

NOVEL DOSAGE REGIMEN FOR AN ONCOLYTIC ADENOVIRAL VECTOR

FIELD

[0001] The present invention relates generally to virology, immunology, and medicine.
5 In particular, the invention relates to a dosage regimen including formulation, route of administration, dose, dosing interval, and treatment duration, of an oncolytic adenovirus in the treatment of cancer.

BACKGROUND

[0002] WO2014170389 relates to oncolytic adenoviral vectors alone or together with
10 therapeutic compositions, particularly adoptive-cell compositions, for therapeutic uses and therapeutic methods for cancer. After years of development, the oncolytic viruses are currently starting to be used as cancer therapeutics, particularly in combination treatments with other cancer drugs. In clinical trials, oncolytic viruses have demonstrated a favorable safety profile and promising efficacy. However, there is still room for improvement in the
15 responses, especially in patients with a significant metastasis burden. The present invention provides a novel dosage regimen for a specific oncolytic adenovirus that surprisingly shows that a monotherapy approach can be effective in cancer patients.

SUMMARY OF THE INVENTION

[0003] An object of the present invention is to provide a dosage regimen for
20 overcoming the problem of inefficient and unpredictable cancer therapies. More specifically, the invention provides a novel approach for oncolytic viral vector therapy. The objects of the invention are achieved by viral vectors, methods, and arrangements, which are characterized by what is stated in the appended independent claims. Some specific embodiments are defined in the dependent claims.

25 [0004] According to an aspect of the present invention, there is provided an oncolytic adenoviral vector coding for at least TNFalpha and/or IL-2 for use in treatment of cancer, wherein the adenoviral vector is to be administered to a subject in an active treatment cycle at least three times, preferably at least 5, 6 or 7 times; and wherein at least one, preferably the first, administration is a systemic administration, wherein said active treatment cycle

preferably does not include administration of an adoptive cell therapeutic composition comprising TILs or administration of an immune checkpoint inhibitor.

BRIEF DESCRIPTION OF THE DRAWINGS

5 [0005] FIGURES illustrate the present invention in accordance with at least some embodiments of the present invention.

[0006] **Figure 1** – TILT-123 treatment regimen in humans. Trial participants were screened up to 2 weeks before initiating TILT-123 treatment, in order to determine eligibility. Eligible patients were planned to receive an intravenous (day 1) and multiple intratumoral administrations of TILT-123 (day 8, 22, 36, 50 and 64). Trial participants entering the
10 treatment extension period (beyond day 78), continued intratumoral or intravenous administrations every 3 weeks. Positron Emission Tomography (PET) coupled to X-ray Computed Tomography (CT) imaging was performed in patients at baseline, day 78, and approximately every 3 months in the treatment extension period.

[0007] **Figure 2** – TILT-123 treatment effect in tumor lesions of an advanced lung
15 cancer patient by Positron Emission Tomography (PET) coupled to X-ray computed tomography imaging. A patient with stage IV non-small cell lung cancer was treated with 6 doses of TILT-123 – one intravenous administration followed by multiple intratumoral administrations. Representative PET-CT images from a TILT-123-injected tumor lesion (left lower neck/shoulder (lymph node)) and a non-injected tumor lesion (left axilla (lymph node)),
20 pre- and post-treatment are shown. Metabolic activity, quantified as maximum standardized uptake value (SUVmax), was measured. Percentage change was calculated comparing SUVmax values from pre-treatment with after TILT-123 values. Data cut-off 1st March 2023.

[0008] **Figure 3** – TILT-123 treatment effect in tumor lesions from a patient with thyroid cancer by Positron Emission Tomography (PET) coupled to X-ray Computed
25 Tomography (CT) imaging. A patient with thyroid anaplastic carcinoma was treated with 10 doses of TILT-123 – one intravenous administration followed by multiple intratumoral administrations. Representative PET-CT images from a TILT-123-injected tumor lesion (lower abdomen) and 2 non-injected tumor lesions (mesenterium lymph nodes and lung solitary left lower lobe), pre- and post-treatment (day 78 and day 167) are shown. Metabolic
30 activity, quantified as maximum standardized uptake value (SUVmax), was measured in the lesions. Computerized Tomography values measurement are shown in table from TL1.

Percentage change was calculated comparing SUVmax or CT values from pre-treatment with after TILT-123 values. Data cut-off 1st March 2023.

[0009] **Figure 4** – Efficacy of TILT-123 treatment. A. Response evaluation in all injected lesions, evaluated by CT. B. Response evaluation in all injected lesions, evaluated by PET. C. Response evaluation in all imaged non-injected lesions, evaluated by CT. D. Response evaluation in all imaged non-injected lesions, evaluated by PET. Best response shown for A-D if patient continued to extension. E. Overall survival in the trial. F. Progression-free survival in the trial. G. Time to progression in the trial. For E-G, disease control defined with PET-based criteria and comparison of disease control and no disease control evaluated with Mantel-Cox Logrank test. *** = $p < 0.001$, **** = $p < 0.0001$. Data from efficacy example #1 and #2 are included in the dataset used to generate the graphs.

[0010] **Figure 5** – Baseline neutralizing antibodies and their development. A. Neutralizing antibodies against TILT-123 at baseline in all patients. B. Neutralizing antibody presence compared to disease control at day 78. Disease control defined as SMD or better at day 78. No disease control defined as PMD or NA at day 78. Groups compared with Fisher's exact test. C. Baseline neutralizing antibody presence compared to overall survival across trial. Groups compared with MaxCombo logrank test. D. Neutralizing antibody titer across trial in all dose cohorts. Baseline defined as day 1 pre-treatment value, for days 1-64 the highest titer shown for each day (pre or post treatment). Data from efficacy example #1 and #2 are included in the dataset used to generate the graphs.

[0011] **Figure 6** – Neutralizing antibodies and antitumor response. A. Neutralizing antibodies across trial in patients with best RECIST1.1 responses. B. Neutralizing antibodies across trial in patients with best PET criteria responses. C. Neutralizing antibodies across trial in patients with longest survival. Data from efficacy example #1 and #2 are included in the dataset to generate the graphs.

EMBODIMENTS

[0012] **Oncolytic Virus**

[0013] In preferred embodiments, the oncolytic virus of the present disclosure is an oncolytic adenovirus.

[0014] As used herein “an oncolytic adenoviral vector” refers to an adenoviral vector capable of infecting and killing cancer cells by selective replication in tumor versus normal cells. The term adenoviral vector may thus refer herein either to the adenovirus or adenoviral particle capable of transferring a nucleic acid into a cell or to the transferred nucleic acid itself. WO2014170389 discloses oncolytic adenoviral vectors encoding TNFalpha and/or IL-2
5 as transgene(s) that can be used in this invention.

[0015] The vectors may be modified in any way known in the art, e.g., by deleting, inserting, mutating or modifying any viral areas. The vectors are made tumor specific with regard to replication. For example, the adenoviral vector may comprise modifications in E1,
10 E3 and/or E4 such as insertion of tumor specific promoters (e.g., to drive E1), deletions of areas (e.g., the constant region 2 of E1 as used in “D24”, E3/gp19k, E3/6.7k) and insertion of transgenes. Furthermore, fiber knob areas of the vector can be modified. In one embodiment of the invention, the adenoviral vector is Ad5/3 comprising an Ad5 nucleic acid backbone and Ad3 fiber knob or Ad5/3 chimeric fiber knob.

15 [0016] As used herein, the expression “adenovirus serotype 5 (Ad5) nucleic acid backbone” refers to the genome of Ad5.

[0017] “Ad5/3 vector” refers to a chimeric vector having parts of both Ad5 and Ad3 vectors. In a specific embodiment of the invention, the capsid modification of the vector is Ad5/3 chimerism. As used herein, “Ad5/3 chimeric fiber knob” refers to a chimerism,
20 wherein the knob part of the fiber is from Ad serotype 3, and the rest of the fiber is from Ad serotype 5. Specifically, in one embodiment, the construct has the fiber knob from Ad3 while the remainder of the genome is from Ad5 (SEQ ID NO:5).

[0018] One approach for generation of a tumor specific oncolytic adenovirus is engineering a 24 base pair deletion (D24) affecting the constant region 2 (CR2) of E1 (SEQ
25 ID NO:4). In wild type adenovirus, CR2 is responsible for binding the cellular Rb tumor suppressor/cell cycle regulator protein for induction of the synthesis (S) phase, i.e. DNA synthesis or replication phase. The interaction between Rb and E1A requires eight amino acids (121 to 127) of the E1A protein conserved region, which are deleted in the present vector. The vector of the present invention comprises a deletion of nucleotides corresponding
30 to amino acids 122-129 of the vector according to Heise C. et al. (2000). Viruses with the D24 are known to have a reduced ability to overcome the G1-S checkpoint and replicate efficiently

only in cells where this interaction is not necessary, e.g., in tumor cells defective in the Rb-p16 pathway, which includes most if not all human tumors.

[0019] It is also possible to replace E1A endogenous viral promoter for example by a tumor specific promoter. In a specific embodiment of the invention, the hTERT or E2F
5 promoter is utilized in the place of E1A endogenous viral promoter.

[0020] In a specific embodiment, the E1B 19K gene (SEQ ID NO:1), generally known to support replication of adenoviral vectors, has a disabling deletion dE1B 19K (SEQ ID NO:2) in the present vectors. Deletion of E1B 19K is known to sensitize cancer cells to TNFalpha and thus it promotes apoptosis.

10 [0021] The sequence for wild-type E1B 19K gene is the following (the deletable region is underlined):

```
atggaggcctt gggagtgttt ggaagatttt tctgctgtgc gtaacttgct
ggaacagagc tctaacagta cctcttggtt ttggagggtt ctgtggggct
catcccaggc aaagttagtc tgcagaatta aggaggatta caagtgggaa
15 tttgaagagc ttttgaaatc ctgtgggtgag ctgtttgatt ctttgaatct
gggtcaccag gcgcttttcc aagagaaggt catcaagact ttggattttt
ccacaccggg gcgcgtgctg gctgctgttg cttttttgag ttttataaag
gataaatgga gcgaagaaac catctgagc ggggggtacc tgctggattt
tctggccatg catctgtgga gagcggttgt gagacacaag aatcgcttgc
20 tactgttgct ttccgtccgc cggcgataa taccgacgga ggagcagcag
cagcagcagg aggaagccag gcggcggcgg caggagcaga gcccatggaa
cccagagacc ggcttgacc ctcggaatg a (SEQ ID NO:1)
```

[0022] Accordingly, in an embodiment, the sequence for dE1B 19K in the present viral vectors is

```
25 atggaggcctt gggagtgttt ggaagatttt tctgctgtgc gtaacttgct
ggaacagctg ggtcaccagg cgcttttcca agagaaggct atcaagactt
tggttttttc cacaccgggg gcgcgtgctg ctgctgttgc ttttttgagt
tttataaagg ataaatggag cgaagaaacc catctgagcg gggggtacct
gctggatttt ctggccatgc atctgtggag agcggttgtg agacacaaga
30 atcgcttgc actgttgtct tccgtccgcc cggcgataat accgacggag
```

gagcagcagc agcagcagga ggaagccagg cggcggcggc aggagcagag
cccatggaac ccgagagccg gcctggaccc tcgggaatga (SEQ ID NO:2)

[0023] The E3 region is nonessential for viral replication in vitro, but the E3 proteins have an important role in the regulation of host immune response i.e. in the inhibition of both innate and specific immune responses. The gp19k/6.7K deletion in E3 refers to a deletion of 965 base pairs from the adenoviral E3A region. In a resulting adenoviral construct, both gp19k and 6.7K genes are deleted (Kanerva A et al. 2005). The gp19k gene product is known to bind and sequester major histocompatibility complex I (MHC1, known as HLA1 in humans) molecules in the endoplasmic reticulum, and to prevent the recognition of infected cells by cytotoxic T-lymphocytes. Since many tumors are deficient in HLA1/MHC1, deletion of gp19k increases tumor selectivity of viruses (virus is cleared faster than wild type virus from normal cells but there is no difference in tumor cells). 6.7K proteins are expressed on cellular surfaces and they take part in downregulating TNF-related apoptosis inducing ligand (TRAIL) receptor 2.

[0024] Both of these deletions provide an advantage. To regain expression of HLA/MHC for presentation of tumor epitopes, e.g., to adoptively transferred T cells, expression of the gp19k protein is counterproductive and in fact, the upregulation of HLA/MHC requires deletion of gp19k. With regard to 6.7k, since an embodiment of our invention is production of TNFalpha from the virus, and one of its anti-tumor activities is a direct anti-tumor proapoptotic effect (on both transduced and non-transduced bystander cells), the presence of 6.7k is counterproductive.

[0025] In one embodiment of the invention, the cytokine transgene or transgenes are placed into a gp19k/6.7k deleted E3 region, under the E3 promoter. This restricts transgene expression to tumor cells that allow replication of the virus and subsequent activation of the E3 promoter. E3 promoter may be any exogenous (e.g. CMV or E2F promoter, SEQ ID NO:3) or endogenous promoter known in the art, specifically the endogenous E3 promoter. Although the E3 promoter is chiefly activated by replication, some expression occurs when E1 is expressed. As the selectivity of D24 type viruses occurs post E1 expression (when E1 is unable to bind Rb), these viruses do express E1 also in transduced normal cells. Thus, it is important to regulate also E1 expression to restrict E3 promoter mediated transgene expression to tumor cells.

[0026] In a specific embodiment of the invention, the oncolytic adenoviral vector is based on an adenovirus serotype 5 (Ad5) nucleic acid backbone comprising a 5/3 chimeric fiber knob, and comprising the following: 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, with a transgene insertion into the deleted region, resulting in replication-associated control of transgene expression under the viral E3 promoter, and a nucleic acid sequence encoding at least one cytokine transgene in the place of the deleted adenoviral genes gp19k/6.7K in the E3 region. In one embodiment of the invention, the adenoviral vector is based on a human adenovirus.

10 [0027] The exact functions of the Early Region (E3) proteins in adenovirus 3 are not known. Generally, in adenoviruses they do not seem to impair replication when deleted and they seem to affect anti-viral host response to adenoviruses. The E3 of the human adenovirus genome contains the highest level of genetic diversity among the six species (A-F) of adenoviruses found in humans. This diversity in genetic content is primarily located between the highly conserved E3-gp19K and E3-RID α open reading frames (ORFs) where species-specific arrays of genes are encoded.

[0028] Cytotoxic T-cell mediated killing of viral-infected cells is modulated by E3-gp19K. This is accomplished by blocking transport of MHC class I to the plasma membrane, and inhibiting the TAP-MHC class I complex formation.

20 [0029] Thus, in one aspect of the invention, the important molecule E3-gp19K is comprised in the adenoviral vector to make virus replication stealthier and enable more time for oncolysis and its beneficial effects. Also, retaining E3-gp19K can reduce induction of anti-adenovirus-cytotoxic T cells, resulting in more anti-tumor T cells.

[0030] Cytokines participate in immune response by acting through various mechanisms including recruitment of T cells towards the tumor. The nucleotide sequence encoding a cytokine transgene may be from any animal such as a human, ape, rat, mouse, hamster, dog or cat, but specifically it is encoded by a human sequence. The nucleotide sequence encoding the transgene may be modified in order to improve its effects, or unmodified i.e., of a wild type.

30 [0031] Particular embodiments of the present invention include viral vectors coding for at least one cytokine. In a specific embodiment of the invention the cytokine is IL-2 or

TNFA α , preferably the viral vectors are coding for both cytokines. In one embodiment of the invention the viral vectors are coding for IL-2 and/or TNFA α and a further cytokine, preferably selected from a group consisting of interferon alpha, interferon beta, interferon gamma, complement C5a, CD40L, IL12, IL23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7 and XCL2.

[0032] Cytokine TNFA α (tumor necrosis factor alpha) functions by attracting and activating the T cells and reducing tumor immunosuppression, while IL-2 (interleukin-2) induces the propagation of T-cells.

[0033] In one embodiment of the invention, the virus vector comprises an internal ribosomal entry site (IRES) or optionally a ribosome shunt site 2A between the two transgenes. Thus, IRES or a ribosome shunt site 2A may be between any cytokines, such as IL-2 and any other cytokine, preferably selected from the above listed cytokine group. As used herein "IRES" refers to a nucleotide sequence that enables initiation of the translation in the middle of a messenger RNA sequence in protein synthesis. IRES can be from any virus, but in one embodiment of the invention IRES is from encephalomyocarditis virus (EMCV). As used herein "a ribosome shunt site 2A" refers to a translation initiation site in which ribosomes physically bypass parts of the 5' untranslated region to reach the initiation codon. Both the IRES and the A2 enable viruses to produce two transgenes from one promoter (the E3 promoter).

[0034] Examples of detailed structures of the oncolytic adenoviral vector encoding TNFA α and/or IL-2 as a transgene are disclosed in WO2014170389.

[0035] In summary, the key advantages of the present invention utilizing viral vectors comprising at least one cytokine transgene are: i) cytokines and virus per se cause a danger signal which recruits T cells and other immune cells to tumors, ii) cytokines induce T-cell proliferation both at the tumor and in local lymphoid organs, iii) cytokines and virus per se are able to induce T cells (natural, innate anti-tumor T cells) to propagate at the tumor, iv)

cytokine and/or virus induce the upregulation of antigen-presenting molecules (HLA) on cancer cells, rendering them sensitive to recognition and killing by T cells, and v) cytokines and virus replication favorably alter tumor microenvironment by reducing immunosuppression and cellular anergy.

5 [0036] The viral vectors utilized in the present inventions may also comprise other modifications than described above. Any additional components or modifications may optionally be used but are not obligatory for the present invention.

[0037] Insertion of exogenous elements may enhance effects of vectors in target cells. The use of exogenous tissue or tumor-specific promoters is common in recombinant vectors
10 and they can also be utilized in the present invention.

[0038] In summary, the replication of oncolytic virus can recruit T cells and induce danger signals at the tumor, reducing immunosuppression and cellular anergy. These effects are mediated through pathogen associated molecular pattern recognition receptors, an evolutionarily conserved mechanism for inducing immunity and not subject to tolerance. An
15 added benefit of the oncolytic platform is the capability of replication in tumors but not normal cells, i.e. self-amplification at the tumor. In addition, the oncolytic effect per se may add to the overall anti-tumor effect in humans.

[0039] **Cancer**

[0040] The recombinant vectors of the present invention are replication competent in
20 tumor cells. In one embodiment of the invention, the vectors are replication competent in cells, which have defects in the Rb-pathway, specifically Rb-p16 pathway. These defective cells include all tumor cells in animals and humans. As used herein “defects in the Rb-pathway” refers to mutations and/or epigenetic changes in any genes or proteins of the pathway. Due to these defects, tumor cells overexpress E2F and thus, binding of Rb by E1A
25 CR2, that is normally needed for effective replication, is unnecessary. Further selectivity is mediated by the E2F promoter, which only activates in the presence of free E2F, as seen in Rb/p16 pathway defective cells. In the absence of free E2F, no transcription of E1A occurs and the virus does not replicate. Inclusion of the E2F promoter is important to prevent expression of E1A in normal tissues, which can cause toxicity both directly and indirectly
30 through allowing transgene expression from the E3 promoter.

[0041] The present invention relates to approaches for treating cancer in a subject. In one embodiment of the invention, the subject is a human or a mammal, specifically a mammal or human patient, more specifically a human or a mammal suffering from cancer.

[0042] The approach can be used to treat any cancers or tumors, including both malignant and benign tumors, both primary tumors and metastases may be targets of the approach. In one embodiment of the invention, the cancer features tumor-infiltrating lymphocytes. The tools of the present invention are particularly appealing for treatment of metastatic solid tumors featuring tumor-infiltrating lymphocytes.

[0043] As used herein, the term “treatment” or “treating” refers to administration of at least oncolytic adenoviral vectors to a subject, preferably a mammal or human subject, for purposes which include not only complete cure but also prophylaxis, amelioration, or alleviation of disorders or symptoms related to a cancer or tumor. Therapeutic effect may be assessed by monitoring the symptoms of a patient, tumor markers in blood, or for example a size of a tumor, metabolic activity of the tumor or the length of survival of the patient.

[0044] In another embodiment of the invention the cancer or tumor 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, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, esophagus cancer, gall bladder cancer, head cancer, eye cancer, neck cancer, Wilms' tumor, Kaposi's sarcoma, prostate cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, 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, nerve cancer, palate

cancer, parotid gland cancer, peritoneum cancer, pharynx cancer, pleural cancer, salivary gland cancer, tongue cancer, and tonsil cancer. Preferably, the cancer or tumor treated is selected from the group consisting of renal cancer, ovarian cancer, bladder cancer, prostate cancer, breast cancer, colorectal cancer, lung cancer (such as small-cell lung carcinoma, non-small-cell lung carcinoma and squamous non-small-cell lung carcinoma), gastric cancer, classical Hodgkin lymphoma, mesothelioma, and liver cancer. In a more preferred embodiment, the cancer or tumor type is melanoma, lung cancer (such as small-cell lung carcinoma, non-small-cell lung carcinoma and squamous non-small-cell lung carcinoma), ovarian cancer, head and neck cancer, or thyroid cancer. In another more preferred embodiment, the cancer or tumor type is soft tissue sarcoma, e.g. leiomyosarcoma or liposarcoma,

[0045] Before classifying a human or animal patient as suitable for the therapy of the present invention, the clinician may examine a patient. Based on the results deviating from the normal and revealing a tumor or cancer, the clinician may suggest treatment of the present invention for a patient.

[0046] In an embodiment of the invention, the subject or patient has already failed at least one previous chemotherapy, radiotherapy or immunotherapy treatment such as a CPI treatment, i.e. the cancer of the patient is an immune checkpoint inhibitor (CPI) refractory tumor. In a preferred embodiment, the present invention is directed to the treatment of a CPI refractory tumor.

[0047] An immune checkpoint inhibitor (also referred to as a CPI) as described herein is any compound capable of inhibiting the function of an immune checkpoint protein. Inhibition includes reduction of function as well as full blockade. In particular, the immune checkpoint protein is a human checkpoint protein. Thus, the immune checkpoint inhibitor is preferably an inhibitor of a human immune checkpoint.

[0048] Checkpoint proteins include, without limitation, CTLA-4, PD-1 (and its ligands PD-L1 and PD-L2), B7-H3, B7-H4, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, BTLA, TIGIT and/or IDO. The pathways involving LAG3, BTLA, B7-H3, B7-H4, TIM3 and KIR are recognized in the art to constitute immune checkpoint pathways similar to the CTLA-4 and PD-1 dependent pathways. The immune checkpoint inhibitor can be an inhibitor of CTLA-4, PD-1 (and its ligands PD-L1 and PD-L2), B7-H3, B7-H4, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, BTLA, TIGIT and/or IDO. In some embodiments, the immune

checkpoint inhibitor is an inhibitor of PD-L1 or PD-1. In some embodiments, the immune checkpoint inhibitor is an antibody.

[0049] Pharmaceutical composition

[0050] A pharmaceutical composition of the invention comprises at least one type of viral vectors of the invention. The present invention also provides said pharmaceutical combination for use in the treatment of cancer. Furthermore, the composition may comprise at least two, three or four different vectors. In addition to the vector, a pharmaceutical composition may also comprise other therapeutically effective agents, any other agents such as pharmaceutically acceptable carriers, buffers, excipients, adjuvants, additives, preservatives, antiseptics, filling, stabilising and/or thickening agents, and/or any components normally found in corresponding products. Selection of suitable ingredients and appropriate manufacturing methods for formulating the compositions belongs to general knowledge of a man skilled in the art.

[0051] The pharmaceutical composition may be in any form, such as solid, semisolid or liquid form, suitable for administration. A formulation can be selected from a group consisting of, but not limited to, solutions, sprays, emulsions, suspensions, tablets, pellets and capsules. The compositions of the current invention are not limited to a certain formulation, instead the composition can be formulated into any known pharmaceutically acceptable formulation. The pharmaceutical compositions may be produced by any conventional processes known in the art.

[0052] A pharmaceutical kit of the present invention comprises an oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene. In an embodiment, the oncolytic adenoviral vector encoding TNFalpha and/or IL-2 as a transgene is formulated in a first formulation and in case of combination treatment with another active pharmaceutical ingredient, the latter is formulated in a second formulation. In another embodiment of the invention the first and the second formulations are for simultaneous or sequential, in any order, administration to a subject. In another embodiment, said kit is for use in the treatment of cancer or tumor.

[0053] Administration and dosage regimen

[0054] The vector or pharmaceutical composition of the invention may be administered to any mammal subject. In a specific embodiment of the invention, the subject is a human. A

mammal may be selected from a group consisting of pets, domestic animals and production animals.

[0055] Any conventional method may be used for administration of the vector or composition to a subject. The route of administration depends on the formulation or form of the composition, the disease, location of tumors, the patient, comorbidities and other factors. Accordingly, the dose amount and dosing frequency of each therapeutic agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated, and patient characteristics. Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the patient consistent with an acceptable level of side effects.

[0056] In one embodiment of the invention, the administration of oncolytic virus is conducted through an intratumoral (i.t.), intra-arterial, intravenous (i.v.), intrapleural (i.pl.), intravesicular, intracavitary or peritoneal (i.p.) injection, or an oral or intranasal administration. In another embodiment of the invention, the administration of oncolytic virus is a systemic administration. Systemic administration is a route of administration of medication into the circulatory system so that the entire body is affected. Systemic administration can take place via parenteral administration (i.e. generally injection or infusion). Any combination of administrations is also possible. The approach can give systemic efficacy despite local injection.

[0057] The effective dose of vectors depends on at least the subject in need of the treatment, tumor type and location of the tumor and stage of the tumor. Each dose may vary for example from about 1×10^8 viral particles (VP) to about 1×10^{14} VP, specifically from about 5×10^9 VP to about 1×10^{13} VP and more specifically from about 3×10^9 VP to about 4×10^{12} VP. In one embodiment, oncolytic adenoviral vectors coding for at least IL-2 and/or TNF α are administered in an amount of 1×10^{10} - 1×10^{14} virus particles. In another embodiment of the invention, the dose is in the range of about 5×10^{10} - 5×10^{11} VP.

[0058] In a special embodiment, the present invention is directed to a dosage regimen, wherein the adenoviral vector is to be administered to a subject in an active treatment cycle at least three times, preferably at least 4, 5, 6 or 7 times; and wherein at least one, preferably the first, administration is a systemic administration, wherein said active treatment cycle preferably does not include administration of an adoptive cell therapeutic composition comprising TILs or administration of an immune checkpoint inhibitor. In a preferred

embodiment, the duration of said active treatment cycle is at least 60 days, preferably 61-81 days. In another preferred embodiment, said active treatment cycle is carried out as a monotherapy treatment of cancer with said adenoviral vector from the onset of the treatment cycle until the last administration of said adenoviral vector.

5 [0059] As used herein “adoptive cell therapeutic composition” refers to any composition comprising cells suitable for adoptive cell transfer (see e.g. WO2014170389). Generally, the adoptive cell therapeutic composition comprises a cell type selected from a group consisting of a tumor infiltrating lymphocyte (TIL), TCR (i.e. heterologous T-cell receptor) modified lymphocytes and CAR (i.e. chimeric antigen receptor) modified
10 lymphocytes. The adoptive cell therapeutic composition may comprise 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. Commonly, TILs, T-cells, CD8⁺ cells, CD4⁺ cells, NK-cells, delta-gamma T-cells, regulatory T-cells or peripheral blood mononuclear cells form the adoptive cell therapeutic composition. In one specific case, the
15 adoptive cell therapeutic composition comprises T cells. As used herein “tumor-infiltrating lymphocytes” (TILs) refer to white blood cells that have left the bloodstream and migrated into a tumor.

[0060] In a preferred embodiment, said systemic administration is given parenterally, preferably intravenously.

20 [0061] In another preferred embodiment, the second and subsequent administrations are given locally, preferably intratumorally, intraperitoneally, or intrapleurally.

[0062] In another preferred embodiment, the second administration of the adenoviral vector is to be administered within 10 days, preferably on the 8th day, after the onset of the treatment, i.e. the first administration of said adenoviral vector.

25 [0063] In another preferred embodiment, a further administration of the adenoviral vector is to be administered within 20 days, preferably on the 15th day, after the onset of the treatment. In a more preferred embodiment, a further administration of the adenoviral vector is to be administered within 20 days after the onset of the treatment so that the previous administration was administered within 10 days after the onset of the treatment.

30 [0064] In another preferred embodiment, a further administration of the adenoviral vector is to be administered within 32 days, preferably on the 22nd day or 29th day, after the

onset of the treatment. In a more preferred embodiment, a further administration of the adenoviral vector is to be administered within 32 days after the onset of the treatment so that the previous administration was administered within 20 days after the onset of the treatment.

[0065] In another preferred embodiment, a further administration of the adenoviral vector is to be administered within 46 days, preferably on the 36th day or 43rd day, after the onset of the treatment. In a more preferred embodiment, a further administration of the adenoviral vector is to be administered within 46 days after the onset of the treatment so that the previous administration was administered within 32 days after the onset of the treatment.

[0066] In another preferred embodiment, a further administration of the adenoviral vector is to be administered within 60 days, preferably on the 50th or 57th day, after the onset of the treatment. In a more preferred embodiment, a further administration of the adenoviral vector is to be administered within 60 days after the onset of the treatment so that the previous administration was administered within 46 days after the onset of the treatment.

[0067] In another preferred embodiment, a further administration of the adenoviral vector is to be administered within 67 days, preferably on the 64th day, after the onset of the treatment. In a more preferred embodiment, a further administration of the adenoviral vector is to be administered within 67 days after the onset of the treatment so that the previous administration was administered within 60 days after the onset of the treatment.

[0068] In another preferred embodiment, a further administration of the adenoviral vector is to be administered within 81 days, preferably on the 71st or 78th day, after the onset of the treatment. In a more preferred embodiment, a further administration of the adenoviral vector is to be administered within 81 days after the onset of the treatment so that the previous administration was administered within 67 days after the onset of the treatment.

[0069] In another preferred embodiment, the second administration of the adenoviral vector is to be administered within 2-6 hours after the onset of the treatment, i.e. the first administration of said adenoviral vector, wherein said administrations are preferably all intravenous in said treatment.

[0070] In another preferred embodiment, two further (preferably the 3rd and the 4th) administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 5 days, preferably on the 3rd day, after the onset of the treatment.

[0071] In another preferred embodiment, two further (preferably the 5th and the 6th) administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 10 days, preferably on the 8th day, after the onset of the treatment. In a more preferred embodiment, two further administrations of the adenoviral vector are to be administered within 5 10 days after the onset of the treatment so that the previous administrations were administered within 5 days after the onset of the treatment.

[0072] In another preferred embodiment, two further (preferably the 7th and the 8th) administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 13 days, preferably on the 10th day, after the onset of the treatment. In a more preferred 10 embodiment, two further administrations of the adenoviral vector are to be administered within 13 days after the onset of the treatment so that the previous administrations were administered within 8 or 10 days after the onset of the treatment.

[0073] In another preferred embodiment, two further (preferably the 9th and the 10th) administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 15 30 days, preferably on the 22nd day, after the onset of the treatment. In a more preferred embodiment, two further administrations of the adenoviral vector are to be administered within 30 days after the onset of the treatment so that the previous administrations were administered within 13 days after the onset of the treatment.

[0074] In another preferred embodiment, two further (preferably the 11th and 12th) 20 administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 46 days, preferably on the 43th day, after the onset of the treatment. In a more preferred embodiment, two further administrations of the adenoviral vector are to be administered within 46 days after the onset of the treatment so that the previous administrations were administered within 30 days after the onset of the treatment.

25 [0075] In another preferred embodiment, the two further (preferably the 13th and 14th) administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 67 days, preferably on the 64th day, after the onset of the treatment. In a more preferred embodiment, two further administrations of the adenoviral vector are to be administered within 67 days after the onset of the treatment so that the previous administrations were administered 30 within 46 days after the onset of the treatment.

[0076] In an embodiment, the treatment period is extended over 60-81 days and at least one further intravenous, intratumoral, intraperitoneal, or intrapleural administration is given after the initial 60-81 days treatment period (see Figure 1).

[0077] In a more preferred embodiment, the adenoviral vector is to be administered to a subject in an active treatment cycle of at least 60 days, at least five times, preferably 6 times; and wherein at least one, preferably the first, administration is given intravenously and the rest of the administrations are given intratumorally.

[0078] An example of the dosage scheme is shown in Figure 1.

[0079] Another example of the dosage scheme is the following (preferably with a viral dose ranging between 1×10^{11} - 2×10^{12} per administration):

Day 1 - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

Day 3 - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

Day 8 (+/- 1 day) - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

Day 10 (+/- 3 days) - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

Day 22 (+/- 3 days) - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

Day 43 (+/- 3 days) - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

Day 64 (+/- 3 days) - 2x i.v. administrations of the oncolytic virus (each injection 4h +/- 2h apart)

[0080] Another example of the dosage scheme is the following (preferably with a viral dose ranging between 3×10^{10} - 2×10^{12} per administration):

Day 1 - 1x i.v. administration of the oncolytic virus

Day 8 (+/- 1 day) - 1x i.t. administration of the oncolytic virus

Day 15 (+/- 3 days) - 1x i.t. administration of the oncolytic virus

Day 29 (+/- 3 days) - 1x i.t. administration of the oncolytic virus

Day 43 (+/- 3 days) - 1x i.t. administration of the oncolytic virus

Day 57 (+/- 3 days) - 1x i.t. administration of the oncolytic virus

Day 71 (+/- 3 days) - 1x i.t. administration of the oncolytic virus

- 5 [0081] Another example of the dosage scheme can be the following (preferably with a viral dose ranging between 1×10^{11} - 4×10^{12} per administration):

Day 1 - 1x i.v. administration of the oncolytic virus

Day 8 (+/- 1 day) - 1x i.t./i.p. administration of the oncolytic virus

Day 22 (+/- 3 days) - 1x i.t./i.p. administration of the oncolytic virus

- 10 Day 36 (+/- 3 days) - 1x i.t./i.p. administration of the oncolytic virus

Day 57 (+/- 3 days) - 1x i.t./i.p. administration of the oncolytic virus

Day 78 (+/- 3 days) - 1x i.t./i.p. administration of the oncolytic virus

- [0082] Any other treatment or combination of treatments may be used in addition to the therapies of the present invention. In a specific embodiment the method or use of the invention further comprises administration of concurrent or sequential radiotherapy, chemotherapy, antiangiogenic agents or targeted therapies, such as alkylating agents, nucleoside analogs, cytoskeleton modifiers, cytostatic agents, monoclonal antibodies, kinase inhibitors or other anti-cancer drugs or interventions (including surgery) to a subject.
- 15

- [0083] Without wishing to be bound by any theory, the experimental results disclosed herein suggest that higher levels of neutralizing antibodies against adenoviral vectors may improve the efficacy of the treatment according to the present invention in some patients. Accordingly, in an embodiment, said active treatment cycle of the present disclosure can be preceded with an immune system stimulation dose of an adenoviral vector in order to raise neutralizing antibodies in the patient, wherein said adenoviral vector preferably comprises an adenovirus serotype 5 (Ad5) backbone (optionally with the fiber knob of adenovirus serotype 3, Ad3), and wherein said vector is in a more preferred embodiment the same which is used in said active treatment cycle.
- 20
- 25

[0084] The present invention is also directed to a method for selecting patients for a cancer treatment with an oncolytic adenoviral vector, the method comprising a step of providing a biological sample of a subject, measuring the level of adenovirus neutralizing antibodies in the sample, and selecting patients based on the level of presence of said adenovirus neutralizing antibodies in said sample. In a preferred embodiment, said biological sample is a blood sample or sample derived from a blood sample (e.g., blood serum sample). In another preferred embodiment, the selection step is performed by comparing the level of adenovirus neutralizing antibodies in the sample to the level of adenovirus neutralizing antibodies in the sample obtained from healthy subjects or patients who have benefited from said cancer treatment with an oncolytic adenoviral vector.

[0085] The terms “treat” or “increase”, as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or increase. Rather, there are varying degrees of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect.

[0086] The term "administration", as used herein, refers to a dosing session wherein one or more injections/infusions of a therapeutic agent can be given to a human or mammal, and in case of more than one injection/infusion, the injections/infusions are given preferably in a time frame of 1-8 hours and can be given through one or more routes of administration.

[0087] **Other embodiments**

[0088] Reference throughout this specification to one embodiment or an embodiment means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases “in one embodiment” or “in an embodiment” in various places throughout this specification are not necessarily all referring to the same embodiment. Where reference is made to a numerical value using a term such as, for example, about or substantially, the exact numerical value is also disclosed.

[0089] It is to be understood that the embodiments of the invention disclosed are not limited to the particular structures, process steps, or materials disclosed herein, but are extended to equivalents thereof as would be recognized by those ordinarily skilled in the relevant arts. It should also be understood that terminology employed herein is used for the purpose of describing particular embodiments only and is not intended to be limiting.

[0090] Reference throughout this specification to “one embodiment” or “an embodiment” means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances of the phrases “in one embodiment” or “in an embodiment” in
5 various places throughout this specification are not necessarily all referring to the same embodiment.

[0091] As used herein, a plurality of items, structural elements, compositional elements, and/or materials may be presented in a common list for convenience. However, these lists should be construed as though each member of the list is individually identified as a separate
10 and unique member. Thus, no individual member of such list should be construed as a de facto equivalent of any other member of the same list solely based on their presentation in a common group without indications to the contrary. In addition, various embodiments and example of the present invention may be referred to herein along with alternatives for the various components thereof. It is understood that such embodiments, examples, and
15 alternatives are not to be construed as de facto equivalents of one another, but are to be considered as separate and autonomous representations of the present invention.

[0092] Furthermore, the described features, structures, or characteristics may be combined in any suitable manner in one or more embodiments. In the following description, numerous specific details are provided, such as examples of lengths, widths, shapes, etc., to
20 provide a thorough understanding of embodiments of the invention. One skilled in the relevant art will recognize, however, that the invention can be practiced without one or more of the specific details, or with other methods, components, materials, etc. In other instances, well-known structures, materials, or operations are not shown or described in detail to avoid obscuring aspects of the invention.

[0093] While the forgoing examples are illustrative of the principles of the present invention in one or more particular applications, it will be apparent to those of ordinary skill in the art that numerous modifications in form, usage and details of implementation can be made without the exercise of inventive faculty, and without departing from the principles and concepts of the invention. Accordingly, it is not intended that the invention be limited, except
25 as by the claims set forth below.

[0094] The verbs “to comprise” and “to include” are used in this document as open limitations that neither exclude nor require the existence of also un-recited features. The

features recited in depending claims are mutually freely combinable unless otherwise explicitly stated. Furthermore, it is to be understood that the use of "a" or "an", i.e. a singular form, throughout this document does not exclude a plurality.

[0095] **Table 1.** The sequences listed in the appended Sequence listing.

SEQ ID NO:	Name:
1	E1B 19K
2	dE1B 19K
3	E2F
4	D24
5	5/3 knob modification

5

EXPERIMENTAL SECTION

EXAMPLE 1

10 **Materials and Methods**

Trial participants

Female or male participants over 18-years-old with pathologically confirmed refractory or recurrent cancer that had previously failed standard therapy, were enrolled in the trial. Additional inclusion criteria comprised having, evaluable disease (not needing to fulfil
15 response evaluation criteria in solid tumors (RECIST 1.1), and at least one injectable tumor available for injections and biopsies. Participants needed to have adequate hepatic and renal values and World health organization (WHO)/Eastern Cooperative Oncology Group (ECOG) performance varying from 0 to 1. Participants were not eligible for the trial if they had been
20 treated with anti-cancer therapies during 30 days prior to the first treatment injection, or treated with immunosuppressive medications (with some exemptions), as well as other exclusion criteria. Among the trial subjects, one patient with non-small cell lung cancer (Efficacy Example #1; data cut-off 1st March 2023), and one patient with thyroid anaplastic

carcinoma (Efficacy example #2; data cut-off 1st March 2023), participated in the trial.

Trial design and treatments

5 This study was an open-label, dose-escalation phase 1 clinical trial conducted in two different clinical sites in Helsinki, Finland. Treatment duration lasted 64 days with the option for extended treatment if potential signs of benefits were observed in enrolled patients.

During the length of the trial patients received a total of 6 doses of oncolytic vector Ad5/3-E2F-D24-TNF α -IRES-IL2 (TILT-123) during the first 64 days of therapy (Figure 1).

10 Treatment with TILT-123 started with an intravenous administration at day 1, followed by intratumoral administrations at day 8, 22, 36, 50, 64, and every 3 weeks thereafter, if the patient entered the treatment extension. Except day 1 visit, all other trial visits had a tolerance of +/- 1-3 days. The patient from efficacy example #1 received 3×10^9 virus particles (VP) of TILT-123 per intravenous and intratumoral dosing occasion, while the patient from efficacy example #2 received 3×10^{11} VPs intravenously and 1×10^{11} VPs intratumorally.

15 Regarding intratumoral administration, the priority was to inject 10 virus deposits over a total of 4 mL volume per each dosing occasion, preferably over 10 different lesions (i.e. one injection per lesion). Multiple injections per tumor was possible, if less than 10 lesions were available for intratumoral injection. Injecting larger lesions multiple times was prioritized over smaller lesions.

20 Study assessments

The primary endpoint of the trial is safety by day 85 based on adverse events, serious adverse events, vital signs, electrocardiogram (ECG), and safety laboratory results. Secondary endpoints, included (but not limited) tumor response by RECIST 1.1 immune(i)RECIST and a PET-based criteria, measured at screening and day 78 using whole body X-ray computerized
25 tomography (CT) and positron emission tomography (PET) scans. While the CT scans provide the material for measuring the actual size of tumor lesions, the PET imaging provides a measurement of the metabolic activity of the tumor via maximum standardized uptake value (SUVmax) of a radiotracer (fluorodeoxyglucose (FDG)).

Results

30 Efficacy Example #1

A subject with progressing stage IV non-small cell lung cancer was enrolled in the trial and received 6 administrations of TILT-123 up to day 64 (Figure 2) as described earlier. By day 78 after treatment, imaging of target lesion 3 demonstrated a reduction in the metabolic activity of a lesion administered with TILT-123 by 61% in the SUVmax value compared to baseline (Figure 2). Similar occurrence was seen in target lesion 4, as evidenced by a 54% reduction in SUVmax compared to baseline. Of note, the latter lesion was not administered with TILT-123, showing the antitumor effect of TILT-123 in distant lesions.

Efficacy Example #2

A subject with progressing stage IV thyroid anaplastic carcinoma was enrolled in the trial and received 10 doses of TILT-123 up to day 167 (Figure 3) as described earlier. After TILT-123 treatment, target lesion 1 demonstrated a reduction in tumor size of up to 69% by day 167, as measured by CT scans. Further antitumor effects were observed by complete ablation of tumor metabolic activity by day 167, in target lesion 1. Other non-target lesions that were present in the patient prior to treatment with TILT-123, became negative by day 78 (non-target lesion 2), and day 167 (non-target lesion 4). The effect of TILT-123 treatments to induce antitumor effects in non-injected lesions reiterates the systemic effect of the therapeutic approach.

EXAMPLE 2

Materials and Methods

20 Patients and methods

Between February 18th 2021 and July 13th 2023, 20 patients were enrolled in the trial. Inclusion criteria included cancer disease where standard therapy had failed or did not exist, at least one tumor available for intratumoral injection, adequate hematological (hemoglobin > 100 g/L, WBC > 3.0 E9/L, platelets > 75 000/mm³), hepatic (AST, ALT < 3 x ULN and bilirubin < 1.5 x ULN) and renal function (GFR > 60 ml/min), WHO/ECOG performance score of 0-1 at screening and life expectancy longer than 3 months. Exclusion criteria included use of immunosuppressive medications (corticosteroids or drugs used in autoimmune diseases), treatment with anti-cancer therapy within 30 days, history of severe liver disease or coagulation disorder, uncontrolled cardiac or vascular disease, or previous therapy with oncolytic virus.

All patients gave written informed consent. The trial protocol and ethics were reviewed by the Finnish Medical Agency (FIMEA) and the Helsinki University Hospital (HUS) Ethics board (approval 49/2020 and statement HUS/1804/2020).

Production of TILT-123

- 5 TILT-123 was manufactured according to Good Manufacturing Practices in A549 cells. Prior to administration, TILT-123 was resuspended in 0.9% saline and administered in 1.0-5.0 mL volume for intratumoral injections and 10.0-40.0 mL for intravenous injections, depending on the dose cohort.

Treatment

- 10 Patients received multiple doses of TILT-123; an intravenous dose on day 1 and intratumoral doses on days 8, 22, 36, 50 and 64. Patients judged as possibly benefiting could continue to receive additional rounds of TILT-123 beyond the primary endpoint. The intravenous dose ranged from 3×10^9 to 4×10^{12} viral particles (VP) and the intratumoral dose ranged from 3×10^9 to 5×10^{11} VPs according to the dose-escalation scheme.
- 15 Intratumoral injections were performed with ultrasound guidance using a 21-gauge needle. At least one tumor was injected during intratumoral dosing, with the agent distributed evenly to multiple locations inside each injected tumor.

Assessment of anti-tumor efficacy, survival and progression-free survival

- Anti-tumor efficacy was assessed on day 78 with contrast-enhanced computer tomography (CT) imaging and positron emission tomography (PET) with ^{18}F -FDG. Maximum tumor diameters and SUVmax readings were obtained from the images by a specialized radiologist. Tumor responses were evaluated by RECIST 1.1, iRECIST, and PET-based criteria as determined in Koski et al. 2013. For individual lesion analysis, CT disease control was defined as lesion size increase less than 20%. For individual lesion PET analysis, metabolic
- 25 disease control was defined as lesion SUVmax increase less than 30%.

Survival data and progression-free survival data were retrieved from the electrical clinical trial system. Data cutoff for survival and progression-free survival was November 26th 2023. Patients not reported as deceased or progressing at data cutoff date were included in analysis as alive or non-progressed until data cutoff date.

Neutralizing antibody Assays analyses

Anti-adenovirus antibodies were measured by neutralizing antibody assay, described in more detail previously in Hemminki et al 2002, and titer of 1:64 was the lowest assayed titer.

5

Statistical analyses

For overall survival and grouped analyses, Mantell-Cox logrank test or MaxCombo test was used to compare groups, as reported in figure legends. For categorical analyses, Fisher's exact tests were used to compare groups. GraphPad Prism 9.4.1 and R-package 'nph' was used for statistical analyses.

Results

15 Efficacy

Anti-tumor efficacy was seen in both injected and non-injected tumors. In injected lesions, disease control was seen in 9/19 lesions by CT and 11/17 by PET (Figure 4A and 4B; including the patients discussed in example 1). Regarding non-injected lesions, disease control was seen in 9/13 lesions by CT and 11/14 lesions by PET (Figure 4C and 4D; including the patients discussed in example 1). Median survival of all patients enrolled in the trial was 124.5 days (Figure 4E; including the patients discussed in example 1). For patients showing disease control, the median survival was 213.5 days whilst for patients not showing disease control the median survival was 109 days ($p=0.165$; Figure 4E; including the patients discussed in example 1). The median progression-free survival (PFS) of all patients enrolled was 87.5 days (Figure 4F; including the patients discussed in example 1). For patients showing disease control at day 78, the median PFS was significantly longer at 181 days, compared to 65 days in patients not showing disease control at day 78 (Figure 4F; $p<0.0001$).

The median time to progression (TTP) for all patients was 97 days (Figure 4G; including the patients discussed in example 1). Patients showing disease control at day 78 had markedly longer median TTP at 518 days, compared to patients without disease control with median TTP of 83 days (Figure 4G, $p=0.0002$). Notably, 4 patients showed markedly long survival, with two patients alive more than 600 days after enrollment (Figure 4F).

30

Four patients surviving more than 1 year after enrollment included a patient with myxoid liposarcoma (20204), anaplastic thyroid carcinoma (20103; efficacy example #1 from example 1 results), leiomyosarcoma (20212) and nodular melanoma (20211). Notably, three
5 of the four patients were heavily pre-treated and resistant to other therapies. Patient 20204 with myxoid liposarcoma had received 10 previous cancer therapies consisting of neo-
adjuvant radiotherapy, 3 surgeries and 6 lines of chemotherapy. The patient completed the trial and received 4 additional rounds of TILT-123 intratumorally, before finally succumbing
10 to the disease 821 days after enrollment to the trial. The patient did not receive other cancer therapies after the trial, aside from palliative radiotherapy to a groin metastasis. Therefore, the patient lived for more than 600 days after the trial without any further systemic therapies administered.

Patient 20212 with leiomyosarcoma had received 6 regimens of previous cancer therapy
15 consisting of 2 radiotherapy regimens and 4 lines of chemotherapy. The patient completed the trial and was evaluated as progressive disease at day 78. The patient received palliative doxorubicin and pazopanib after the trial, and is alive at the time of the data cutoff, 654 days after enrollment.

20 Another patient with long survival was a patient with nodular melanoma (20211). The patient was markedly treatment resistant, having had 4 rounds of surgery, 2 lines of nivolumab, paclitaxel combined with carboplatin and an investigational immune checkpoint inhibitor BMS-986218 targeting CTLA-4. The patient was evaluated as progressive disease on day 78, and received palliative temozolomide and radiation therapy after the trial. The patient
25 survived 295 days after the last dose of TILT-123.

Immunostimulatory effects

At baseline, 9/20 patients had a low or no neutralizing antibody titer against TILT-123
30 (defined as titer less than 1:64), while 11/20 patients had detectable titers (Figure 5A; including the patients discussed in example 1). No patients had high titers at baseline, consistent with the lack of a 5/3 chimeric adenovirus in nature. The baseline level of neutralizing antibodies did not correlate with disease control (Figure 5B; including the

patients discussed in example 1), but patients with baseline neutralizing antibodies presented a tail of long-term survivors although not reaching significance due to small sample size ($p=0.258$, Figure 5C; including the patients discussed in example 1).

- 5 Additionally, the two best responders according to RECIST 1.1 (20103 and 20108) both developed the highest measurable titer of neutralization (Figure 6A; including efficacy example #1 from example 1 results). Similarly, both best PET responders and 2/4 longest survivors developed the highest measurable titer of neutralization (Figure 6B and 6C; including the patients discussed in example 1). Neutralizing antibody titers increased in all
- 10 dose cohorts across trial without clear association to dose (Figure 5D; including the patients discussed in example 1).

CITATIONS

15

Non-patent documents

Heise C et al., 2000, Nature Med 6, 1134-1139.

Kanerva A et al., 2005, Gene Therapy 12, 87-94.

- 20 Koski A et al., 2013, Hum Gene Ther 24(12), 1029-41.

Hemminki A et al., 2002, Hum Gene Ther 13(12), 1505-14.

Patent documents

25

WO2014170389

CLAIMS:

1. An oncolytic adenoviral vector coding for at least TNFalpha and/or IL-2 for
5 use in treatment of cancer,

wherein the adenoviral vector is to be administered to a subject in an active treatment cycle
at least three times, preferably at least 5, 6 or 7 times; and

wherein at least one, preferably the first, administration is a systemic administration, wherein
said active treatment cycle preferably does not include administration of an adoptive cell
10 therapeutic composition comprising TILs or administration of an immune checkpoint
inhibitor.

2. The oncolytic adenoviral vector for use according to claim 1, wherein said
systemic administration is given parenterally, preferably intravenously.

3. The oncolytic adenoviral vector for use according to claim 1 or 2, wherein
15 the second and subsequent administrations are given locally, preferably intratumorally,
intraperitoneally, and/or intrapleurally.

4. The oncolytic adenoviral vector for use according to any one of claims 1-3,
wherein a further administration of the adenoviral vector is to be administered within 10
days, preferably on the 8th day, after the onset of the treatment, i.e., the first administration of
20 said adenoviral vector. 5. The oncolytic adenoviral vector for use according to claim 4,
wherein a further administration of the adenoviral vector is to be administered within 20
days, preferably on the 15th day, after the onset of the treatment, and wherein the previous
administration was preferably administered within 10 days after the onset of the treatment.

6. The oncolytic adenoviral vector for use according to claim 4 or 5, wherein a
25 further administration of the adenoviral vector is to be administered within 32 days,
preferably on the 22nd or 29th day, after the onset of the treatment, and wherein the previous
administration was preferably administered within 10 or 20 days after the onset of the
treatment.

7. The oncolytic adenoviral vector for use according to claim 6, wherein a
30 further administration of the adenoviral vector is to be administered within 46 days,

preferably on the 36th or 43rd day, after the onset of the treatment, and wherein the previous administration was preferably administered within 32 days after the onset of the treatment.

8. The oncolytic adenoviral vector for use according to claim 7, wherein a further administration of the adenoviral vector is to be administered within 60 days,
5 preferably on the 50th or 57th day, after the onset of the treatment, and wherein the previous administration was preferably administered within 46 days after the onset of the treatment.

9. The oncolytic adenoviral vector for use according to claim 7 or 8, wherein a further administration of the adenoviral vector is to be administered within 67 days,
preferably on the 64th day, after the onset of the treatment, and wherein the previous
10 administration was preferably administered within 46 or 60 days after the onset of the treatment.

10. The oncolytic adenoviral vector for use according to claim 8, wherein a further administration of the adenoviral vector is to be administered within 81 days,
preferably on the 71st or 78th day, after the onset of the treatment, and wherein the previous
15 administration was preferably administered within 67 days after the onset of the treatment.

11. The oncolytic adenoviral vector for use according to any one of claims 4-10, wherein each administration is either intratumoral, intraperitoneal, or intrapleural.

12. The oncolytic adenoviral vector for use according to any one of claims 1-2, wherein the second administration of the adenoviral vector is to be administered within 2-6
20 hours after the onset of the treatment, i.e. the first administration of said adenoviral vector.

13. The oncolytic adenoviral vector for use according to claim 12, wherein two further administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 5 days, preferably on the 3rd day, after the onset of the treatment.

14. The oncolytic adenoviral vector for use according to claim 13, wherein two
25 further administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 10 days, preferably on the 8th day, after the onset of the treatment, and wherein the previous administrations were preferably administered within 5 days after the onset of the treatment.

15. The oncolytic adenoviral vector for use according to claim 14, wherein two
30 further administrations of the adenoviral vector are to be administered with a 2-6 hours

interval within 13 days, preferably on the 10th day, after the onset of the treatment, wherein the previous administrations were preferably administered within 8 or 10 days after the onset of the treatment.

16. The oncolytic adenoviral vector for use according to claim 15, wherein two
5 further administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 30 days, preferably on the 22nd day, after the onset of the treatment, wherein the previous administrations were preferably administered within 13 days after the onset of the treatment.

17. The oncolytic adenoviral vector for use according to claim 16, wherein two
10 further administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 46 days, preferably on the 43rd day, after the onset of the treatment, wherein the previous administrations were preferably administered within 30 days after the onset of the treatment.

18. The oncolytic adenoviral vector for use according to claim 17, wherein two
15 further administrations of the adenoviral vector are to be administered with a 2-6 hours interval within 67 days, preferably on the 64th day, after the onset of the treatment, wherein the previous administrations were preferably administered within 46 days after the onset of the treatment.

19. The oncolytic adenoviral vector for use according to any one of claims 12-
20 18, wherein all administrations during the treatment are systemic administrations, preferably given parenterally, more preferably intravenously.

20. The oncolytic adenoviral vector for use according to any of the preceding
claims, wherein in each administration said oncolytic adenoviral vector is administered in an amount of 1×10^9 - 1×10^{13} virus particles, preferably in an amount between 3×10^9 - 4×10^{12}
25 virus particles.

21. The oncolytic adenoviral vector for use according to any of the preceding
claims, wherein the cancer or tumor 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
30 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, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of
5 gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, esophagus cancer, gall bladder cancer, head cancer, eye cancer, neck cancer, Wilms' tumor, Kaposi's sarcoma, prostate cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, parathyroid cancer, penis cancer, pituitary cancer, soft
10 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, nerve cancer, palate cancer, parotid gland cancer, peritoneum cancer, pharynx
15 cancer, pleural cancer, salivary gland cancer, tongue cancer and tonsil cancer.

22. The oncolytic adenoviral vector for use according to claim 21, wherein said tumor is melanoma, lung cancer, head and neck cancer, ovarian cancer, or thyroid cancer.

23. The oncolytic adenoviral vector for use according to claim 21, wherein
20 said soft tissue sarcoma is preferably leiomyosarcoma or liposarcoma.

24. The oncolytic adenoviral vector for use according to any of claims 1-23, wherein the subject has failed at least one previous cancer treatment such as chemotherapy or radiotherapy treatment.

25. The oncolytic adenoviral vector for use according to any of the preceding claims, wherein said oncolytic adenoviral vector comprises an adenovirus serotype 5 (Ad5) backbone with the fiber knob of adenovirus serotype 3 (Ad3).

26. The oncolytic adenoviral vector for use according to claim 25, wherein said nucleic acid sequence encoding TNFalpha and/or IL-2 is in the place of a deleted nucleic acid sequence in the E3 region of said oncolytic adenoviral vector.

27. The oncolytic adenoviral vector for use according to claim 26, wherein the deletion of a nucleic acid sequence in the E3 region is a deletion of viral *gp19k* and *6.7k* reading frames.

28. The oncolytic adenoviral vector for use according to any of claims 25-27,
5 wherein the vector comprises a 24 bp deletion ($\Delta 24$) in the adenoviral E1 sequence of said oncolytic adenoviral vector.

29. The oncolytic adenoviral vector for use according to any of claims 1-28,
wherein said active treatment cycle is preceded with an immune system stimulation dose of an adenoviral vector in order to raise adenovirus neutralizing antibodies in the subject,
10 wherein said adenoviral vector preferably comprises an adenovirus serotype 5 (Ad5) backbone, wherein said vector is more preferably the same which is used in said active treatment cycle.

30. The oncolytic adenoviral vector for use according to any of claims 1-29,
wherein said active treatment cycle is carried out as a monotherapy treatment of cancer with
15 said adenoviral vector from the onset of the treatment cycle until the last administration of said adenoviral vector.

31. The oncolytic adenoviral vector for use according to any of claims 1-30,
wherein said subject who is treated has been selected for the treatment based on the level of adenovirus neutralizing antibodies present in said subject before the treatment or after
20 administration of an immune system stimulation dose of an adenoviral vector preceding the treatment.

32. Method of treating cancer in a subject, the method comprising a step of administered an oncolytic adenoviral vector coding for at least TNFalpha and/or IL-2 to a subject in an active treatment cycle at least three times, preferably at least 5, 6 or 7 times;
25 and

wherein at least one, preferably the first, administration is a systemic administration, wherein said active treatment cycle preferably does not include administration of an adoptive cell therapeutic composition comprising TILs or administration of an immune checkpoint inhibitor.

33. Use of an oncolytic adenoviral vector coding for at least TNFalpha and/or IL-2 in manufacturing a medicine to be used in an active treatment cycle at least three times, preferably at least 5, 6 or 7 times; and

5 wherein at least one, preferably the first, administration of said medicine is a systemic administration, wherein said active treatment cycle preferably does not include administration of an adoptive cell therapeutic composition comprising TILs or administration of an immune checkpoint inhibitor.

10 34. A method for selecting patients for a cancer treatment with an oncolytic adenoviral vector, the method comprising a step of providing a biological sample of a subject, measuring the level of adenovirus neutralizing antibodies in the sample, and selecting patients based on the level of presence of said adenovirus neutralizing antibodies in said sample.