Anti-viral immunity has been considered restrictive for virotherapy approaches including oncolytic adenoviruses. One example of anti-viral immunity is anti-viral T-cells. However, the present disclosure surprisingly reveals that when an oncolytic adenovirus is used for production of a BiTE at the tumor, anti-viral T-cells can be retargeted against the tumor. This effect amplifies during treatment, as replication of the oncolytic virus results in further anti-viral T-cells, which then are also targeted towards the tumor through the BiTE produced by the virus (Figure 7).

In one example, the present disclosure relates to enhancement of T-cell therapy with an oncolytic adenovirus coding for a BiTE. Oncolytic adenovirus is the optimal platform for using a BiTe for enhancing T-cell therapy, because of the unexpected synergy between the anti-immunosuppressive effects of oncolysis and BiTE expression at the tumor.

The present specification describes construction of recombinant adenoviral vectors, methods related to the adenoviral vectors, and their different uses. Furthermore, the adenoviral vectors of the present disclosure coding for T-cell engagers may be combined with adoptive cell therapeutics for cancer treatment.

Advantages of the present disclosure are achieved by a method of treating malignancy, comprising administering an effective amount of an adenoviral vector of the present disclosure (e.g. alone or together with TILs) to a patient afflicted with cancer to cause regression or stabilization of the cancer.

The present disclosure relates to an oncolytic adenoviral vector comprising

a deletion of a nucleic acid sequence in the E3 region, and

a nucleic acid sequence encoding a bispecific monoclonal antibody in the place of the deleted nucleic acid sequence in E3 region.

In one aspect, there is provided an oncolytic adenoviral vector comprising

a deletion of a nucleic acid sequence in the E3 region, wherein the nucleic acid sequence deletion is a deletion of viral *gp19k* and *6.7k* reading frames, and

a nucleic acid sequence encoding a bipartite molecule in the place of the deleted nucleic acid sequence in E3 region, wherein the bipartite molecule comprises a single chain variable fragment (scFv) specific for a cell surface molecule on a T lymphocyte and a scFv specific for a tumor antigen, ~~wherein the cell surface molecule is selected from CD3, CD8 and CD4,~~ wherein the backbone of

the vector is adenovirus serotype 5 (Ad5) nucleic acid backbone and the vector further comprises an Ad3 fiber knob, E2F1 promoter for tumor specific expression of E1A, and a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1, wherein the tumor antigen is EpCAM1 and the cell surface molecule is CD3; or the tumor antigen is MUC1 and the cell surface molecule is CD3.

In another aspect, there is provided a pharmaceutical composition comprising an oncolytic adenoviral vector of the disclosure.

Also, the present disclosure relates to a pharmaceutical composition comprising an oncolytic adenoviral vector comprising a deletion of a nucleic acid sequence in the E3 region, and a nucleic acid sequence encoding a bispecific monoclonal antibody in the place of the deleted nucleic acid sequence in E3 region.

Furthermore, the present disclosure relates to a combination of an oncolytic adenoviral vector comprising a deletion of a nucleic acid sequence in the E3 region and a nucleic acid sequence encoding a bispecific monoclonal antibody in the place of the deleted nucleic acid sequence in E3 region, and an adoptive cell therapeutic composition.

In one aspect, there is provided an oncolytic adenoviral vector of the disclosure for use in treatment of cancer.

Furthermore, the present disclosure relates to a combination of an oncolytic adenoviral vector of the disclosure and an adoptive cell therapeutic composition for use in treatment of cancer.

Furthermore, the present disclosure relates to an oncolytic adenoviral vector of the disclosure together with an adoptive cell therapeutic composition for use in treatment of cancer.

Furthermore, the present disclosure relates to an oncolytic adenoviral vector of the disclosure for use in treatment of cancer together with an adoptive cell therapeutic composition.

Still, the present disclosure relates to a method of treating cancer in a subject, wherein the method comprises administration of an oncolytic adenoviral vector of the disclosure to a subject.

Still, the present disclosure relates to an oncolytic adenoviral vector comprising a deletion in the E3 region and a nucleic acid sequence encoding a bispecific monoclonal antibody in the place of the deleted region of E3, for use in increasing the efficacy of adoptive cell therapy in a subject.

Still, the present disclosure relates to a method of increasing the efficacy of adoptive cell therapy in a subject by administering an oncolytic adenoviral vector comprising a deletion in the E3 region and a nucleic acid sequence encoding a bispecific monoclonal antibody in the place of the deleted region of E3, to a subject in need thereof, wherein the subject has been administered or is to be administered with adoptive cell therapy.

In one aspect, there is provided use of an oncolytic adenoviral vector of the present disclosure in the manufacture of a medicament for treating cancer in a subject.

~~In one aspect, there is provided an oncolytic adenoviral vector of the present disclosure for use in increasing the efficacy of adoptive cell therapy in a subject.~~ Also, the present disclosure relates to a use of an oncolytic adenoviral

vector of the disclosure in the manufacture of a medicament for increasing the efficacy of adoptive cell therapy in a subject. ~~In one aspect, there is provided use of an oncolytic adenoviral vector of the present disclosure in the manufacture of a medicament for increasing the efficacy of adoptive cell therapy in a subject in need thereof, wherein the subject has been administered or is to be administered with adoptive cell therapy.~~

The advantages of the arrangements of the present disclosure include but are not limited to enhanced therapeutic effect and reduced side effects. Severe adverse events, even deaths are prevented, because enhancements in efficacy, and the anti-suppressive effects of our approach, may reduce the need for preconditioning chemotherapy and/or radiation used in the prior art methods to "make room" for transferred cells and reduce tumor immunosuppression.

BRIEF DESCRIPTION OF THE DRAWINGS

In the following the invention will be described in greater detail by means of specific embodiments with reference to the attached drawings, in which

Figure 1 shows the mechanism of action of T-cell therapy with oncolytic adenovirus coding for bi-specific T-cell engager BiTE.

Figure 2 shows that treatment with adenovirus induces danger signals in tumors. Treatment with 5/3 chimeric adenovirus (Ad5 based vector having fiber knob from Ad3) induces danger signals in B16.OVA tumors as demonstrated by interferon gamma expression. Binding of adenoviral pathogen-associated molecular patterns (PAMP) to toll-like receptors (TLR) on host cells can induce secretion of interferon-γ, which leads to rapid activation of innate and adaptive immune responses. Consequently, adenovirus can be used to generate an immunogenic tumor phenotype that is effectively recognized by the immune system.

Figure 3 shows that adenovirus has anti-immunosuppressive effects in the tumor microenvironment. 5/3 chimeric adenovirus has anti-immunosuppressive effects on B16.OVA tumor microenvironment. Tumors are highly resistant to immune attack and even high numbers of adoptively transferred tumor-specific OT-I T-cells cannot overcome tumor immunosuppression. However, if mice are simultaneously treated with 5/3 chimeric adenovirus, immunosuppressive molecules (such as TIM-3) are downregulated in the tumors.

Figure 4 reveals that lifting of immunosuppression alone is not sufficient to induce trafficking of T-cells to tumors: BiTEs are needed. Lifting of immunosuppression is not sufficient to induce trafficking of T-cells to B16.OVA tumors. Intratumoral injection of 5/3 chimeric adenovirus can induce CD8+ T-cells in peripheral blood but these cells cannot infiltrate the tumors efficiently. This

CLAIMS

1. An oncolytic adenoviral vector comprising

a deletion of a nucleic acid sequence in the E3 region, wherein the nucleic acid sequence deletion is a deletion of viral gp19k and 6.7k reading frames, and

a nucleic acid sequence encoding a bipartite molecule in the place of the deleted nucleic acid sequence in E3 region, wherein the bipartite molecule comprises a single chain variable fragment (scFv) specific for a cell surface molecule on a T lymphocyte and a scFv specific for a tumor antigen~~, wherein the cell surface molecule is selected from CD3, CD8 and CD4~~,

wherein the backbone of the vector is adenovirus serotype 5 (Ad5) nucleic acid backbone and the vector further comprises an Ad3 fiber knob, E2F1 promoter for tumor specific expression of E1A, and a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1~~.~~

~~2. The oncolytic adenoviral vector according to claim 1, wherein the tumor antigen is selected from Table 1 or from the group consisting of mesothelin, EpCAM1 and MUC1.~~

~~3. The oncolytic adenoviral vector according to claim 1 or 2, wherein the tumor antigen is mesothelin and the cell surface molecule is CD3;~~ wherein the tumor antigen is EpCAM1 and the cell surface molecule is CD3; or the tumor antigen is MUC1 and the cell surface molecule is CD3.

~~4. The oncolytic adenoviral vector according to any one of the previous claims, wherein the oncolytic adenoviral vector codes for two or more transgenes.~~

~~5~~2. The oncolytic adenoviral vector according to ~~any one of the previous claims~~claim 1, further comprising IL-2, TNFalpha or CD40L transgene.

~~6~~3. A pharmaceutical composition comprising an oncolytic adenoviral vector according to ~~any one of claims 1-5~~claim 1 or 2.

~~7~~4. An oncolytic adenoviral vector according to ~~any one of claims 1-5~~claim 1 or 2 for use in treatment of cancer.

~~8~~5. An oncolytic adenoviral vector for use according to claim ~~7~~4 ~~together~~ in combinatorial therapy with an adoptive T-cell therapeutic composition,

wherein the oncolytic adenoviral vector and the adoptive T-cell therapeutic composition are to be administered to a subject simultaneously or consecutively~~, in any order~~.

~~9~~6. Use of an oncolytic adenoviral vector according to ~~any one of claims 1-5~~claim 1 or 2 in the manufacture of a medicament for treating cancer in a subject.

~~10~~7. The use according to claim ~~9~~6, wherein the medicament is to be administered in combination with an adoptive T-cell therapeutic composition to the subject.

~~11. The use or the oncolytic adenoviral vector for use according to claim 8 or 10, wherein the adoptive cell therapeutic composition comprises a cell type selected from a group consisting of a tumor infiltrating lymphocyte (TIL), T-cell receptor modified lymphocytes and chimeric antigen receptor modified lymphocytes.~~

~~12. The use or the oncolytic adenoviral vector for use according to any one of claims 8 or 10-11, wherein the adoptive cell therapeutic composition comprises a cell type selected from a group consisting of T-cells, CD8+ cells, CD4+ cells, NK-cells, delta-gamma T-cells, regulatory T-cells, and peripheral blood mononuclear cells.~~

~~13. The use or the oncolytic adenoviral vector for use according to any one of claims 8 or 10-12, wherein the adoptive cell therapeutic composition comprises T-cells.~~

~~14~~8. The use or the oncolytic adenoviral vector for use according to any one of claims ~~7-13~~4-7, wherein the cancer is selected from a group consisting of nasopharyngeal cancer, synovial cancer, hepatocellular cancer, renal cancer, cancer of connective tissues, melanoma, lung cancer, bowel cancer, colon cancer, rectal cancer, colorectal cancer, brain cancer, throat cancer, oral cancer, liver cancer, bone cancer, pancreatic cancer, choriocarcinoma, gastrinoma, pheochromocytoma, prolactinoma, T-cell leukemia/lymphoma, neuroma, von Hippel-Lindau disease, Zollinger-Ellison syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, ~~brain cancer,~~ oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, bone cancer, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer,

Paget's disease, cervical cancer, colorectal cancer, rectal cancer, esophagus cancer, gall bladder cancer, head cancer, eye cancer, neck cancer, kidney cancer, Wilms' tumor, ~~liver cancer,~~ Kaposi's sarcoma, prostate cancer, ~~lung cancer,~~ testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, oral cancer, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, pancreatic cancer, parathyroid cancer, penis cancer, pituitary cancer, soft tissue sarcoma, retinoblastoma, small intestine cancer, stomach cancer, thymus cancer, thyroid cancer, trophoblastic cancer, hydatidiform mole, uterine cancer, endometrial cancer, vagina cancer, vulva cancer, acoustic neuroma, mycosis fungoides, insulinoma, carcinoid syndrome, somatostatinoma, gum cancer, heart cancer, lip cancer, meninges cancer, mouth cancer, nerve cancer, palate cancer, parotid gland cancer, peritoneum cancer, pharynx cancer, pleural cancer, salivary gland cancer, tongue cancer and tonsil cancer.

~~15. The use according to any one of claims 10-14, wherein the oncolytic viral vectors and an adoptive cell therapeutic composition are to be administered to a subject simultaneously or consecutively, in any order.~~

~~16~~9. The use or the oncolytic adenoviral vector for use according to any one of claims ~~7-15~~4-8, wherein the medicament or oncolytic adenoviral vector is to be administered with concurrent or sequential radiotherapy~~, monoclonal antibodies,~~ or chemotherapy ~~or other anti-cancer drugs or interventions to a subject~~.

~~17. The oncolytic adenoviral vector according to any one of claims 1-5 for use in increasing the efficacy of adoptive cell therapy in a subject.~~

~~18. Use of an oncolytic adenoviral vector according to any one of claims 1-5 in the manufacture of a medicament for increasing the efficacy of adoptive cell therapy in a subject in need thereof, wherein the subject has been administered or is to be administered with adoptive cell therapy.~~