

Claims

1. A mutant of lymphocytic choriomeningitis virus, wherein the mutant is capable of undergoing a stronger propagation in a tumor cell as compared to the wild type lymphocytic choriomeningitis virus strain WE, wherein the mutant comprises a nucleic acid encoding a glycoprotein, wherein said glycoprotein comprises at least one of the mutations Arg 185 → Trp and Ile 181 → Met as compared to the wild type glycoprotein sequence set forth in SEQ ID NO: 10.
2. The mutant of claim 1, wherein the mutant comprises a nucleic acid encoding a glycoprotein that has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.5%, preferably at least about 99.7% sequence identity, to the wild type glycoprotein sequence set forth in SEQ ID NO: 10.
3. The mutant of claim 1 or 2, wherein the mutant comprises a nucleic acid encoding a glycoprotein, wherein said glycoprotein comprises the mutations Arg 185 → Trp and Ile 181 → Met as compared to the wild type glycoprotein set forth in SEQ ID NO: 10.
4. The mutant any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a glycoprotein, wherein said glycoprotein has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.5%, preferably at least about 99.7% sequence identity, or is preferably identical, to a sequence set forth in any one of SEQ ID NOs: 18, 26, 34, 42, 50, and 58.
5. The mutant of any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a glycoprotein, wherein said nucleic acid comprises a sequence that has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at

least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7% sequence identity, or is preferably identical, to a sequence set forth in SEQ ID NOs: 17, 25, 33, 41, 49, and 57.

6. The mutant of claim 1, wherein the mutant comprises a nucleic acid encoding a L-protein, wherein said L-protein has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7% sequence identity to the wild type L-protein sequence set forth in SEQ ID NO: 16.
7. The mutant any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a L-protein, wherein said L-protein has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7%, preferably at least about 99.8%, preferably at least about 99.9% sequence identity, or is preferably identical, to a sequence set forth in any one of SEQ ID NOs: 24, 32, 40, 48, 56, and 64.
8. The mutant of any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a L-protein, wherein said L-protein comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the following mutations: Lys 253 → Arg; Lys 1512 → Met; Lys 1513 → Glu; Ser 1758 → Phe; Phe 1995 → Ser; Ile 2094 → Val; Lys 2115 → Glu; Thr 2141 → Ala; Arg 2175 → Lys; Thr 2185 → Ala as compared to the wild type L-protein sequence set forth in SEQ ID NO: 16.
9. The mutant of any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a L-protein, wherein said L-protein comprises one of the following sets of mutations:
 - (a) Ser 1758 → Phe;

- (b) Phe 1995 → Ser; optionally Ile 2094 → Val; and optionally Thr 2141 → Ala;
- (c) Lys 1513 → Glu; Phe 1995 → Ser; and optionally Arg 2175 → Lys;
- (d) Lys 253 → Arg; Lys 1512 → Met; Lys 2115 → Glu; optionally Thr 2141 → Ala; Thr 2185 → Ala
- (e) Phe 1995 → Ser; or
- (f) Lys 2115 → Glu,

as compared to the wild type L-protein sequence set forth in SEQ ID NO: 16.

10. The mutant of any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding an L-protein, wherein said nucleic acid is complementary to a sequence that has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7%, preferably at least about 99.8%, preferably at least about 99.9% sequence identity, or that is preferably identical, to a sequence set forth in SEQ ID NOs: 17, 25, 33, 41, 49, and 57.
11. The mutant any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding an nucleoprotein, wherein said nucleoprotein has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7%, sequence identity, or that is preferably identical, to a nucleoprotein set forth in any one of SEQ ID NOs: 12, 20, 28, 36, 44, 52, and 60.
12. The mutant any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding an nucleoprotein, wherein said nucleic acid is complementary to a sequence that has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at

least about 99.7%, sequence identity, or that is preferably identical, to a nucleoprotein set forth in any one of SEQ ID NOs: 11, 19, 27, 35, 43, 51, and 59.

13. The mutant any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a Z-protein, wherein said Z-protein has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7%, sequence identity, or that is preferably identical, to a sequence set forth in any one of SEQ ID NOs: 14, 22, 30, 38, 46, 54, and 62.
14. The mutant any one of the preceding claims, wherein the mutant comprises a nucleic acid encoding a Z-protein, wherein said nucleic acid comprises a sequence that has at least about 95%, preferably at least about 96%, preferably at least about 97%, preferably at least about 98% preferably at least about 99%, preferably at least about 99.1%, preferably at least about 99.2%, preferably at least about 99.3%, preferably at least about 99.4%, preferably at least about 99.5%, preferably at least about 99.6%, preferably at least about 99.7%, sequence identity, or that is identical, to a sequence set forth in any one of SEQ ID NOs: 13, 21, 29, 37, 45, 53, and 61.
15. A method of producing an antitumoral arenavirus, in particular an arenavirus, which has improved antitumor properties over an original arenavirus, the process comprising the following steps:
 - a) plating primary tumor cells or cells of the cell line H1975, C643 or Tramp-C2 onto and/or into a nutrient medium,
 - b) inoculating the plated primary tumor cells or plated cells of the cell line H1975, C643 or Tramp-C2 with an original arenavirus,
 - c) incubating the inoculated primary tumor cells or inoculated cells of the cell line H1975, C643 or Tramp-C2 under conditions which are suitable for infecting at least a portion of the inoculated primary tumor cells or the inoculated cells of the cell line H1975, C643 or Tramp-C2 with the original arenavirus,

- d) extracting an arenavirus-containing cell culture supernatant from a cell culture containing the incubated primary tumor cells or the incubated cells of the cell line H1975, C643 or Tramp-C2,
- wherein the step sequence a) to d) is repeated a plurality of times, wherein the primary tumor cells or the cells of the cell line H1975, C643 or Tramp-C2 when performing the first repetition of step sequence a) to d), for performing step b) is inoculated with the arenavirus-containing cell culture supernatant or a part thereof extracted when performing step d) before the first repetition of the step sequence a) to d), and wherein the primary tumor cells or the cells of cell line H1975, C643 or Tramp-C2 when performing each further repetition of the step sequence a) to d), for performing step b) is inoculated with the arenavirus-containing cell culture supernatant or a part thereof extracted when performing step d) of a previous repetition of the step sequence a) to d).
16. The method of claim 15, characterized in that the primary tumor cells are passaged at most 1000 times, in particular at most 100 times, preferably at most 10 times, before performing step a).
17. The method of claim 15 or 16, characterized in that step c) is performed
- within a period from 1 hour to 1000 hours, in particular 3 hours to 300 hours, preferably 12 hours to 96 hours, and/or
 - at a temperature of 4 °C to 50 °C, in particular 20 °C to 42 °C, preferably 34 °C to 39 °C and/or
 - in a nutrient medium selected from the group consisting of RPMI-1640, DMEM and IMDM, wherein the nutrient medium in particular additionally comprises serum, such as fetal calf serum or human serum, and/or amino acids, such as glutamate and/or glutamine, and/or antibiotics, and/or
 - under a carbon dioxide atmosphere of 0 % to 20 %, in particular 2 % to 10 %, preferably 4 % to 6 %.
18. The method of any one of claims 15 to 17, characterized in that the sequence of steps is repeated 3 to 1000 times, in particular 10 to 100 times, preferably 20 to 50 times.
19. The method of any one of claims 15 to 18, characterized in that
- the primary tumor cells or the cells of the cell line H1975, C643 or Tramp-C2 and/or

- the original arenavirus and/or the arenavirus contained in the extracted cell culture supernatant or a part thereof are treated with at least one chemotherapeutic agent and/or subjected to a radiation treatment before performing step d) and/or
 - primary tumor cells or cells of the cell line H1975, C643 or Tramp-C2 which are resistant to at least one chemotherapeutic agent are used, and/or
 - primary tumor cells or the cells of the cell line H1975, C643 or Tramp-C2 and/or the original arenavirus and/or the arenavirus contained in the extracted cell culture supernatant or a part thereof before performing step d) are treated with at least one antiviral compound, in particular with alpha-interferon and/or gamma-interferon.
20. The method of any one of claims 15 to 19, characterized in that the primary tumor cells are selected from the group consisting of primary choroidal melanoma cells, primary anal carcinoma cells, primary angiosarcoma cells, primary astrocytoma cells, primary basal cell carcinoma cells, primary cervical carcinoma cells, primary chondrosarcoma cells, primary chorionic carcinoma cells, primary dermal squamous cell carcinoma cells, primary small intestine carcinoma cells, primary endometrial carcinoma cells, primary Ewing sarcoma cells, primary fibrosarcoma cells, primary gallbladder carcinoma cells, primary bile duct carcinoma cells, primary glioblastoma cells, primary bladder carcinoma cells, primary ureter carcinoma cells, primary urethral carcinoma cells, primary hepatocellular carcinoma cells, primary testicular tumor cells, primary hypopharyngeal carcinoma cells, primary pituitary carcinoma cells, primary Kaposi sarcoma cells, primary small-cell bronchial carcinoma cells, primary colon carcinoma cells, primary colorectal carcinoma cells, primary laryngeal carcinoma cells, primary leiomyosarcoma cells, primary liposarcoma cells, primary gastric carcinoma cells, primary malignant fibrous histiocytoma cells, primary breast carcinoma cells, primary medulloblastoma cells, primary melanoma cells, primary oral floor carcinoma cells, primary sinus carcinoma cells, primary nasopharyngeal carcinoma cells, primary adrenocortical carcinoma cells, primary parathyroid carcinoma cells, primary neurogenic sarcoma cells, primary non-small-cell bronchial carcinoma cells, primary renal carcinoma cells, primary oropharyngeal carcinoma cells, primary osteosarcoma cells, primary ovarian carcinoma cells, primary pancreatic tumor cells, primary penile carcinoma cells, primary pheochromocytoma cells, primary pleural mesothelioma cells, primary prostate carcinoma cells, primary rectal carcinoma cells, primary retinoblastoma cells, primary rhabdomyosarcoma cells, primary thyroid carcinoma cells, primary salivary gland carcinoma cells, primary esophageal carcinoma cells, primary

tonsil carcinoma cells, primary vaginal carcinoma cells, primary vulvar carcinoma cells, primary Wilms tumor cells, primary cells of neuroendocrine tumors, and primary tongue carcinoma cells.

21. The method of any one of claims 15 to 20, characterized in that as original arenavirus a wild-type arenavirus is used, in particular a wild-type lymphocytic choriomeningitis virus.
22. A lymphocytic choriomeningitis virus mutant comprising
 - a glycoprotein with at least one mutation, wherein the at least one mutation is an amino acid substitution of the isoleucine at position 181 of the glycoprotein by another amino acid, preferably methionine, and/or an amino acid substitution of the arginine at position 185 of the glycoprotein by another amino acid, preferably tryptophan, and/or
 - an L-protein having at least one mutation, wherein the at least one mutation is an amino acid substitution of the lysine at position 1513 of the L-protein by another amino acid, preferably glutamate, and/or an amino acid substitution of the phenylalanine at position 1995 of the L-protein by another amino acid, preferably serine, and/or an amino acid substitution of isoleucine at position 2094 of the L-protein by another amino acid, preferably valine, and/or an amino acid substitution of threonine at position 2141 of the L-protein by another amino acid, preferably alanine, and/or an amino acid substitution of arginine at position 2175 of the L-protein by another amino acid, preferably lysine.
23. A lymphocytic choriomeningitis virus mutant, in particular according to claim 22, characterized in that the lymphocytic choriomeningitis virus mutant comprises a protein or peptide, in particular a glycoprotein or L-protein, having or consisting of an amino acid sequence according to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 18, SEQ ID NO: 26, SEQ ID NO: 34, SEQ ID NO: 42, SEQ ID NO: 50, SEQ ID NO: 58, SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 40, SEQ ID NO: 48, SEQ ID NO: 56, or SEQ ID NO: 64.
24. The lymphocytic choriomeningitis virus mutant, in particular according to claim 22 or 23, characterized in that the lymphocytic choriomeningitis virus mutant comprises a nucleic acid which is coding for a protein or peptide comprising or consisting of an amino acid

sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 18, SEQ ID NO: 26, SEQ ID NO: 34, SEQ ID NO: 42, SEQ ID NO: 50, SEQ ID NO: 58, SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 40, SEQ ID NO: 48, SEQ ID NO: 56, or SEQ ID NO: 64, and/or the lymphocytic choriomeningitis virus mutant comprising a nucleic acid which has a nucleic acid sequence or consists of a nucleic acid sequence which is or is complementary to a nucleic acid sequence according to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 17, SEQ ID NO: 25, SEQ ID NO: 33, SEQ ID NO: 41, SEQ ID NO: 49, SEQ ID NO: 57, SEQ ID NO: 23, SEQ ID NO: 31, SEQ ID NO: 39, SEQ ID NO: 47, SEQ ID NO: 55, or SEQ ID NO: 63.

25. A lymphocytic choriomeningitis virus mutant of any one of claims 1 to 14 and 22 to 24 for use in medicine, in particular for use in the treatment and/or prevention of a tumor, preferably a tumor selected from the group consisting of anal carcinoma, bronchial carcinoma, lung carcinoma, endometrial carcinoma, gallbladder carcinoma, bladder carcinoma, hepatocellular carcinoma, testicular carcinoma, colon carcinoma, colorectal carcinoma, colorectal carcinoma, hepatocellular carcinoma, testicular carcinoma, colon carcinoma, tumor of the bladder, bladder carcinoma, tumor of the bladder, hepatocellular carcinoma, lung carcinoma, lung carcinoma, endometrial carcinoma, colorectal carcinoma, rectal carcinoma, laryngeal carcinoma, esophageal carcinoma, gastric carcinoma, breast carcinoma, renal carcinoma, ovarian carcinoma, pancreatic carcinoma, pharyngeal carcinoma, oropharyngeal carcinoma, prostate carcinoma, thyroid carcinoma, Cervical cancer, angiosarcoma, chondrosarcoma, Ewing sarcoma, fibrosarcoma, Kaposi sarcoma, liposarcoma, leiomyosarcoma, malignant fibrous histiocytoma, lymphoma, leukemia, neurogenic sarcoma, osteosarcoma and rhabdomyosarcoma.
26. A medicament or pharmaceutical composition comprising a lymphocytic choriomeningitis virus mutant of any one of claims 1 to 14 and 22 to 24.
27. The medicament according to claim 26, characterized in that the medicament further comprises a checkpoint blocker, such as PD-1, and/or an apoptosis modulator, in particular an apoptosis inhibitor, such as SMAC mimeticum.
28. An isolated protein or peptide, in particular glycoprotein, L-protein, nucleoprotein, or Z-protein, comprising or consisting of an amino acid sequence according to SEQ ID NO: 1,

SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, , SEQ ID NO: 10, SEQ ID NO: 18, SEQ ID NO: 26, SEQ ID NO: 34, SEQ ID NO: 42, SEQ ID NO: 50, SEQ ID NO: 58, SEQ ID NO: 24, SEQ ID NO: 32, SEQ ID NO: 40, SEQ ID NO: 48, SEQ ID NO: 56, or SEQ ID NO: 64, SEQ ID NO: 12, SEQ ID NO: 20, SEQ ID NO: 28, SEQ ID NO: 36, SEQ ID NO: 44, SEQ ID NO: 52, SEQ ID NO: 60, SEQ ID NO: 14, SEQ ID NO: 22, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 46, SEQ ID NO: 54, or SEQ ID NO: 62.

29. An isolated nucleic acid, in particular encoding for a glycoprotein, L-protein, nucleoprotein, or Z-protein, wherein the nucleic acid has a nucleic acid sequence or consists of a nucleic acid sequence which is or is complementary to a nucleic acid sequence according to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 17, SEQ ID NO: 25, SEQ ID NO: 33, SEQ ID NO: 41, SEQ ID NO: 49, SEQ ID NO: 57, SEQ ID NO: 23, SEQ ID NO: 31, SEQ ID NO: 39, SEQ ID NO: 47, SEQ ID NO: 55, SEQ ID NO: 63, SEQ ID NO: 11, SEQ ID NO: 19, SEQ ID NO: 27, SEQ ID NO: 35, SEQ ID NO: 43, SEQ ID NO: 51, SEQ ID NO: 59, SEQ ID NO: 13, SEQ ID NO: 21, SEQ ID NO: 29, SEQ ID NO: 37, SEQ ID NO: 45, SEQ ID NO: 53, or SEQ ID NO: 61 .