neutralization potential is decreasing. C57BL/6 mice 30 were primed i.m. at wk 0 and boosted i.m. at wk 3 with 1 µg/mouse of mRNA-1273, defined as the optimal dose of this vaccine in mice (*Nature*, 2020, Vol. 586, 567–571) (**Figure 2A**). Longitudinal serological follow-up demonstrated that at 3 wks post prime, cross-neutralization activities against both S_{D614G} and S_{Alpha} were readily detectable (**Figure 2B**). Cross sero-neutralization was also detectable, although to a lesser degree, 35 against S_{Gamma}, but not against S_{Beta}, S_{Delta} or S_{Delta+}. At wk 6, i.e., 3 wks post boost, cross sero-

neutralization activities against all S_{CoV-2} variants were detectable, although at significantly lesser extents against S_{Beta}, S_{Delta} and S_{Delta+}. From wk 6 to wk 10, cross sero-neutralization against S_{Beta}, S_{Delta}, or S_{Delta+} gradually and significantly decreased.

At wk 15, groups of mRNA-1273-primed and -boosted mice received i.n. escalating doses of 1×10^6 , 1×10^7 , 1×10^8 , or 1×10^9 TU/mouse of LV::S_{Beta-2P} (**Figure 2A**). Control mRNA-1273-primed and -boosted mice received i.n. 1×10^9 TU of an empty LV (LV Ctrl). In parallel, at this time point, mRNA-1273-primed and -boosted mice were injected i.m. with 1 μ g of mRNA-1273 or PBS. Unprimed, age-matched mice received i.n. 1×10^9 TU of LV::S_{Beta-2P} or PBS.

In mRNA-1273-primed mice, serum anti-S_{CoV-2} and anti-RBD IgG were detected at wk 3, increased after mRNA-1273 boost as studied at wk 6 and 10, and then decreased at wk 17 in the absence of additional boost (**Figure 8A**). In the mRNA-1273-primed and -boosted mice, boosted at wk 15, a significant increase was observed in the titer of anti-S_{CoV-2} IgG in the mice injected with 1×10^8 or 1×10^9 TU of LV::S_{Beta-2P} or a third dose of mRNA-1273 (**Figure 2C**). The titers of anti-S_{CoV-2} IgA were higher in the mice injected with 1×10^9 TU of LV::S_{Beta-2P} than those injected with a third dose of mRNA-1273 (**Figure 2C**). At the mucosal level, at this time point, titers of anti-S_{CoV-2} and anti-RBD IgG in the total lung extracts increased in a dose-dependent manner in LV::S_{Beta-2P}-injected mice, with the highest dose of LV::S_{Beta-2P} was comparable to the third i.m. dose of mRNA-1273 (**Figure 8B**). Importantly, significant titers of lung anti-S_{CoV-2} IgA were only detected in LV::S_{Beta-2P}-boosted mice but were barely detectable in their counterparts boosted late via i.m. with mRNA-1273 (**Figure 8B**).

At the mucosal cellular level, inside the lung IgM⁺/IgD⁺ CD19⁺ (Ig switched) B-cell population, increased percentages of CD38⁺ CD62L⁺ CD69⁺ CD73⁺ CD80⁺ cells, which may constitute resident memory B cells, were detected in a dose-dependent manner in mice boosted i.n. with LV::S_{Beta-2P}, but not in their mRNA-1273 i.m.-boosted counterparts (**Figure 3A, B**).

Features of systemic and mucosal T-cell immunity after i.n. LV::S_{Beta-2P} boost in previously mRNA-1273-primed and -boosted mice

Systemic anti-S_{CoV-2} T-cell immunity was assessed by IFN- γ -specific ELISPOT in the spleen of individual mice immunized following the above-mentioned regimen (**Figure 2A**) after *in vitro* stimulation with individual S:256-275, S:536-550 or S:576-590 peptide, encompassing immunodominant S_{CoV-2} regions for CD8⁺ T cells in H-2^b mice (*Ku MW, et al. Cell Host Microbe, 29(2), 236-249 e236, 2021*). Importantly, the weak anti-S CD8⁺ T-cell immunity, detectable in the spleen of mRNA-1273 primed-boosted mice at wk 17, largely

increased following i.n. boosting with 1×10^8 and 1×10^9 TU/mouse of LV::S_{Beta-2P} (**Figure 4**). The booster effect of the 1×10^9 TU dose of LV::S_{Beta-2P} i.n. was comparable, or had a tendency to be higher than, the additional dose of mRNA-1273 i.m. on the systemic T-cell immunity.

In parallel, in the same animals, the mucosal anti-S_{CoV-2} T-cell immunity was assessed by intracellular Tc1 and Tc2 cytokine staining in T-cell enriched fraction from individual mice, after *in vitro* stimulation with autologous bone-marrow-dendritic cells loaded with a pool of S:256-275, S:536-550 and S:576-590 peptides (**Figure 5A**). In previously mRNA-1273-primed and -boosted mice, only a few S_{CoV-2}-specific IFN- γ /TNF/IL-2 CD8⁺ T-cell responses were detected in the lungs (**Figure 5A**). The i.n. administration of LV::S_{Beta-2P} boosted, in a dose dependent manner, these Tc1 responses with sizable percentages of such Tc1 cells induced with the doses of 1×10^8 or 1×10^9 TU. The mRNA-1273 i.m. administration had a substantially lower boost effect on mucosal T cells (**Figure 5A, B**). Tc2 responses (IL-4, IL-5, IL-10 and IL-13) were not detected in any experimental group (**Figure 9**).

Importantly, the proportions of lung resident memory CD8⁺ T cells (Trm) were significantly increased in mice boosted i.n. with escalating doses of LV::S_{Beta-2P}, in net contrast to their counterparts which received i.m. a third dose of mRNA-1273. In fact, the latter did not have significant proportions of this Trm cell population which is very frequently correlated with protective potential in numerous infectious diseases (**Figure 6A, B**).

20 **Full protective potential of a late LV::S_{Beta-2P} i.n. boost in mRNA-1273-primed and -boosted mice**

The inventors then evaluated the protective vaccine efficacy of LV::S_{Beta-2P} i.n. boost in mRNA-1273-primed and -boosted mice, following a vaccination regimen comparable to the above-mentioned one. At wk 15, mRNA-1273-primed and -boosted mice received i.n. the suboptimal dose of 1×10^8 TU of LV::S_{Beta-2P} or control empty LV (**Figure 7A**). The choice of such suboptimal dose was based on numerous previous observations from the inventors with this dose which was effective in protection in a homologous LV prime-boost experiment (*Ku MW, et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021; *Ku MW, et al. EMBO Mol Med*, e14459, 2021). Control mRNA-1273-immunized mice received mRNA-1273 i.m. or PBS. Unvaccinated, age-and sex-matched controls were left unimmunized. Four weeks after the late boost, *i.e.* wk 20, all mice were pre-treated with 3×10^8 Infectious Genome Units (IGU) of an adenoviral vector serotype 5 encoding hACE2164(Ad5::hACE2) (*Ku MW, et al. Cell Host Microbe*, 29(2), 236-249 e236, 2021) to render their lungs permissive to SARS-CoV-2 replication (**Figure 7B**). Four days later, mice were challenged with SARS-CoV-2 Delta variant,

which, at the time of the present invention, *i.e.*, November 2021, was the most expanded SARS-CoV-2 variant worldwide.

At day 3 post infection (dpi), analysis of the total lung RNA first showed that hACE-2 was expressed homogeneously in all mice after Ad5::hACE2 *in vivo* transduction (**Figure 7B**). Lung viral loads were then determined at 3 dpi by assessing total E RNA and sub-genomic (Esg) E_{CoV-2} RNA qRT-PCR, the latter being an indicator of active viral replication (*Chandrashekar A, et al. Science, 369(6505), 812-817, 2020; Tostanoski LH, et al. Nat Med, 26(11), 1694-1700, 2020; Wolfel R, Corman VM, Guggemos W et al. Virological assessment of hospitalized patients with COVID-2019. Nature, 581(7809), 465-469, 2020*). In mice initially primed and boosted with mRNA-1273, and then injected i.n. with the control LV or i.m. with PBS alone, no significant protection potential was detectable against the challenge with SARS-CoV-2 Delta variant. In net contrast, the LV::S_{Beta-2P} i.n. boost drastically reduced the total E_{CoV-2} RNA content of SARS-CoV-2 and no copies of the replication-related Esg E_{CoV-2} RNA were detected in this group (**Figure 7B**). The content of total E_{CoV-2} RNA was also significantly reduced in the group which received a late mRNA-1273 i.m. boost. The content of Esg E_{CoV-2} RNA was undetected in 3 out of 5 in this group.

Claims

1. A pseudotyped lentiviral vector particle encoding a Spike (S) protein of a Severe
5 Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2) or a derivative thereof for use as a
heterologous boost or target immunization agent in a vaccine regimen for administration to the
upper respiratory tract of a subject, in particular a human subject, who received a prime
immunization with a vaccine composition against SARS-CoV-2 infection or disease selected
10 from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a
protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a
protein or an mRNA vaccine composition against SARS-CoV-2 infection or disease.

2. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or
a derivative thereof for use according to claim 1, wherein the administration is performed as
15 intranasal mucosal boost or target immunization in a subject who received a prime
administration with a vaccine composition against SARS-CoV-2 infection or disease selected
from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a
protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a
protein or an mRNA vaccine composition against SARS-CoV-2 infection or disease.

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3. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or
a derivative thereof for use according to claim 1 or 2, wherein:

- the S protein is from a SARS-CoV-2 virus which is pathogenic for a human host, in
particular (i) a S protein from a SARS-CoV-2 virus selected from the group consisting
25 of SARS-CoV-2 Ancestral strain, D614G strain, Alpha strain, Beta strain, Gamma
strain, Delta strain and Omicron strain, preferably from Beta strain or Omicron strain,
more preferably from Beta strain, or (ii) a S protein from a variant of said Ancestral,
D614G, Alpha, Beta, Gamma, Delta or Omicron strains, wherein such variants encode
a Spike protein with an amino acid sequence at least 90% identical to SEQ ID NO: 1
30 or,
- the S protein is a derivative of the native S protein of one of the Ancestral, D614G,
Alpha, Beta, Gamma, Delta or Omicron strains by mutation of 1 to 12, especially 1 to 6
amino acid residues, in particular by substitution and/or deletion of 1 to 12, especially
1 to 6 amino acid residues, in particular (a) is a stabilized form of the S protein
35 characterized by substitution of two consecutive amino acid residues for Proline
residues in the S2 domain of the S protein at positions provided as residues 986 and

987 by reference to SEQ ID NO: 1 in the amino acid sequence of the Spike protein or (b) is a prefusion form of the S protein by deletion of the furin site located from residue 675 to residue 685 by reference to SEQ ID NO: 1 in the amino acid sequence of the S protein or (c) is a stabilized prefusion form of the S protein by deletion of the furin site and substitution of two consecutive amino acid residues for Proline residues in the S2 domain at positions 986 and 987 by reference to SEQ ID NO: 1.

4. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 3, wherein the amino acid sequence of the S protein is SEQ ID NO: 1 or is a derivative thereof having an amino acid sequence at least 90% identical to SEQ ID NO: 1, and wherein the derivative of the S protein of SARS-CoV-2 comprises at least five amino acid mutations including (i) a mutation of the lysine residue to the asparagine residue at position 417 of the amino acid sequence of SEQ ID NO: 1 (K417N), (ii) a mutation of the glutamic acid residue to the lysine residue at position 484 of the amino acid sequence of SEQ ID NO: 1 (E484K) or a mutation of the glutamic acid residue to the alanine residue at position 484 of the amino acid sequence of SEQ ID NO: 1 (E484A), (iii) a mutation of the asparagine residue to the tyrosine residue at position 501 of the amino acid sequence of SEQ ID NO: 1 (N501Y), (iv) a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence of SEQ ID NO: 1 (K986P) and (v) a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P).

5. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 4, wherein the subject receiving administration of the composition is selected from the group consisting of (a) a subject that has previously received a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease as a systemic prime and/or boost administration(s) such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular prime and/or boost administration(s), (b) a subject that has received a systemic prime administration such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular prime administration, of a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an

mRNA-based vaccine against SARS-CoV-2 infection or disease and has then recovered from coronavirus disease such as coronavirus disease 2019 (COVID-19), (c) a subject that has first recovered from coronavirus disease such as COVID-19 and has then received a systemic prime administration such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular administration, of a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease, and (d) a subject that has received more than two, in particular more than three, systemic administrations such as intramuscular, intradermal or sub-cutaneous administration(s), in particular an intramuscular administration, of a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease.

6. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 5, wherein the S protein of SARS-CoV-2 further comprises amino acid mutations selected from the group consisting of (vi) a mutation of the glycine residue to the serine residue at position 446 of the amino acid sequence of SEQ ID NO: 1 (G446S), (vii) a mutation of the threonine residue to the lysine residue at position 478 of the amino acid sequence of SEQ ID NO: 1 (T478K), (viii) a mutation of the glutamine residue to the arginine residue at position 493 of the amino acid sequence of SEQ ID NO: 1 (Q493R) and (ix) a mutation of the glutamine residue to the arginine residue at position 498 of the amino acid sequence of SEQ ID NO: 1 (Q498R).

7. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 6, wherein the encoded mutated S protein of SARS-CoV-2 has the amino acid sequence of SEQ ID NO: 10 or SEQ ID NO: 18, preferably of SEQ ID NO: 10.

8. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 7, wherein the pseudotyped lentiviral vector particle is pseudotyped with a Vesicular Stomatitis Virus glycoprotein G (VSV-G) protein.

9. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 8, in a prime/boost or target immunization regimen for elicitation of a long-lasting protective mucosal humoral immune response and/or a long-lasting mucosal cellular immune response against SARS-CoV-2 infection or disease, wherein said response protects the respiratory system and/or the central nervous system of the subject.

10. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 9, wherein the pseudotyped lentiviral vector particle elicits a CD8⁺ T-cell response against SARS-CoV-2.

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11. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 10, wherein the pseudotyped lentiviral vector particle elicits lung-resident memory CD8⁺ T cells (Trm) and/or effector CD8⁺ T cells (Tc1) specific to Spike and produce Interferon-gamma (IFN-γ)/Tumor Necrosis Factor (TNF)/Interleukin-2 (IL-2).

10

12. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 11, wherein the pseudotyped lentiviral vector particle is non-integrative, non-cytopathic and non-replicative.

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13. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 12, wherein the subject has a waning immunity from week 12 after the first injection of the initial vaccination with a vaccine composition against SARS-CoV-2 infection or disease selected from the group consisting of a protein, an mRNA, an adenovirus, an inactivated virus and a protein subunit vaccine composition against SARS-CoV-2 infection or disease, in particular a protein- or an mRNA-based vaccine against SARS-CoV-2 infection or disease or post SARS-CoV-2 disease recovery, in particular post-COVID-19 recovery.

20

14. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 13, wherein the pseudotyped lentiviral vector particle is formulated as a liquid composition or a dry powder for an administration as intranasal aerosols, intranasal drops or intranasal insufflations.

25

15. The pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof for use according to any one of claims 1 to 14, wherein the administration regimen comprises administration of one or more dosage form(s) of the pseudotyped lentiviral vector particle, wherein the dose of each dosage form is from 10⁷ to 10⁹ Transduction Unit (TU).

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16. An immunogenic composition comprising a pseudotyped lentiviral vector particle encoding a S protein of a SARS-CoV-2 or a derivative thereof and a pharmaceutically acceptable carrier, wherein the pseudotyped derivative of the S protein of SARS-CoV-2 comprises at least nine amino acid mutations including (i) a mutation of the lysine residue to the asparagine residue at position 417 of the amino acid sequence of SEQ ID NO: 1 (K417N),
5 (ii) a mutation of the glutamic acid residue to the alanine residue at position 484 of the amino acid sequence of SEQ ID NO: 1 (E484A), (iii) a mutation of the asparagine residue to the tyrosine residue at position 501 of the amino acid sequence of SEQ ID NO: 1 (N501Y), (iv) a mutation of the lysine residue to the proline residue at position 986 of the amino acid sequence
10 of SEQ ID NO: 1 (K986P), (v) a mutation of the valine residue to the proline residue at position 987 of the amino acid sequence of SEQ ID NO: 1 (V987P), (vi) a mutation of the glycine residue to the serine residue at position 446 of the amino acid sequence of SEQ ID NO: 1 (G446S), (vii) a mutation of the threonine residue to the lysine residue at position 478 of the amino acid sequence of SEQ ID NO: 1 (T478K), (viii) a mutation of the glutamine residue to the arginine
15 residue at position 493 of the amino acid sequence of SEQ ID NO: 1 (Q493R) and (ix) a mutation of the glutamine residue to the arginine residue at position 498 of the amino acid sequence of SEQ ID NO: 1 (Q498R).

17. The immunogenic composition according to claim 16, wherein the encoded mutated
20 S protein of SARS-CoV-2 has the amino acid sequence of SEQ ID NO: 18.

18. The immunogenic composition according to claim 16 or 17, which is formulated for intranasal administration.