

especially in patients with a significant metastasis burden. Further characterization of pathways related to the activity of oncolytic viruses could reveal potential targets for improving the efficacy of virotherapy.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure is based on a discovery that co-administration of an oncolytic adenoviral virus coding for cytokines TNFalpha and/or IL-2 and the immune checkpoint inhibitor anti-PD-L1 or PD-1 to clinically relevant cancer models results in a significant shift towards immune activity against the treated cancer concomitant with a high survival benefit relative to administration of either agent alone.

In one aspect, the present disclosure provides a 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.

In another aspect, the present disclosure provides 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 pharmaceutical composition comprising a checkpoint inhibitor, and the package insert comprises instructions for treating an individual having cancer using the pharmaceutical composition(s).

In yet another aspect, the present disclosure provides a use of an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene and an immune checkpoint inhibitor in the manufacture of a medicament for combination therapy to treat cancer or tumor.

Accordingly, in several ~~embodiment~~examples, the present application provides a combination therapy for use in the treatment and/or prevention of cancer and/or the establishment of metastases in a mammal and/or for use in initiating, enhancing or prolonging an anti-tumor response in a mammal comprising co-administering to the mammal (a) an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene and (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1.

In certain ~~aspect~~examples, co-administration of an oncolytic virus and immune checkpoint inhibitor to a subject with cancer provides an enhanced and even synergistic anti-tumor immunity compared to either treatment alone.

In other related [aspectexamples](#), a method for enhancing, potentiating or prolonging the effects of the checkpoint inhibitor or enabling the toxicity or dose or number of treatments of the checkpoint inhibitor to be reduced, comprising administering to a mammal in need thereof (a) an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene and (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, is provided.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Urological cancer patient-derived samples respond to oncolytic virotherapy despite T-cell infiltration and suppression differences. Patient sample 1 is urothelial carcinoma, patient samples 2 and 3 are Clear cell renal cell carcinoma. (A) Hematoxylin and Eosin staining performed on the paraffin-embedded samples. (B) CD8 immunohistochemistry. (C) PD-L1 immunohistochemistry. (D) MTS viability assay after *ex vivo* treatment of the histocultures. Statistical significances are shown for day 7 calculated by unpaired t-test with Welch's correction (** $p < 0.01$; *** $p < 0.001$). Mean and standard error of the mean (SEM) are shown.

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 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.~~
- ~~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.~~
- ~~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.~~
- ~~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 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 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, KLRG2 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 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 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.~~

~~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,~~

~~-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.~~

~~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.~~

211. 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.

222. The composition according to claim **211**, wherein said one or more immune checkpoint inhibitors selectively binds to PD-L1 or PD-1.

233. The pharmaceutical composition of claim **211**, 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
- 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.

244. 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 pharmaceutical composition comprising a checkpoint inhibitor, and the package insert comprises instructions for treating an individual having cancer using the pharmaceutical composition(s).

255. The kit according to claim **244**, 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
- 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.

266. The composition of claim 21-1 or the kit of claim 244, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene further comprises a deletion of E1B19k.

277. Use of a ~~An oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene~~ together with ~~and~~ an immune checkpoint inhibitor in the manufacture of a medicament for combination therapy to use in the treatment of ~~treat~~ cancer or tumor.

288. The ~~oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for~~ use according to claim 277, wherein said immune checkpoint inhibitor selectively binds to PD-L1 or PD-1.

299. The ~~oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for~~ use according to claim 277 or 288, wherein the oncolytic adenoviral vector is to be administered intratumorally, intravenously, intra-arterially, or intraperitoneally.

3010. 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 277-299, wherein the oncolytic adenoviral vector is to be administered intratumorally.

3111. 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 277-3010, 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.

3212. 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 277-3010, 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).

3313. 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 277-3212, wherein the virus is to be 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.

3414. 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 277-3313, wherein the checkpoint inhibitor is to be administered in an amount from about 2 mg/kg to 25 mg/kg.

~~3515~~. 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 ~~277-3414~~, 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 cancer, sarcoma, mesothelioma, melanoma, pancreatic cancer, head and neck cancer, ovarian cancer, breast cancer, cervical cancer and liver cancer.

~~3616~~. The ~~oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for~~ use according to claim ~~3515~~, wherein the subject has renal cell carcinoma or head and neck cancer.

~~3717~~. 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 ~~277-3616~~, wherein the subject has failed at least one previous chemotherapy or immunotherapy treatment such as a treatment with a checkpoint inhibitor.

~~3818~~. The ~~oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for~~ use according to claim ~~3717~~, wherein the subject has a cancer capable of mediating dysfunctionality of CD8+ T-cells.

~~3919~~. The ~~oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for~~ use according to claim ~~3717~~ or ~~3818~~, 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.

~~4020~~. 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 ~~3717-3919~~, wherein the subject has an anti-PD-1 refractory cancer, said cancer preferably being melanoma.

~~4121~~. 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 ~~277-4020~~, comprising ~~administering to that~~ the subject is to be administered 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.

~~4222. 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 ~~277-4121~~, wherein the subject is a human.

~~4323. 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 ~~277-4222~~, wherein a first dose of the oncolytic adenoviral vector and a first dose of the immune checkpoint inhibitor are to be simultaneously administered to the subject.

~~4424. 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 ~~277-4323~~, wherein a first dose of the oncolytic adenoviral vector and a first dose of the immune checkpoint inhibitor are to be administered sequentially in 24 hours.

~~4525. 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 ~~277-4424~~, 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
- 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.

~~4626. The oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene together with an immune checkpoint inhibitor for~~ use according to claim ~~4525~~, wherein said oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene further comprises a deletion of E1B19k.