[0014] The disclosure provides a recombinant vesicular stomatitis virus encoding in its genome at least one CCL21 protein or a functional variant thereof, preferably human CCL21, selected from the group comprising: (i) plasmin processed CCL21 protein, (ii) c-terminally truncated CCL21 protein, (iii) a protein comprising SEQ ID NO:2 or having at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 99% identity to SEQ ID NO:2, (iv) a protein comprising SEQ ID NO:3 or having at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 99% identity to SEQ ID NO:3, (v) a protein comprising SEQ ID NO:4 or having at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 99% identity to SEQ ID NO:4, (vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence, (vii) a protein comprising SEQ ID NO:1 or having at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 99% identity to SEQ ID NO:1, or (viii) a protein comprising SEQ ID NO:5 or having at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 99% identity to SEQ ID NO:5, wherein the gene coding for the glycoprotein G of the recombinant vesicular stomatitis virus is replaced by the gene coding for the glycoprotein GP of lymphocyte choriomeningitis virus (LCMV), and/or the glycoprotein G is replaced by the glycoprotein GP of LCMV.

[0014a] In one aspect, the disclosure provides a recombinant vesicular stomatitis virus encoding in its genome at least one CCL21 protein, preferably human CCL21, selected from the group consisting of:

(i) a plasmin processed CCL21 protein comprising the amino acids 1-79, 1-81, 1-88, 1-91, or 1-104 as shown in SEQ ID NO:2,

(ii) a c-terminally truncated CCL21 protein comprising the sequence of SEQ ID NO:2 with the proviso that the protein lacks at least one amino acid residue indicated at positions 80-111 of SEQ ID NO:2,

(iii) a protein comprising SEQ ID NO:2,

(iv) a protein comprising SEQ ID NO:3,

(v) a protein comprising SEQ ID NO:4,

(vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence,

(vii) a protein comprising SEQ ID NO:1,

(viii) a protein comprising SEQ ID NO:5,

wherein the gene coding for the glycoprotein G of the recombinant vesicular stomatitis virus is replaced by the gene coding for the glycoprotein GP of Lymphocyte choriomeningitis virus (LCMV), and/or the glycoprotein G is replaced by the glycoprotein GP of LCMV.

[0015] The present disclosure relates to a recombinant vesicular stomatitis virus, encoding in its genome at least for a vesicular stomatitis virus nucleoprotein (N), large protein (L), phosphoprotein (P), matrix protein (M), glycoprotein (G) and at least one CCL21 protein or a functional variant thereof, preferably human CCL21.

[0015a] In another aspect, the present disclosure relates to a recombinant vesicular stomatitis virus, encoding in its genome at least for a vesicular stomatitis virus nucleoprotein (N), large protein (L), phosphoprotein (P), matrix protein (M), glycoprotein (G) and at least one CCL21 protein, preferably human CCL21, wherein

(i) the gene coding for the glycoprotein G is replaced by the gene coding for the glycoprotein GP of Lymphocyte choriomeningitis virus (LCMV), and/or

(ii) the glycoprotein G is replaced by the glycoprotein GP of LCMV.

[0016] In one example, the nucleoprotein (N) comprises an amino acid sequence as set forth in SEQ ID NO:7 or a functional variant at least 80%, 85%, 90%, 92%, 94%, 96%, 98% identical to SEQ ID NO:7.

(M), glycoprotein (G) and at least one CCL21 protein or a functional variant thereof, preferably human CCL21, wherein the CCL21 protein or functional variant thereof is selected from the group comprising: (i) plasmin processed CCL21 protein, (ii) c-terminally truncated CCL21 protein, (iii) a protein comprising SEQ ID NO:2 or having at least 80% identity to SEQ ID NO:2, (iv) a protein comprising SEQ ID NO:3 or having at least 80% identity to SEQ ID NO:3, (v) a protein comprising SEQ ID NO:4 or having at least 80% identity to SEQ ID NO:4, (vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence, (vii) a protein comprising SEQ ID NO:1 or having at least 80% identity to SEQ ID NO:1, or (viii) a protein comprising SEQ ID NO:5 or having at least 80%, 85%, 90%, 92%, 94%, 96%, 98% or 99% identity to SEQ ID NO:5, wherein, the gene coding for the glycoprotein G of the vesicular stomatitis virus is replaced by the gene coding for the glycoprotein GP of lymphocyte choriomeningitis virus (LCMV), and/or the glycoprotein G is replaced by the glycoprotein GP of LCMV, and wherein the nucleoprotein (N) comprises an amino acid as set forth in SEQ ID NO:7 or a functional variant at least 80%, 85%, 90%, 92%, 94%, 96%, 98% identical to SEQ ID NO:7, the phosphoprotein (P) comprises an amino acid as set forth in SEQ ID NO:8 or a functional variant at least 80%, 85%, 90%, 92%, 94%, 96%, 98% identical to SEQ ID NO:8, the large protein (L) comprises an amino acid as set forth in SEQ ID NO:9 or a functional variant at least 80%, 85%, 90%, 92%, 94%, 96%, 98% identical to SEQ ID NO:9, and the matrix protein (M) comprises an amino acid as set forth in SEQ ID NO:10 or a functional variant at least 80%, 85%, 90%, 92%, 94%, 96%, 98% identical to SEQ ID NO:10.

[0024a] In another aspect, the present disclosure provides a recombinant vesicular stomatitis virus encoding in its genome a vesicular stomatitis virus nucleoprotein (N), large protein (L), phosphoprotein (P), matrix protein (M), glycoprotein (G) and at least one CCL21 protein, preferably human CCL21, wherein the CCL21 protein is selected from the group comprising:

(i) a plasmin processed CCL21 protein comprising the amino acids 1-79, 1-81, 1-88, 1-91, or 1-104 as shown in SEQ ID NO:2,

(ii) a c-terminally truncated CCL21 protein comprising the sequence of SEQ ID NO:2 with the proviso that the protein lacks at least one amino acid residue indicated at positions 80-111 of SEQ ID NO:2,

(iii) a protein comprising SEQ ID NO:2,

(iv) a protein comprising SEQ ID NO:3,

(v) a protein comprising SEQ ID NO:4,

(vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence,

(vii) a protein comprising SEQ ID NO:1,

(viii) a protein comprising SEQ ID NO:5,

wherein, the gene coding for the glycoprotein G of the vesicular stomatitis virus is replaced by the gene coding for the glycoprotein GP of lymphocyte choriomeningitis virus (LCMV), and/or the glycoprotein G is replaced by the glycoprotein GP of LCMV, and wherein

- the nucleoprotein (N) comprises an amino acid as set forth in SEQ ID NO:7
- wherein the phosphoprotein (P) comprises an amino acid as set forth in SEQ ID NO:8
- wherein the large protein (L) comprises an amino acid as set forth in SEQ ID NO:9
- the matrix protein (M) comprises an amino acid as set forth in SEQ ID NO:10.

composition for the use in the treatment of cancer, preferably solid cancers. In another aspect, the present disclosure provides use of the recombinant vesicular stomatitis virus according to the disclosure or a pharmaceutical composition according to the disclosure in the manufacture of a medicament for the treatment of solid cancers. In a preferred example, the solid cancer is selected from the list comprising: reproductive tumor, an ovarian tumor, a pancreatic tumor, a testicular tumor, an endocrine tumor, a gastrointestinal tumor, a liver tumor, a kidney tumor, a colon tumor, a colorectal tumor, a bladder tumor, a prostate tumor, a skin tumor, melanoma, a respiratory tumor, a lung tumor, a breast tumor, a head & neck tumor, a head and neck squamous-cell carcinoma (HNSCC), and a bone tumor.

[0028] In one example, the recombinant rhabdovirus, the recombinant vesicular stomatitis virus, or the pharmaceutical composition is to be administered intratumorally or intravenously. In another related example, the recombinant rhabdovirus, the recombinant vesicular stomatitis virus or the pharmaceutical composition is to be administered at least once intratumorally and subsequently intravenously. In a further related example, the subsequent intravenous administration of the recombinant rhabdovirus, recombinant vesicular stomatitis virus or the pharmaceutical composition is given 1day, 2days, 3days, 4days, 5days, 6days, 7days, 8days, 9days, 10days, 11days, 12days, 13days, 14days, 15days, 16days, 17days, 18days, 19days, 20days, 21days, 22days, 23days, 24days, 25days, 26days, 27days, 28days, 29, days, 30days or 31days after the initial intratumoral administration.

[0029] The present disclosure provides for a composition comprising a recombinant rhabdovirus according to the disclosure or any of its examples, or a recombinant vesicular stomatitis virus according to the disclosure or any of its examples and further an inhibitor, wherein the inhibitor is a PD-1 pathway inhibitor or a SMAC mimetic.

(i) Infecting a host cell with a recombinant rhabdovirus, preferably a vesicular stomatitis virus,

(ii) Culturing the host cell under conditions allowing replication of the recombinant rhabdovirus,

(iii) Harvesting the recombinant rhabdovirus from the cell culture,

(iv) Optionally, enzyme treatment of the virus harvest, preferably with benzonase,

(v) Capturing the rhabdovirus harvest by loading on a cation exchange monolith membrane adsorber or resin followed by elution,

(vi) Polish rhabdovirus by subjecting the eluate of step (v) to size exclusion, multi modal size exclusion/ion exchange or tangential flow filtration,

(vii) Buffer change of polished rhabdovirus by ultrafiltration/diafiltration,

(viii) Sterile filtration of rhabdovirus.

[0037] In one example, the host cell is a HEK293 cell.

[0038] In one example, the host cell is cultured in suspension.

[0039] In one example, the recombinant rhabdovirus is formulated into a pharmaceutical composition. In a preferred example, the recombinant rhabdovirus according to the disclosure or any of its examples, or a recombinant vesicular stomatitis virus according to the disclosure or any of its examples is formulated into a pharmaceutical composition.

[0040] The present disclosure provides a recombinant rhabdovirus encodes in its RNA genome at least one CCL21 protein or a functional variant thereof, preferably human CCL21, wherein the RNA genome of the recombinant rhabdovirus comprises or consists of a coding sequence identical or at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 24.

[0040a] In a further aspect, the present disclosure provides a recombinant rhabdovirus encoding in its RNA genome at least one CCL21 protein, preferably human CCL21, wherein the RNA genome of the recombinant rhabdovirus comprises or consists of a coding sequence identical to SEQ ID NO: 24.

Claims

1. A recombinant vesicular stomatitis virus encoding in its genome at least one CCL21 protein, preferably human CCL21, selected from the group consisting of:
   (i) a plasmin processed CCL21 protein comprising the amino acids 1-79, 1-81, 1-88, 1-91, or 1-104 as shown in SEQ ID NO:2,
   (ii) a c-terminally truncated CCL21 protein comprising the sequence of SEQ ID NO:2 with the proviso that the protein lacks at least one amino acid residue indicated at positions 80-111 of SEQ ID NO:2,
   (iii) a protein comprising SEQ ID NO:2,
   (iv) a protein comprising SEQ ID NO:3,
   (v) a protein comprising SEQ ID NO:4,
   (vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence,
   (vii) a protein comprising SEQ ID NO:1,
   (viii) a protein comprising SEQ ID NO:5,
   wherein the gene coding for the glycoprotein G of the recombinant vesicular stomatitis virus is replaced by the gene coding for the glycoprotein GP of Lymphocyte choriomeningitis virus (LCMV), and/or the glycoprotein G is replaced by the glycoprotein GP of LCMV.

2. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a plasmin processed CCL21 protein comprising the amino acids 1-79, 1-81, 1-88, 1-91, or 1-104 as shown in SEQ ID NO:2.

3. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a c-terminally truncated CCL21 protein comprising the sequence of SEQ ID NO:2 with the proviso that the protein lacks at least one amino acid residue indicated at positions 80-111 of SEQ ID NO:2.

4. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a protein comprising SEQ ID NO:2.

5. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a protein comprising SEQ ID NO:3.

6. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a protein comprising SEQ ID NO:4.

7. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a protein comprising a protein according to any of (i) – (v) further comprising a signal peptide sequence.

8. The recombinant vesicular stomatitis virus according to claim 1, wherein the genome encodes for a protein comprising SEQ ID NO:1.

9. The recombinant vesicular stomatitis virus according to claim 1 wherein the genome encodes for a protein comprising SEQ ID NO:5.

10. A recombinant vesicular stomatitis virus, encoding in its genome at least for a vesicular stomatitis virus nucleoprotein (N), large protein (L), phosphoprotein (P), matrix protein (M), glycoprotein (G) and at least one CCL21 protein, preferably human CCL21, wherein
(i) the gene coding for the glycoprotein G is replaced by the gene coding for the glycoprotein GP of Lymphocyte choriomeningitis virus (LCMV), and/or
(ii) the glycoprotein G is replaced by the glycoprotein GP of LCMV.

11. The recombinant vesicular stomatitis virus according to claim 10, wherein the nucleoprotein (N) comprises an amino acid sequence as set forth in SEQ ID NO:7.

12. The recombinant vesicular stomatitis virus according to any of claims 10 or 11, wherein the phosphoprotein (P) comprises an amino acid sequence as set forth in SEQ ID NO:8.

13. The recombinant vesicular stomatitis virus according to any of claims 10 to 12, wherein the large protein (L) comprises an amino acid sequence as set forth in SEQ ID NO:9.

14. The recombinant vesicular stomatitis virus according to any of claims 10 to 13, wherein the matrix protein (M) comprises an amino acid sequence as set forth in SEQ ID NO:10.

15. The recombinant vesicular stomatitis virus according to any of claims 10 to 14, wherein:
- the nucleoprotein (N) comprises an amino acid sequence as set forth in SEQ ID NO:7
- wherein the phosphoprotein (P) comprises an amino acid sequence as set forth in SEQ ID NO:8
- wherein the large protein (L) comprises an amino acid sequence as set forth in SEQ ID NO:9, and
- the matrix protein (M) comprises an amino acid sequence as set forth in SEQ ID NO:10.

16. The recombinant vesicular stomatitis virus according to any of claims 10 to 15, which is replication-competent.

17. The recombinant vesicular stomatitis virus according to any of claims 10 to 16, wherein the CCL21 protein is selected from the group consisting of:
    (i) a plasmin processed CCL21 protein comprising the amino acids 1-79, 1-81, 1-88, 1-91, or 1-104 as shown in SEQ ID NO:2,
    (ii) a c-terminally truncated CCL21 protein comprising the sequence of SEQ ID NO:2 with the proviso that the protein lacks at least one amino acid residue indicated at positions 80-111 of SEQ ID NO:2,
    (iii) a protein comprising SEQ ID NO:2,
    (iv) a protein comprising SEQ ID NO:3,
    (v) a protein comprising SEQ ID NO:4,
    (vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence,
    (vii) a protein comprising SEQ ID NO:1,
    (viii) a protein comprising SEQ ID NO:5.

18. A recombinant vesicular stomatitis virus encoding in its genome a vesicular stomatitis virus nucleoprotein (N), large protein (L), phosphoprotein (P), matrix protein (M), glycoprotein (G) and at least one CCL21 protein, preferably human CCL21, wherein the CCL21 protein is selected from the group consisting of:
    (i) a plasmin processed CCL21 protein comprising the amino acids 1-79, 1-81, 1-88, 1-91, or 1-104 as shown in SEQ ID NO:2,
    (ii) a c-terminally truncated CCL21 protein comprising the sequence of SEQ ID NO:2 with the proviso that the protein lacks at least one amino acid residue indicated at positions 80-111 of SEQ ID NO:2,
    (iii) a protein comprising SEQ ID NO:2,
    (iv) a protein comprising SEQ ID NO:3,
    (v) a protein comprising SEQ ID NO:4,
    (vi) a protein according to any of (i) – (v) further comprising a signal peptide sequence,
    (vii) a protein comprising SEQ ID NO:1,
    (viii) a protein comprising SEQ ID NO:5,
    wherein, the gene coding for the glycoprotein G of the vesicular stomatitis virus is replaced by the gene coding for the glycoprotein GP of lymphocyte choriomeningitis virus (LCMV), and/or the glycoprotein G is replaced by the glycoprotein GP of LCMV, and wherein
- the nucleoprotein (N) comprises an amino acid as set forth in SEQ ID NO:7
- wherein the phosphoprotein (P) comprises an amino acid as set forth in SEQ ID NO:8
- wherein the large protein (L) comprises an amino acid as set forth in SEQ ID NO:9
- the matrix protein (M) comprises an amino acid as set forth in SEQ ID NO:10.

19. A pharmaceutical composition, characterized in that the composition comprises a recombinant vesicular stomatitis virus according to any of claims 1 to 18.

20. A recombinant vesicular stomatitis virus according to any of claims 1 to 18 or a pharmaceutical composition according to claim 19 for use as a medicament.

21. Use of the recombinant vesicular stomatitis virus according to any of claims 1 to 18 or a pharmaceutical composition according to claim 19 in the manufacture of a medicament for the treatment of solid cancers.

22. The use according to claim 21, wherein the solid cancer is selected from the list comprising: glioblastoma, non-small cell lung cancer, reproductive tumor, an ovarian tumor, a testicular tumor, an endocrine tumor, a gastrointestinal tumor, a pancreatic tumor, a liver tumor, a kidney tumor, a colon tumor, a colorectal tumor, a bladder tumor, a prostate tumor, a skin tumor, melanoma, a respiratory tumor, a lung tumor, a breast tumor, a head & neck tumor, a head and neck squamous-cell carcinoma (HNSCC) and a bone tumor.

23. The use according to claim 21 or 22, wherein the recombinant vesicular stomatitis virus, or the pharmaceutical composition is to be administered intratumorally or intravenously.

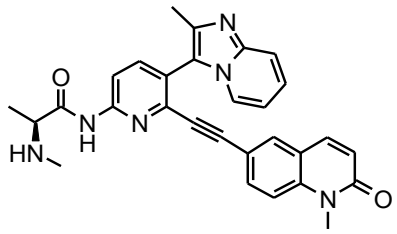
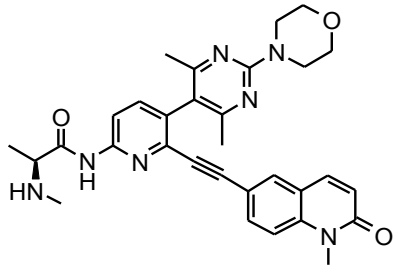
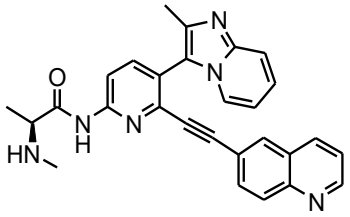
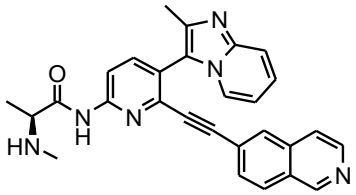
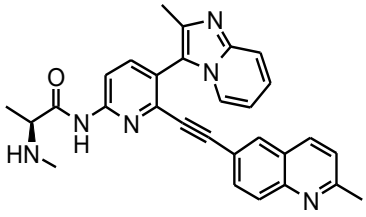
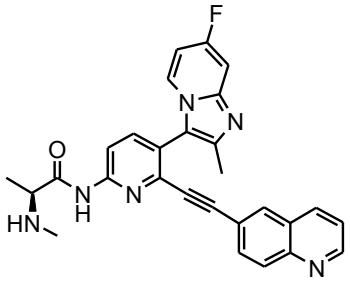
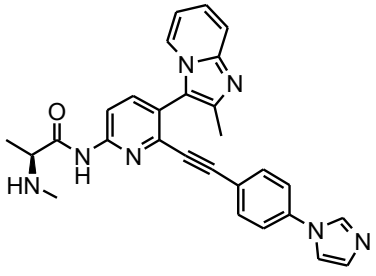
24. The use according to any of claims 21 to 23, wherein the recombinant vesicular stomatitis virus or the pharmaceutical composition is to be administered at least once intratumorally and subsequently intravenously.

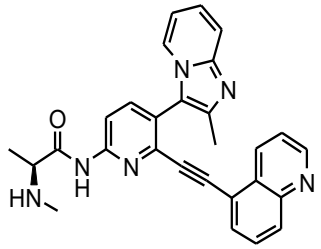
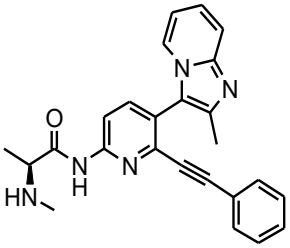
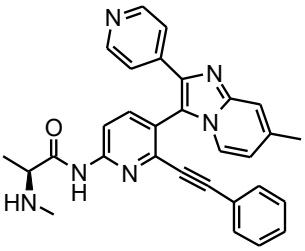
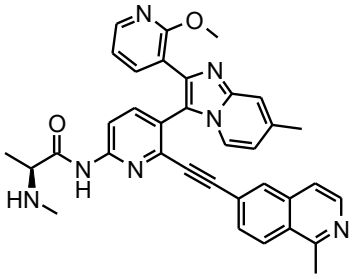
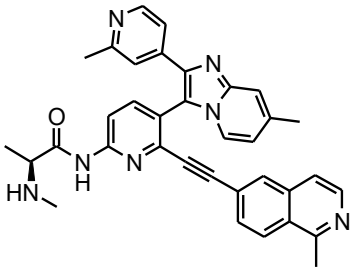
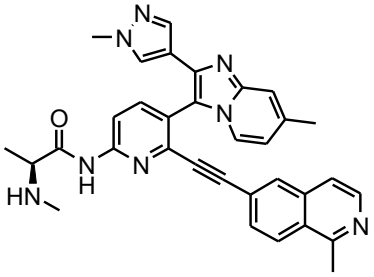
25. The use according to claim 24, wherein the subsequent intravenous administration is to be given 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 14 days, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days or 31 days after the initial intratumoral administration.

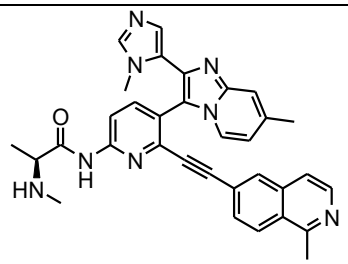
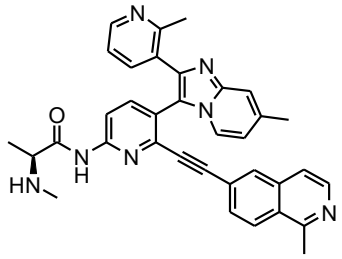
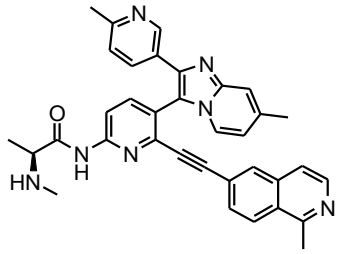
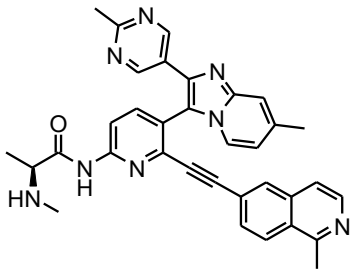
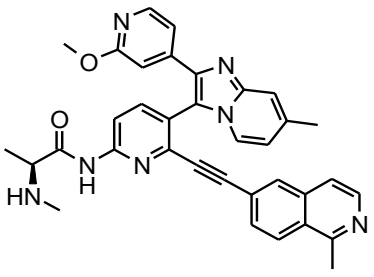
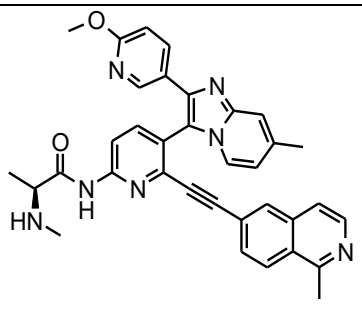
26. A composition comprising a recombinant vesicular stomatitis virus according to any of the preceding claims and further a PD-1 pathway inhibitor or a SMAC mimetic.

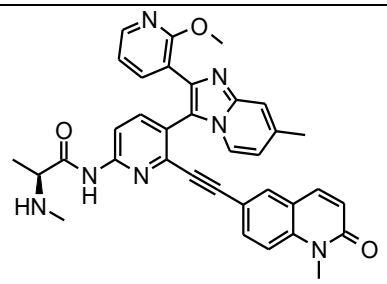
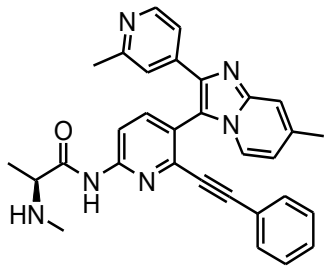
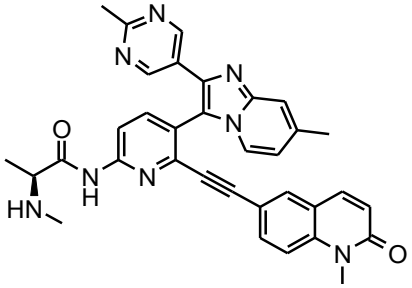
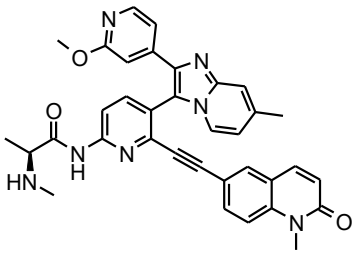
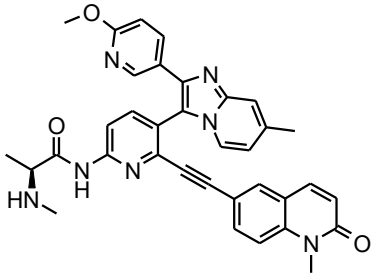
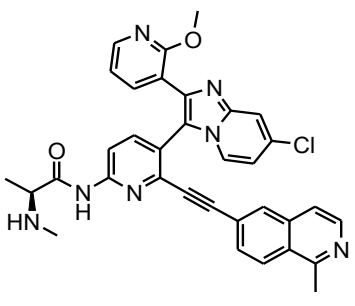
27. The composition according to claim 26, wherein the PD-1 pathway inhibitor is an antagonistic antibody, which is directed against PD-1 or PD-L1.

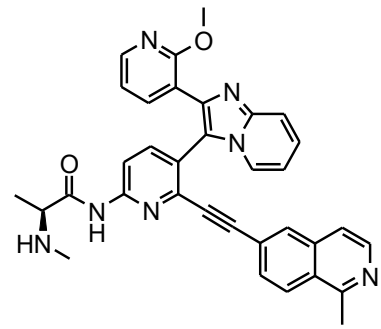
28. The composition according to claim 26, wherein the SMAC mimetic is selected from the group consisting of any one of compounds 1 to 26:

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or a pharmaceutically acceptable salt of one of these compounds.

29. The composition according to claim 26, wherein the PD-1 pathway inhibitor is an antagonist selected from the group consisting of pembrolizumab, nivolumab, pidilizumab, atezolizumab, avelumab, durvalumab, PDR-001, PD1-1, PD1-2, PD1-3, PD1-4 and PD1-5.

30. A kit of parts comprising:
    a) a recombinant vesicular stomatitis virus or a pharmaceutical composition as defined in any of the preceding claims, and
    b) a PD-1 pathway inhibitor as defined in claim 29 or a SMAC mimetic as defined in claim 28.

31. A recombinant vesicular stomatitis virus, or a pharmaceutical composition for use according to claim 20, or the use according to claim 21 or 22, in combination with a PD-1 pathway inhibitor or a SMAC mimetic.

32. The recombinant vesicular stomatitis virus, or the pharmaceutical composition for use, or the use according to claim 31, wherein the recombinant vesicular stomatitis virus, or the pharmaceutical composition is to be administered concomittantly, sequentially or alternately with the PD-1 pathway inhibitor or the SMAC mimetic.

33. The recombinant vesicular stomatitis virus, or the pharmaceutical composition for use, or the use according to claims 31 to 32, wherein the SMAC mimetic is selected from the group consisting of any one of compounds 1 to 26 according to claim 28 or a pharmaceutically acceptable salt of one of these compounds.

34. The recombinant vesicular stomatitis virus, or the pharmaceutical composition for use, or the use according to claims 31 to 32, wherein the PD-1 pathway inhibitor is selected from the group consisting of pembrolizumab, nivolumab, pidilizumab, atezolizumab, avelumab, durvalumab, PDR-001, PD1-1, PD1-2, PD1-3, PD1-4 and PD1-5.

35. The recombinant vesicular stomatitis virus, or the pharmaceutical composition for use, or the use according to any of claims 31 to 33, wherein the recombinant vesicular stomatitis virus, or the pharmaceutical composition is to be administered via a different administration route than the PD-1 pathway inhibitor or the SMAC mimetic.

36. The recombinant vesicular stomatitis virus, or the pharmaceutical composition for use, or the use according to any of claims 31 to 33, wherein the recombinant vesicular stomatitis virus, or the pharmaceutical composition are to be administered at least once intratumorally and the PD-1 pathway inhibitor or the SMAC mimetic is to be administered intravenously.

37. A virus producing cell, characterized in that the cell produces a recombinant vesicular stomatitis virus according to any of the preceding claims.

38. The virus producing cell of claim 37, characterized in that the cell is a Vero cell, a HEK cell, a HEK293 cell, a Chinese hamster ovary cell (CHO), or a baby hamster kidney (BHK) cell.

39. A recombinant rhabdovirus encoding in its RNA genome at least one CCL21 protein, preferably human CCL21, wherein the RNA genome of the recombinant rhabdovirus comprises or consists of a coding sequence identical to SEQ ID NO: 24.