

Yu et al. (2014, Mol Ther 22(1):102-11) describe oncolytic vaccinia viruses coding for an anti-EphA2 BiTe. With regard to vectored delivery of BiTEs, single-chain molecules, including dual-single-chain constructs such as BiTEs, are not automatically secreted from mammalian cells. In fact, the poor secretion of single-chain molecules and construct such as BiTEs has formed an obstacle in their gene therapy use. Antibodies are normally produced by B-cell lineage plasma cells and thus it is no surprise their production and release from epithelial tumor cells is problematic.

With regard to efficacy of oncolytic viral vectors, either alone or together with other therapies, room is left for improvement. Increased specificity and sufficient tumor killing ability of therapies in general are warranted.

The present invention provides efficient tools and methods for cancer therapeutics by utilizing specific viral vectors, e.g. with adoptive cell therapies.

SUMMARY

An object of the present disclosure is to provide simple methods and tools for overcoming the above problems of inefficient, unsafe and unpredictable cancer therapies. In one example, the disclosure provides novel methods and means for cell therapy. The objects of the disclosure are achieved by specific viral vectors, methods and arrangements, which are characterized by what is stated in the independent claims. The specific examples of the disclosure are disclosed in the dependent claims.

The present disclosure proposes use of specific oncolytic adenoviruses to resolve the issues of highly immunosuppressive tumor microenvironment, which inactivates and anergizes the T-cell graft, inhibits local propagation of the graft, and hinders trafficking of the adoptively transferred T-cells to the tumor. The disclosure is based on the surprising realization that oncolytic adenoviruses coding for bi-specific T-cell engagers (BiTE) can resolve said issues (Figure 1). In particular, data related to the present disclosure indicates that adenovirus can induce danger signals in tumors of mice and in humans, as exemplified by interferon gamma production (Figure 2), which leads to reduction in TIM3 (TIM3 is a key indicator of tumor immunosuppression) expression (Figure 3). Importantly, even if adenovirus alone is able to produce danger signals at the tumor, this is not sufficient to recruit T-cells to the tumor (Figure 4). Thus, for optimal enhancement of adoptive cell therapy, arming of oncolytic adenovirus with BiTE is required (Figure 1).

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.

CLAIMS

1. An oncolytic adenoviral vector comprising
a deletion of a nucleic acid sequence in the E3 region, wherein the
5 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 mole-
cule comprises a single chain variable fragment (scFv) specific for a cell sur-
10 face 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
15 (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 meso-
thelin, EpCAM1 and MUC1.
3. The oncolytic adenoviral vector according to claim 1 or 2, wherein
20 the tumor antigen is mesothelin and the cell surface molecule is CD3; the tu-
mor 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 previ-
ous claims, wherein the oncolytic adenoviral vector codes for two or more
25 transgenes.
5. The oncolytic adenoviral vector according to any one of the previ-
ous claims, further comprising IL-2, TNFalpha or CD40L transgene.
6. A pharmaceutical composition comprising an oncolytic adenoviral
vector according to any one of claims 1-5.
- 30 7. An oncolytic adenoviral vector according to any one of claims 1-5
for use in treatment of cancer.
8. An oncolytic adenoviral vector for use according to claim 7 togeth-
er with an adoptive cell therapeutic composition, wherein the oncolytic adenovi-
ral vector and the adoptive cell therapeutic composition are to be administered
35 to a subject simultaneously or consecutively, in any order.
9. Use of an oncolytic adenoviral vector according to any one of
claims 1-5 in the manufacture of a medicament for treating cancer in a subject.

10. The use according to claim 9, wherein the medicament is to be administered with an adoptive 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
5 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
10 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
15 comprises T-cells.

14. The use or the oncolytic adenoviral vector for use according to any one of claims 7-13, 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
20 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, oligodendrogloma, neuroblastoma, meningioma, spinal cord tumor, bone cancer, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary
25 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
30 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
35 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.

5 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.

10 16. The use of the oncolytic adenoviral vector for use according to any one of claims 7-15, wherein the medicament or oncolytic adenoviral vector is to be administered with concurrent or sequential radiotherapy, monoclonal antibodies, 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.

15 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.