# Treatment using a one-to-stop attenuated SARS-CoV-2 Virus

## Technical Field

The invention relates to pharmaceutical product comprising a polynucleotide for use in the prevention or treatment of a SARS-CoV-2 virus infection wherein said SARS-CoV-2 virus is not a Wuhan wild-type SARS-CoV-2 virus.

## Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in December 2019 as the causative agent of coronavirus disease 2019 (COVID-19). The virus is highly transmissible among humans. It has spread rapidly around the world within a matter of weeks and the world is still battling with the ongoing COVID-19 pandemic.

The rapid development and availability of vaccines are crucial in combating many viruses and bacteria. The production of suitable vaccines is a multi-stage, complex process and is not always successful despite often high investments. Typically, the development of a suitable vaccine takes years. These long development times consist of a major problem, especially with regard to new emerging pathogens, or mutated pathogens, as from an epidemiological point of view it is only possible to react too late, if at all, to the emergence of new diseases. In contrast, the analysis, identification and further detection of new or heavily mutated pathogens are now possible within weeks or even days, which is a huge improvement over the last century.

In this context, viruses are of special interest, as they harbor high mutation rates causing the spread from other species to humans. Rapid spreading of these viruses makes them a major challenge for modern medicine. The usual time between the detection/identification of a newly emerging virus and the development of a vaccine is typically years. In a few cases, with sufficient prior knowledge, experimental vaccines could be provided within months. However, this period is much longer than the typical time until thousands or millions of people are infected. Such rapid spread is also a direct consequence of the high mobility of today's society.

Ideally, immediately after the identification of a new virus, a vaccine would be available in sufficient quantity and of the highest quality and would allow for a nationwide vaccination of all persons who have somehow come close to the initial outbreak site of the new virus. Furthermore, an ideal method for such a vaccine would be capable of reacting to the evolution and adaptation of the virus. Such an ideal production possibility seems utopian to the person skilled in the art today.

In the recent past, in particular, the corona pandemic has dramatically increased the relevance of developing suitable tools for vaccine production. There is unanimous agreement that the

development of a vaccine against the coronavirus SARS-CoV-2 is the only proven means of containing the pandemic and the associated global crisis in the long term.

The emergence of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led not only to global spread, but also to the evolution of various concerning virus variants (https://www.ecdc.europa.eu/en/covid-19/variants-concern). Despite the rapid development of vaccines, the current vaccines primarily target the spike protein antigen, providing limited protection against infection and viral transmission. Consequently, SARS-CoV-2 can evade immunity through spike gene mutations, hindering consistent interruption of infection chains. Therefore, there is an urgent need for more robust and adaptable vaccination strategies.

SARS-CoV-2 variant strains are often more contagious or pathogenic than the original wild-type SARS-CoV-2 strain. Such new emerging SARS-CoV-2 strains may lead to a reduced efficiency of first-generation vaccines that were developed against the wild-type SARS-CoV-2 strain. Further, it is unclear whether a vaccination against SARS-CoV-2 to protective immune responses in case a SARS-CoV-2 infection occurs after a long period.

Thus, there is a need to provide a vaccine against variants of coronavirus SARS-CoV-2 and vaccines having a long term effect.

**Summary**

The above technical problem is solved by the examples disclosed herein and as defined in the claims. Accordingly, the disclosure relates to, inter alia, the following examples:

1. A polynucleotide encoding an attenuated human coronavirus or a fragment thereof, wherein the polynucleotide comprises at least 20 one-to-stop codons, wherein a one-to-stop codon is

    i) a different but synonymous codon compared to a corresponding codon in a natural human coronavirus genome or a fragment thereof; and

    ii) differs by only one nucleotide from a STOP codon.

2. The polynucleotide of example 1, wherein the fragment of the polynucleotide when combined with corresponding human coronavirus parts encodes a coronavirus particle that induces an immune response after immunization of mice with 5000 PFU coronavirus particle after 15 days and an increased immune response upon challenge with WT human coronavirus after 21 days measured after 35 days.

3. A method for producing a polynucleotide of example 1 or 2, the method comprising the steps of:

a) providing the CDS of a natural human coronavirus genome, a fragment or cDNA clone thereof; and

b) modifying the natural human coronavirus genome, the fragment or the retro-transcribed cDNA sequence of the cDNA clone, respectively,

wherein said modification comprises replacing at least 20 codons in the natural human coronavirus genome, the fragment or the retro-transcribed cDNA sequence, by at least 20 one-to-stop codons, wherein a one-to-stop codon is

i) a different but synonymous codon compared to a corresponding codon in the natural human coronavirus genome, the fragment or the retro-transcribed cDNA sequence; and

ii) differs by only one nucleotide from a STOP codon.

4. The polynucleotide of example 1 or 2 or the method of example 3, wherein the natural human coronavirus genome or a fragment thereof is

a) a SARS-CoV-2 sequence comprised in or consisting of a sequence as defined by SEQ ID NO: 7 or

b) a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7, preferably a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7 which maintains the ability to encode one or more SARS-CoV-2 virus proteins.

5. The polynucleotide of any one of the examples 1, 2 or 4 or the method of example 3 or 4, wherein the fragment has a minimum length of 500 nucleotides.

6. The polynucleotide of any one of the examples 1, 2, 4 or 5 or the method of any one of the examples 3 to 5, wherein the human coronavirus is SARS-CoV-2 and wherein at least one of the one-to-stop codons is comprised in a sequence part or fragment corresponding to a sequence part of ORF1ab of the natural SARS-CoV-2, a sequence part encoding a structure protein of the natural SARS-CoV-2 or a sequence part encoding an accessory protein of the natural SARS-CoV-2.

7. The polynucleotide of example 6 or the method of example 6, wherein at least one of the one-to-stop codons is comprised in a sequence part or fragment corresponding to a sequence part of ORF1ab of the natural SARS-CoV-2.

8. The polynucleotide of example 7 or the method of example 7, wherein at least one of the one-to-stop codons is comprised in a sequence part or fragment corresponding to an Nsp2 to Nsp15 encoding sequence part of the natural SARS-CoV-2 genome.

9. The polynucleotide of example 8 or the method of example 8, wherein at least one of the one-to-stop codons is comprised in a sequence part or fragment corresponding to an Nsp2 to Nsp7 or an Nsp13 to Nsp15 encoding sequence part of the natural SARS-CoV-2 genome.

10.1. The polynucleotide of example 8 or 9 or the method of example 8 or 9, wherein the one-to-stop codons comprise at least one one-to-stop codon having a position selected from Table 1 corresponding to a position on the natural SARS-CoV-2 genome.

10.2. The polynucleotide of example 8 or 9 or the method of example 8 or 9, wherein the one-to-stop codons comprise at least one one-to-stop codon having a position selected from Table 1 or supplementary Table 3 corresponding to a position on the natural SARS-CoV-2 genome.

11. The polynucleotide of any one of examples 1, 2, 4 to 10 or the method of any one of examples 3 to 10, wherein the amino acids encoded by the at least 20 one-to-stop codons consist of Leu, Ser, Arg and/or Gly.

12. The polynucleotide of example 11 or the method of example 11, wherein the amino acids encoded by the one-to-stop codons consist of Leu and/or Ser.

13. The polynucleotide of any one of examples 1, 2, 4 to 12 or the method of any one of examples 3 to 12, wherein the at least 20 one-to-stop codons are at least 50 one-to-stop codons.

14. The polynucleotide of any one of examples 1, 2, 4 to 13, wherein the human coronavirus is SARS-CoV-2 and wherein the polynucleotide comprises no sequence encoding a protein having an Nsp1 functionality of the natural SARS-CoV-2 or a sequence encoding a protein having a reduced Nsp1 functionality compared to the Nsp1 of a natural SARS-CoV-2, preferably wherein the polynucleotide comprises a sequence

encoding a protein having a reduced Nsp1 functionality compared to the Nsp1 of a natural SARS-CoV-2, and polynucleotide comprises a mutation compared to the Nsp1 encoding sequence of natural SARS-CoV-2, wherein the mutation is K164A and/or H165A.

15. The polynucleotide of any one of examples 1, 2, 4 to 14, wherein the human coronavirus is SARS-CoV-2 and wherein the polynucleotide comprises no sequence encoding a protein having the functionality of a protein encoded by the ORF6 gene of the natural SARS-CoV-2 or a sequence encoding a protein having a reduced functionality of a protein encoded by the ORF6 gene of the natural SARS-CoV-2.

16. The polynucleotide of any one of examples 1, 2, 4 to 15, wherein the human coronavirus is SARS-CoV-2 and wherein the polynucleotide comprises no sequence encoding a protein having the functionality of a protein encoded by the ORF7a gene of the natural SARS-CoV-2 or a sequence encoding a protein having a reduced functionality of a protein encoded by the ORF7a gene of the natural SARS-CoV-2.

17. The polynucleotide of any one of examples 1, 2, 4 to 16, wherein the human coronavirus is SARS-CoV-2 and wherein the polynucleotide comprises no sequence encoding a protein having the functionality of a protein encoded by the ORF7b gene of the natural SARS-CoV-2 or a sequence encoding a protein having a reduced functionality of a protein encoded by the ORF7b gene of the natural SARS-CoV-2.

18. The polynucleotide of any one of examples 1, 2, 4 to 17, wherein the human coronavirus is SARS-CoV-2 and wherein the polynucleotide comprises no sequence encoding a protein having the functionality of a protein encoded by the ORF8 gene of the natural SARS-CoV-2 or a sequence encoding a protein having a reduced functionality of a protein encoded by the ORF8 gene of the natural SARS-CoV-2.

19. The polynucleotide of any one of examples 1, 2, 4 to 18, wherein the human coronavirus is SARS-CoV-2 and wherein the polynucleotide comprises a sequence part encoding a spike protein, wherein the spike protein comprises a modified or removed cleavage site compared to the cleavage site of the spike protein of the natural SARS-CoV-2.

20. The polynucleotide according to example 19, wherein the polynucleotide consists of or comprises a sequence as defined SEQ ID NO: 6.

21. A vector comprising the polynucleotide of any one of the examples 1, 2, 4 to 20.

22. A genetically modified cell comprising the polynucleotide of any one of examples 1, 2, 4 to 20.

23. A method for production of an attenuated virus, the method comprising a step of culturing the genetically modified cell of example 22.

24. An attenuated virus comprising the polynucleotide of any one of examples 1, 2, 4 to 20.

25. A pharmaceutical product comprising the vector of example 21, the genetically modified cell of example 22 and/or the attenuated virus of example 24 for use as a medicament.

26. A pharmaceutical product comprising the vector of example 21, the genetically modified cell of example 22 and/or the attenuated virus of example 24 for use in treatment and/or prevention of a human coronavirus infection, preferably a SARS-CoV-2 infection.

27. The pharmaceutical product for use of example 25 to 26, wherein the pharmaceutical product further comprises a mutagen.

28. A method of treatment and/or prevention comprising the step of: Administering a pharmaceutical product in a therapeutically effective amount to a subject, wherein the pharmaceutical product comprises the vector of example 21, the genetically modified cell of example 22 and/or the attenuated virus of example 24.

29. The method of example 28, wherein the treatment and/or prevention is a treatment and/or prevention of a human coronavirus infection, preferably a SARS-CoV-2 infection.

30. The method of example 28 or 29, wherein the method further comprises administering a mutagen in a therapeutically effective amount to a subject.

31. The pharmaceutical product for use of example 27 or the method of example 30, wherein the mutagen is 5-Fluorouracil or Molnupiravir.

32. The polynucleotide of the disclosure, wherein said polynucleotide encodes an attenuated human coronavirus or a fragment thereof, wherein the polynucleotide comprises at least 20 one-to-stop codons, wherein a one-to-stop codon is i) a different but synonymous

codon compared to the corresponding codon in a natural human coronavirus genome and ii) differs by one nucleotide from a STOP codon.

33. The polynucleotide of the disclosure, wherein the natural human coronavirus genome is a natural SARS-CoV-2 genome, preferably a) a SARS-CoV-2 sequence comprised in or consisting of a sequence as defined by SEQ ID NO: 7 or b) a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7, preferably a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7 which maintains the ability to encode one or more SARS-CoV-2 virus proteins.

34. The polynucleotide of the disclosure, wherein at least one of the one-to-stop codons is in a sequence encoding non-structural proteins; preferably the natural human coronavirus genome is a natural SARS-CoV-2 genome, and at least one of the one-to-stop codons is in a sequence corresponding to ORF1ab in the natural SARS-CoV-2 genome.

34. The polynucleotide of the disclosure, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp1 to Nsp15, preferably Nsp3 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome.

36. The polynucleotide of the disclosure, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome.

37. The polynucleotide of the disclosure, wherein the natural human coronavirus genome is a natural SARS-CoV-2 genome, and wherein at least one of the one-to-stop codons has a CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7.

38. The polynucleotide of the disclosure, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome and at least one of the one-to-stop codons has a CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7.

39. The polynucleotide of the disclosure, wherein the one-to-stop codons are defined by CDS codon numbers corresponding each to a CDS codon number from 2023 to 6614 as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7;

preferably, the one-to-stop codons are defined by codon changes and CDS codon numbers corresponding each to a CDS codon number from 2023 to 6614 as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7.

40. The polynucleotide of the disclosure, wherein the polynucleotide consists of or comprises a sequence as defined in SEQ ID NO: 3-6 or 9-23, preferably SEQ ID NO: 4-6, more preferably SEQ ID NO: 5 or 6.

41. A pharmaceutical product comprising a polynucleotide for use in the prevention or treatment of a SARS-CoV-2 virus infection,

wherein said polynucleotide encodes an attenuated human coronavirus or a fragment thereof, wherein the polynucleotide comprises at least 20 one-to-stop codons, wherein a one-to-stop codon is i) a different but synonymous codon compared to the corresponding codon in a natural human coronavirus genome and ii) differs by one nucleotide from a STOP codon, and wherein said SARS-CoV-2 virus is not a Wuhan wild-type SARS-CoV-2 virus.

42. The pharmaceutical product for use according to example 41, wherein said SARS-CoV-2 virus is a variant of the Wuhan wild-type SARS-CoV-2 virus.

43. The pharmaceutical product for use according to example 42, wherein said variant is of lineage B, preferably B.1, more preferably B.1.1 or B.1.617, again more preferably B.1.1.529 or B.1.617.

44. The pharmaceutical product for use according example 42 or 43, wherein the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Gamma (lineage P.1), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Zeta (lineage P.2), Eta (lineage B.1.525), Theta (lineage P.3), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), Lambda (lineage C.37), Mu (lineage B.1.621) and a missense variant of a Wuhan wild-type SARS-CoV-2 virus, wherein the genome of said missense variant comprises at least one missense mutation;

preferably the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Eta (lineage B.1.525), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), Mu (lineage

B.1.621) and a missense variant of a Wuhan wild-type SARS-CoV-2 virus comprising at least one missense mutation;

more preferably the variant is Delta (lineage B.1.617.2), Omicron (B.1.1.529) or a missense variant of a Wuhan wild-type SARS-CoV-2 virus, wherein the genome of said missense variant comprises at least one missense mutation; and

again more preferably the variant is Delta (B.1.617.2), Omicron BA.2, Omicron BA.5 or a variant of a Wuhan wild-type SARS-CoV-2 virus, wherein the genome of said missense variant comprises at least one missense mutation.

45. The pharmaceutical product for use according to example 44, wherein said missense mutation is in an ORF encoding a SARS-CoV-2 spike protein, preferably said missense mutation is D614G.

46. The pharmaceutical product for use according to examples 42-45, wherein the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Gamma (lineage P.1), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Zeta (lineage P.2), Eta (lineage B.1.525), Theta (lineage P.3), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), Lambda (lineage C.37), and Mu (lineage B.1.621);

preferably the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Eta (lineage B.1.525), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), and Mu (lineage B.1.621);

more preferably the variant is Delta (lineage B.1.617.2) or Omicron (B.1.1.529); and

again more preferably the variant is Delta (B.1.617.2), Omicron BA.2 or Omicron BA.5.

47. The pharmaceutical product for use according to the any one of the preceding examples 41-46, wherein the pharmaceutical product is administered intranasally or intramuscularly.

48. The pharmaceutical product for use according to any one of the preceding examples 41-47, wherein the natural human coronavirus genome is a natural SARS-CoV-2 genome, preferably

a) a SARS-CoV-2 sequence comprised in or consisting of a sequence as defined by SEQ ID NO: 7 or

b) a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7, preferably a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7 which maintains the ability to encode one or more SARS-CoV-2 virus proteins.

49. The pharmaceutical product for use according to any one of the preceding examples 41-48, wherein at least one of the one-to-stop codons is in a sequence encoding non-structural proteins; preferably the natural human coronavirus genome is a natural SARS-CoV-2 genome, and at least one of the one-to-stop codons is in a sequence corresponding to ORF1ab in the natural SARS-CoV-2 genome.

50. The pharmaceutical product for use according to example 49, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp1 to Nsp15, preferably Nsp3 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome.

51.1 The pharmaceutical product for use according to example 49 or 50, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome.

51.2 The pharmaceutical product for use according to example 49 or 50, wherein the one-to-stop codons are in sequences corresponding to Nsp3 to Nsp7 and Nsp12 to Nsp15 encoding sequences in the natural SARS-CoV-2 genome.

52. The pharmaceutical product for use according to any one of the preceding examples 41-51, wherein the natural human coronavirus genome is a natural SARS-CoV-2 genome, and wherein at least one of the one-to-stop codons has a CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for (or relative to) SEQ ID NO: 7.

53.1 The pharmaceutical product for use according to example 52, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome and at least one of the one-to-stop codon location is defined by a CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for (or relative to) SEQ ID NO: 7.

53.2 The pharmaceutical product for use according to example 52, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome and at least one of the one-to-stop codon location is defined by a codon change and CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for (or relative to) SEQ ID NO: 7.

54. The pharmaceutical product for use according to example 52 or 53, wherein the one-to-stop codon locations are defined by CDS codon numbers, each corresponding to a CDS codon number from 2023 to 6614 as indicated in Table 1 or supplementary Table 3 for (or relative to) SEQ ID NO: 7;

preferably, the one-to-stop codons are defined by codon changes and CDS codon numbers, each corresponding to a CDS codon number from 2023 to 6614 as indicated in Table 1 or supplementary Table 3 for or relative to SEQ ID NO: 7.

55. The pharmaceutical product for use according to any one of the preceding examples 41-54, wherein the polynucleotide consists of or comprises a sequence as defined in SEQ ID NO: 3-6 or 9-23, preferably SEQ ID NO: 3-6, more preferably SEQ ID NO: 4-6, again more preferably SEQ ID NO: 5 or 6.

56. The pharmaceutical product of the disclosure and according to any one of the preceding examples comprising the polynucleotide of the disclosure, vector of the disclosure, genetically modified cell of the disclosure and/or attenuated virus of the disclosure, for use in the prevention or treatment of a corona virus infection in a human subject,

wherein said polynucleotide encodes an attenuated human coronavirus or a fragment thereof, wherein the polynucleotide comprises at least 20 one-to-stop codons, wherein a one-to-stop codon is i) a different but synonymous codon compared to a corresponding codon in a natural human coronavirus genome or a fragment thereof; and ii) differs by only one nucleotide from a STOP codon, and

wherein said human subject is challenged by corona virus infection.

57. The pharmaceutical product for use according to example 56, wherein the corona virus infection is a SARS-CoV-2 virus infection.

58. The pharmaceutical product for use according to example 56 or 57, wherein said human subject is challenged by a SARS-CoV-2 virus more than 21 days after vaccination with the pharmaceutical product of the disclosure comprising the polynucleotide, vector, genetically modified cell and/or attenuated virus according to the disclosure.

59.1 The pharmaceutical product for use according to examples 56-58, wherein the human subject is at increased risk of developing severe COVID-19.

59.2 The pharmaceutical product for use according to examples 56-58, wherein the human subject is at increased risk of developing severe COVID-19 or acute respiratory distress syndrome.

60. The pharmaceutical product for use according to examples 56-59, wherein said SARS-CoV-2 virus infection is a severe COVID-19 infection or an acute respiratory distress syndrome, preferably said SARS-CoV-2 virus infection is a severe COVID-19 infection.

In one aspect, the present disclosure refers to the use of a pharmaceutical product comprising a polynucleotide in the manufacture of a medicament for the prevention or treatment of a SARS-CoV-2 virus infection,

wherein said polynucleotide encodes an attenuated human coronavirus or a fragment thereof, wherein the polynucleotide comprises at least 20 one-to-stop codons, wherein a one-to-stop codon is i) a different but synonymous codon compared to the corresponding codon in a natural human coronavirus genome and ii) differs by one nucleotide from a STOP codon, and

wherein said SARS-CoV-2 virus is not a Wuhan wild-type SARS-CoV-2 virus.

**Detailed Description**

The inventors developed a safe and effective live-attenuated SARS-CoV-2 vaccines (LAVs, herein also called OTS mutants) based on the one-to-stop (OTS) approach. By introducing synonymous codon changes into the open-reading-frame (ORF) 1ab, the inventors maintained identical amino acid sequences to the wild-type virus while increasing the probability of premature termination codons. This compromises viral fitness and pathogenicity, contributing to attenuation.

The inventors demonstrated that the level of attenuation can be adjusted by enriching specific regions of the viral genome with one-to-stop codons. Through stepwise modifications, the inventors achieved significant attenuation in mice, resulting in 100% survival in a lethal SARS-CoV-2 animal model. Furthermore, the inventors disarmed the virus by introducing changes in specific genes known to interfere with antiviral cellular responses.

To enhance safety and antigenicity, non-structural protein 1 (NSP1) can be modified and specific ORFs, preferably 6 to 8 and the polybasic spike S1/S2 cleavage site can be deleted. By deleting these ORFs, the inventors promote early interferon responses, enhance LAV attenuation, and improve immunogenicity. Furthermore, the inventors removed the PRRAR motif from the polybasic spike S1/S2 cleavage site. Several vaccine candidates were generated

**Claims**

1. Use of a pharmaceutical product comprising a polynucleotide in the manufacture of a medicament for the prevention or treatment of a SARS-CoV-2 virus infection,

   wherein said polynucleotide encodes an attenuated human coronavirus or a fragment thereof, wherein the polynucleotide comprises at least 20 one-to-stop codons, wherein a one-to-stop codon is i) a different but synonymous codon compared to the corresponding codon in a natural human coronavirus genome and ii) differs by one nucleotide from a STOP codon, and

   wherein said SARS-CoV-2 virus is not a Wuhan wild-type SARS-CoV-2 virus.

2. The use according to claim 1, wherein said SARS-CoV-2 virus is a variant of the Wuhan wild-type SARS-CoV-2 virus.

3. The use according to claim 2, wherein said variant is of lineage B, preferably B.1, more preferably B.1.1 or B.1.617, again more preferably B.1.1.529 or B.1.617.

4. The use according claim 2 or 3, wherein the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Gamma (lineage P.1), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Zeta (lineage P.2), Eta (lineage B.1.525), Theta (lineage P.3), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), Lambda (lineage C.37), Mu (lineage B.1.621) and a missense variant of a Wuhan wild-type SARS-CoV-2 virus, wherein the genome of said missense variant comprises at least one missense mutation;

   preferably the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Eta (lineage B.1.525), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), Mu (lineage B.1.621) and a missense variant of a Wuhan wild-type SARS-CoV-2 virus wherein the genome of said missense variant comprises at least one missense mutation;

   more preferably the variant is Delta (lineage B.1.617.2), Omicron (B.1.1.529) or a missense variant of a Wuhan wild-type SARS-CoV-2 virus, wherein the genome of said missense variant comprises at least one missense mutation; and

again more preferably the variant is Delta (B.1.617.2), Omicron BA.2, Omicron BA.5 or a missense variant of a Wuhan wild-type SARS-CoV-2 virus, wherein the genome of said missense variant comprises at least one missense mutation.

5. The use according to claim 4, wherein said missense mutation is in an ORF encoding a SARS-CoV-2 spike protein, preferably said missense mutation is D614G.

6. The use according to claims 2-5, wherein the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Gamma (lineage P.1), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Zeta (lineage P.2), Eta (lineage B.1.525), Theta (lineage P.3), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), Lambda (lineage C.37), and Mu (lineage B.1.621);

   preferably the variant is selected from the group comprising, or preferably consisting of, Alpha (lineage B.1.1.7), B.1.1.7 with E484K, Beta (lineage B.1.351), Delta (lineage B.1.617.2), Omicron (B.1.1.529), Epsilon (lineages B.1.429, B.1.427, CAL.20C), Eta (lineage B.1.525), Iota (lineage B.1.526), Kappa (lineage B.1.617.1), and Mu (lineage B.1.621);

   more preferably the variant is Delta (lineage B.1.617.2) or Omicron (B.1.1.529); and

   again more preferably the variant is Delta (B.1.617.2), Omicron BA.2 or Omicron BA.5.

7. The use according to any one of the preceding claims, wherein the pharmaceutical product is to be administered intranasally or intramuscularly.

8. The use according to any one of the preceding claims, wherein the natural human coronavirus genome is a natural SARS-CoV-2 genome, preferably

   a) a SARS-CoV-2 sequence comprised in or consisting of a sequence as defined by SEQ ID NO: 7 or

   b) a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of a sequence as defined by SEQ ID NO: 7, preferably a SARS-CoV-2 sequence being 80% identical to a sequence comprised in or consisting of sequence as defined by SEQ ID NO: 7 which maintains the ability to encode one or more SARS-CoV-2 virus proteins.

9. The use according to any one of the preceding claims, wherein at least one of the one-to-stop codons is in a sequence encoding non-structural proteins; preferably the natural human coronavirus genome is a natural SARS-CoV-2 genome, and at least one of the one-to-stop codons is in a sequence corresponding to ORF1ab in the natural SARS-CoV-2 genome.

10. The use according to claim 9, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp1 to Nsp15, preferably Nsp3 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome.

11. The use according to claim 9 or 10, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 and/or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome.

12. The use according to any one of the preceding claims, wherein the natural human coronavirus genome is a natural SARS-CoV-2 genome, and wherein at least one of the one-to-stop codons has a CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7.

13. The use according to claim 12, wherein at least one of the one-to-stop codons is in a sequence corresponding to an Nsp3 to Nsp7 or an Nsp12 to Nsp15 encoding sequence in the natural SARS-CoV-2 genome and at least one of the one-to-stop codons has a CDS codon number corresponding to a CDS codon number as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7.

14. The use according to claim 12 or 13, wherein the one-to-stop codons are defined by CDS codon numbers corresponding each to a CDS codon number from 2023 to 6614 as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7;

    preferably, the one-to-stop codons are defined by codon changes and CDS codon numbers corresponding each to a CDS codon number from 2023 to 6614 as indicated in Table 1 or supplementary Table 3 for SEQ ID NO: 7.

15. The use according to any one of the preceding claims, wherein the polynucleotide consists of or comprises a sequence as defined in SEQ ID NO: 3-6 or 9-23, preferably SEQ ID NO: 3-6.