

CLAIMS

1. A method for treating cancer in a subject in need of such treatment comprising administering to the subject an effective amount of (a) an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene and (b) one or more immune
5 checkpoint inhibitors.
2. The method of claim 1, wherein said one or more immune checkpoint inhibitors selectively binds to PD-L1 or PD-1.
3. The method of claim 1, wherein the oncolytic adenoviral vector is administered intratumorally, intravenously, intra-arterially, or intraperitoneally.
- 10 4. The method of claim 3, wherein the oncolytic adenoviral vector is administered intratumorally.
5. The method of claim 1, wherein the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-L1, preferably selected from the group consisting of: BMS-936559, LY3300054, atezolizumab, durvalumab and avelumab.
- 15 6. The method of claim 1, wherein the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-1, preferably selected from the group consisting of: pembrolizumab (MK-3475) and nivolumab (BMS-936558).
7. The method of claim 1, wherein the virus is administered in an amount from about 10^6 - 10^{14} VP, 10^6 - 10^{12} VP, 10^8 - 10^{14} VP, 10^8 - 10^{12} VP or 10^{10} - 10^{12} VP.
- 20 8. The method of claim 1, wherein the checkpoint inhibitor is administered in an amount from about 2 mg/kg to 25 mg/kg.
9. The method of claim 1, wherein the subject has a cancer selected from hepatocellular carcinoma, colorectal cancer, renal cell carcinoma, bladder cancer, lung cancer (including non-small cell lung cancer), stomach cancer, esophageal
25 cancer, sarcoma, mesothelioma, melanoma, pancreatic cancer, head and neck cancer, ovarian cancer, breast cancer, cervical cancer and liver cancer.
10. The method of claim 9, wherein the subject has renal cell carcinoma or head and neck cancer.
11. The method of claim 1, wherein the subject has failed at least one previous
30 chemotherapy or immunotherapy treatment such as a treatment with a checkpoint inhibitor.

12. The method of claim 11, wherein the subject has a cancer capable of mediating dysfunctionality of CD8+ T-cells.

13. The method of claim 11, wherein the subject has a cancer wherein at least one of genes related to immune activity selected from a group consisting of GZMG, GMZF,
5 KLRC2 and CD46, and particularly to T-cell activity regulators selected from a group consisting of TNFSF18/GITRL and EAR2 is downregulated.

14. The method of claim 11, wherein the subject has an anti-PD-1 refractory cancer, said cancer preferably being melanoma.

15. The method of claim 1, comprising administering to the subject an additional
10 therapy selected from radiotherapy, chemotherapy, antiangiogenic agents or targeted therapies, such as alkylating agents, nucleoside analogs, cytoskeleton modifiers, cytostatic agents, monoclonal antibodies, kinase inhibitors.

16. The method of claim 1, wherein the subject is a human.

17. The method of claim 1, wherein a first dose of the oncolytic adenoviral vector and
15 a first dose of the immune checkpoint inhibitor are simultaneously administered to the subject.

18. The method of claim 1, wherein a first dose of the oncolytic adenoviral vector and a first dose of the immune checkpoint inhibitor are administered sequentially in 24 hours.

20. 19. The method of claim 1, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene comprises

- a 5/3 chimeric fiber knob,
- E2F1 promoter for tumor specific expression of E1A,
- a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1,
- 25 - a nucleic acid sequence deletion of viral *gp19k* and *6.7k* reading frames, and
- a nucleic acid sequence encoding at least TNFalpha and/or IL-2 as transgene(s) in the place of the deleted *gp19k/6.7K* in the E3 region resulting in replication-associated control of transgene expression under the viral E3 promoter.

30. 20. The method of claim 19, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene further comprises a deletion of E1B19k.

21. Pharmaceutical composition comprising (a) an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene and (b) one or more immune checkpoint inhibitors.

22. The composition according to claim 21, wherein said one or more immune
5 checkpoint inhibitors selectively binds to PD-L1 or PD-1.

23. The pharmaceutical composition of claim 21, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene comprises

- a 5/3 chimeric fiber knob,
- E2F1 promoter for tumor specific expression of E1A,
- 10 - a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1,
- a nucleic acid sequence deletion of viral *gp19k* and *6.7k* reading frames, and
- a nucleic acid sequence encoding at least TNFalpha and/or IL-2 as transgene(s) in the place of the deleted *gp19k/6.7K* in the E3 region resulting in replication-associated control of transgene expression under the viral E3 promoter.

15 24. A kit which comprises a first container, a second container and a package insert, wherein the first container comprises at least one dose of a pharmaceutical composition containing an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene, the second container comprises at least one dose of a
20 pharmaceutical composition comprising a checkpoint inhibitor, and the package insert comprises instructions for treating an individual having cancer using the pharmaceutical composition(s).

25. The kit according to claim 24, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene comprises

- a 5/3 chimeric fiber knob,
- 25 - E2F1 promoter for tumor specific expression of E1A,
- a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1,
- a nucleic acid sequence deletion of viral *gp19k* and *6.7k* reading frames, and
- a nucleic acid sequence encoding at least TNFalpha and/or IL-2 as transgene(s) in the place of the deleted *gp19k/6.7K* in the E3 region resulting in replication-associated
30 control of transgene expression under the viral E3 promoter.

26. The composition of claim 21 or the kit of claim 24, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene further comprises a deletion of E1B19k.

27. An oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene
5 together with an immune checkpoint inhibitor for use in the treatment of cancer or tumor.

28. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to claim 27, wherein said immune checkpoint inhibitor selectively binds to PD-L1 or PD-1.

10 29. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to claim 27 or 28, wherein the oncolytic adenoviral vector is administered intratumorally, intravenously, intra-arterially, or intraperitoneally.

30. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene
15 together with an immune checkpoint inhibitor for use according to any one of claims 27-29, wherein the oncolytic adenoviral vector is administered intratumorally.

31. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-30, wherein the immune checkpoint inhibitor is a monoclonal antibody that
20 selectively binds to PD-L1, preferably selected from the group consisting of: BMS-936559, LY3300054, atezolizumab, durvalumab and avelumab.

32. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-30, wherein the immune checkpoint inhibitor is a monoclonal antibody that
25 selectively binds to PD-1, preferably selected from the group consisting of: pembrolizumab (MK-3475) and nivolumab (BMS-936558).

33. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-32, wherein the virus is administered in an amount from about 10^6 - 10^{14} VP, 10^6 -
30 10^{12} VP, 10^8 - 10^{14} VP, 10^8 - 10^{12} VP or 10^{10} - 10^{12} VP.

34. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-33, wherein the checkpoint inhibitor is administered in an amount from about 2 mg/kg to 25 mg/kg.

35. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-34, wherein the subject has a cancer selected from hepatocellular carcinoma, colorectal cancer, renal cell carcinoma, bladder cancer, lung cancer (including non-
5 small cell lung cancer), stomach cancer, esophageal cancer, sarcoma, mesothelioma, melanoma, pancreatic cancer, head and neck cancer, ovarian cancer, breast cancer, cervical cancer and liver cancer.
36. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to claim 35, wherein
10 the subject has renal cell carcinoma or head and neck cancer.
37. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-36, wherein the subject has failed at least one previous chemotherapy or immunotherapy treatment such as a treatment with a checkpoint inhibitor.
- 15 38. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to claim 37, wherein the subject has a cancer capable of mediating dysfunctionality of CD8+ T-cells.
39. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to claim 37 or 38,
20 wherein the subject has a cancer wherein at least one gene related to immune activity selected from a group consisting of GZMG, GMZF, KLRC2 and CD46 and particularly to T-cell activity regulators selected from a group consisting of TNFSF18/GITRL and EAR2 is downregulated.
40. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims
25 37-39, wherein the subject has an anti-PD-1 refractory cancer, said cancer preferably being melanoma.
41. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims
30 27-40, comprising administering to the subject an additional therapy selected from radiotherapy, chemotherapy, antiangiogenic agents or targeted therapies, such as alkylating agents, nucleoside analogs, cytoskeleton modifiers, cytostatic agents, monoclonal antibodies, kinase inhibitors.

42. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-41, wherein the subject is a human.

5 43. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-42, wherein a first dose of the oncolytic adenoviral vector and a first dose of the immune checkpoint inhibitor are simultaneously administered to the subject.

10 44. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-43, wherein a first dose of the oncolytic adenoviral vector and a first dose of the immune checkpoint inhibitor are administered sequentially in 24 hours.

15 45. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to any one of claims 27-44, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene comprises

- a 5/3 chimeric fiber knob,
- E2F1 promoter for tumor specific expression of E1A,
- a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1,
- a nucleic acid sequence deletion of viral *gp19k* and *6.7k* reading frames, and
- 20 - a nucleic acid sequence encoding at least TNFalpha and/or IL-2 as transgene(s) in the place of the deleted *gp19k/6.7K* in the E3 region resulting in replication-associated control of transgene expression under the viral E3 promoter.

25 46. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for use according to claim 45, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene further comprises a deletion of E1B19k.