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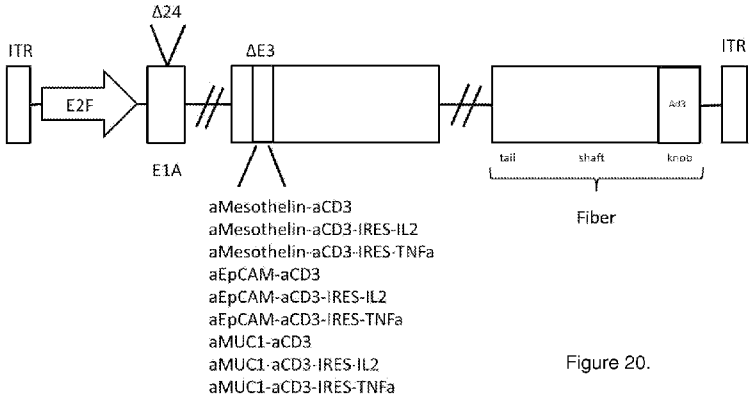

Figure 20.

(57) Abstract: The present invention relates to the fields of life sciences and medicine. Specifically, the invention relates to cancer therapies of humans. More specifically, the present invention relates to an oncolytic adenoviral vector encoding a bispecific monoclonal antibody. Furthermore, the present invention relates to methods and uses utilizing the oncolytic adenoviral vectors, also together with adoptive cell therapies.

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ONCOLYTIC ADENOVIRUSES CODING FOR BI-SPECIFIC ANTIBODIES AND METHODS AND USES RELATED THERETO

FIELD OF THE INVENTION

The present invention relates to the fields of life sciences and medicine. Specifically, the invention relates to cancer therapies of humans. More specifically, the present invention relates to an oncolytic adenoviral vector encoding a bispecific monoclonal antibody. Furthermore, the present invention relates to methods and uses utilizing the oncolytic adenoviral vectors, also together with adoptive cell therapies.

BACKGROUND OF THE INVENTION

Novel therapies are constantly developed for cancer treatment. Adoptive cell therapies (ACT) are a potent approach for treating cancer but also for treating other diseases such as infections and graft versus host disease. Adoptive cell transfer is the passive transplantation of ex vivo grown cells, most commonly immune-derived cells, into a host with the goal of transferring the immunologic functionality and characteristics of the transplant. Adoptive cell transfer can be autologous, as is common in adoptive T-cell therapies, or allogeneic as typical for treatment of infections or graft-versus-host disease. Clinically, common embodiments of this approach include transfer of either immune-promoting or tolerogenic cells such as lymphocytes to patients to either enhance immunity against viruses and cancer or to promote tolerance in the setting of autoimmune disease, such as type I diabetes or rheumatoid arthritis.

The adoptive transfer of autologous tumor infiltrating lymphocytes (TILs) or genetically re-directed peripheral blood mononuclear cells has been used to successfully treat patients with melanoma as well as patients with CD19-expressing hematologic malignancies. In ACT, the most commonly used cell types are T-cells, sometimes sorted for CD8+, but other variations include CD4+ cells, NK-cells, delta-gamma T-cells, regulatory T-cells and peripheral blood mononuclear cells. Cells can be unmodified such as in TIL therapy or genetically modified. In TIL therapy unsorted polyclonal cells are used. There are two common ways to achieve genetic targeting of T-cells to tumor specific targets. One is transfer of a T-cell receptor with known specificity (TCR therapy) and with matched human leukocyte antigen (HLA, known as major histocompatibility

complex in rodents) type. The other is modification of cells with artificial molecules such as chimeric antigen receptors (CAR). This approach is not dependent on HLA and is more flexible with regard to targeting cell surface molecules. For example, single chain antibodies can be used and CARs can also incorporate costimulatory domains. However, the targets of CAR cells need to be on the membrane of target cells, while TCR modifications can utilize intracellular targets. In TCR and CAR therapy, T-cells are obtained from peripheral blood of the patient.

Despite of the development of adoptive cell therapies, the clinical results of adoptive T-cell therapy on non-melanoma solid tumors, constituting more than 90% of human cancers, and 95% of cancer mortality, has been disappointing. The main reason for this is that the tumor microenvironment is highly immunosuppressive, 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. Currently there are no effective tools for resolving said issues.

T-cell engagers have been used for cancer treatment. The main classes are trifunctional antibody, chemically linked Fab and bi-specific T-cell engager (BiTE), the latter being most advanced clinically (Baeuerle PA, Reinhardt C. Cancer Res. 2009 Jun 15;69(12):4941-4). While several BiTEs have been studied preclinically, and two (blinatumomab, an anti-CD19 BiTE, and solitomab, and anti-EpCAM Bite) have been in clinical trials, a number of problems have emerged. A major issue is on-target-off-tumor toxicity, which has resulted in a high adverse event rate including a toxic death rate of 12% in blinatumomab clinical trials (Topp MS et al. 2011, J Clin Oncol. Jun 20;29(18):2493-8). Another issue is insufficient concentrations of the BiTE at the target (the tumor), which is especially problematic in the context of solid tumors whose bulk forms an obstacle to BiTE penetration and concentration. This probably explains why no formal responses (reductions in tumor size fulfilling RECIST criteria) have been seen in trials with solitomab. The best responses were transient stable disease which was achieved in 38% of patients (Walter M et al. 2012, J Clin Oncol 30, (suppl; abstr 2504)). Still a further problem with BiTEs is the short half-life in humans, which has necessitated continuous infusion, which is not a practical solution for routine use.

Oncolytic viral vectors armed with a T-cell engager have been suggested for cancer treatment. **WO 2014138314 A1** (PCT/US2014/020935) and

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.

BRIEF DESCRIPTION OF THE INVENTION

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

The present invention 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 invention 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 invention indicates that adenovirus can induce danger signals in tumors of mice and in humans, as exemplified by interferon gamma production (Figure 2), which leads ro 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).

Of note, we have human data showing that TIM3 expression, and the ability of oncolytic adenovirus to downregulate TIM3, correlates with patient survival. This is potent data indicating that the danger signaling caused by adenovirus results in down-regulation of tumor immunosuppression, which correlates with clinical benefits in patients (Figure 10). Importantly, not all oncolytic viruses are alike, and in fact vaccinia virus is not able to produce danger signals in tumors, and is therefore not comparable with adenovirus for tumor immunotherapy via local production of BiTE (Figure 5-6).

Issues of systemic toxicity and poor local efficacy as well as the short half-life of BiTEs in humans are resolved by the present invention, namely by local production of the BiTE by an adenoviral vector at the tumor, a feature which is advantageous especially in the context of solid tumors (Figure 9).

Also, the present invention resolves the problem of poor secretion of single-chain BiTE molecules in a surprising manner: when using an oncolytic adenovirus, which replicates only in tumor cells, and the last step of replication is lysis of the cell, the BiTE is released into the tumor microenvironment (Figure 8). In other words, the present invention resolves the problem of BiTE secretion in a surprising manner, by utilizing oncolysis as the release device. According to the present invention secretion of BiTEs is not required, and in fact not preferred as a further approach is for restricting BiTE expression to the tumor (only tumor cells are lysed by the virus).

Production of the BiTE at the tumor can recruit the adoptive T-cell graft to the tumor (Figure 1). Binding to the cell surface molecule receptor (e.g. CD3 receptor) activates cells of the graft at the tumor. Moreover, adenoviral oncolysis causes danger signals which counteract tumor immunosuppression. Together, these components achieve an anti-immunosuppressive effect which could not be achieved with any component alone. Of note, adenovirus is unique among oncolytic viruses with regard to its ability to induce anti-immunosuppressive danger signals, through binding to pathogen associated pattern recognition receptors. Moreover, adenovirus has outstanding effects on T-cells, while many other oncolytic viruses such as vaccinia virus are rather stealthy in this regard. In other words, vaccinia cannot be used for enhancing adoptive cell therapy. Finally, the present specification represents data showing that vaccinia is not a good platform for enhancing adaptive cell therapy, while adenovirus is the optimal device for counteracting tumor immunosuppression.

Anti-viral immunity has been considered restrictive for virotherapy approaches including oncolytic adenoviruses. One embodiment of anti-viral immunity is anti-viral T-cells. However, the present invention 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 embodiment, the present invention 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 invention coding for T-cell engagers may be combined with adoptive cell therapeutics for cancer treatment.

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

The present invention 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.

The present invention also 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,

wherein the bispecific monoclonal antibody comprises a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen.

Also, the present invention 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 invention 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.

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

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

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

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

Still, the present invention 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 invention 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.

Also, the present invention relates to a use of an oncolytic adenoviral vector of the present invention in the manufacture of a medicament for treating cancer in a subject.

Also, the present invention relates to a use of an oncolytic adenoviral

vector of the invention in the manufacture of a medicament for increasing the efficacy of adoptive cell therapy in a subject.

The advantages of the arrangements of the present invention 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

poor tumor-trafficking of T-cells highlights the shortcomings of oncolytic adenovirus and adoptive T-cell therapies used as single agents, supporting the invention to enhance the trafficking of adoptively transferred T-cells by BiTe-expressing oncolytic adenovirus.

Figure 5 reveals that adenovirus is superior to vaccinia in inducing cellular anti-tumor immunity; a critical feature for enhancing adoptive cell therapy. Comparison between adenovirus (Ad) and vaccinia virus (VV) immunogenicity. Levels of splenic and B16.OVA tumor-infiltrating CD8+ T-cells were higher in 5/3 chimeric adenovirus treated mice compared to mice treated with double-deleted oncolytic Western reserve vaccinia virus (this strain was used by Yu et al Mol Ther 2014). Thus, oncolytic adenovirus appears to be an ideal expression platform for BiTe due to its inherent immunogenicity, especially in context of adoptive T-cell therapy.

Figure 6 shows that adenovirus is more effective than vaccinia in inducing anti-tumor immunity. Mice bearing syngeneic B16.OVA tumors were injected intratumorally with PBS, adenovirus or vaccinia virus. Tumor cell samples were stained with pentamer-APC detecting T-cell receptors specific for SIINFEKL residues of ovalbumin and assessed by flow cytometry (n=3). Data indicates change in anti-tumor T-cells following adenovirus or vaccinia virus injection; adenovirus is much more effective in inducing anti-tumor immunity while vaccinia was in fact immune suppressive in the context of anti-tumor T-cells.

Figure 7 reveals that BiTE delivered by oncolytic adenovirus targets all classes of T-cells against tumors, including anti-viral T-cells. In many patients, anti-viral T-cells are much more numerous than anti-tumor T-cells (Kanerva A et al. Clin Cancer Res. 2013 May 15;19(10):2734-44). They are generally considered counterproductive in the context of tumor therapy, because a) they consume a major part of a finite amount of immune response available, and b) they can limit replication of the oncolytic virus. In contrast, our invention surprisingly takes advantage of pre-existing and induced anti-adenoviral T-cell immunity as anti-viral T-cell are targeted towards tumors (Figure 7). As TILs of adenovirus-treated tumors contain both anti-tumor and anti-viral T-cells, CD3-scFV of BiTe will activate these T-cells regardless of their endogenous specificity (MHC I-independently). Consequently, tumor-specific killing by these T-cells is achieved by scFV specific for tumor cell surface antigen (such as mesothelin, EpCAM1, MUC1) and no off-tumor/off-target reactivity is expected to be seen.

Thus, this approach re-directs all CD8+ TILs (=anti-tumoral and anti-viral) into anti-tumor T-cells via binding of virus-produced BiTe.

Figure 8 shows that oncolytic adenovirus, but not non-replicating adenovirus, coding for functional antibody results in efficient antibody production and release from cancer cells. Cells were infected with indicated adenoviruses at 100 virus particles (VP)/cell, and several days later analyzed for antibody expression by human IgG ELISA (A) or Western blot (B). At each indicated time point after infection, (A) oncolytic virus Ad5/3-OV-Ab (grey and black bars) showed high production of functional antibody from ovarian cancer SKOV-3 cells: Antibody levels decreased in cell lysate (LYS) during progressive infection and cancer cell killing, and showed significant accumulation in the supernatant (SN). In contrast, non-replicating virus Ad5/3-Ab failed to produce detectable antibody in the supernatant, even though cell lysate showed evidence of antibody at day 7 post-infection (white bars). Of note, non-replicating Ad5/3-Ab virus treated cells were viable throughout the experiment, indicating the lack of active antibody secretion by cancer cells. (B) Supernatant of breast cancer BT-474 cells (left) and human embryonic 293 cells (right) was analyzed by Western blot 6 days after infection with indicated viruses. Under reducing conditions, heavy-chain (HC), light-chain (LC), and the full-length antibody produced by the oncolytic virus Ad5/3-OV-Ab were visualized in supernatant of both cell lines, whereas non-replicating Ad5-Ab and Ad5/3-Ab viruses failed to show antibody release from BT-474 cells that do not allow their replication. To confirm antibody expression by the non-replicating viruses, we used human embryonic 293 cells (right), which allow replication of also E1A-deleted adenoviruses, followed by cell lysis and release of the antibody, readily detected by Western blot. A non-replicating control virus Ad5/3-Luc coding for luciferase was used as a negative control. HC and LC were detected using polyclonal goat anti-human IgG and donkey anti-goat IgG-HRP antibodies, respectively. The antibody affinity was lower to the LC than to the HC resulting in a weaker signal. Bars represent the mean ± SEM. **, $P < 0.01$; *, $P < 0.05$; all Student's T tests.

Figure 9 shows that oncolytic adenovirus coding for antibody shows higher intratumoral while lower systemic antibody levels than after systemic antibody treatment. Subcutaneous N87 gastric cancer xenograft bearing nude/NMRI mice (n = 5 per group) were treated with intratumoral injections of oncolytic Ad5/3-OV-Ab virus (2×10^8 VP/tumor) or intraperitoneal injections of commercial antibody (Ab; 0.3 µg/g) on days 0, 4, 8, and 15. Health of the animals

was monitored and tumors and blood samples were collected from mice sacrificed on days 32 and 40 (systemic Ab), day 46 (systemic Ab and Ad5/3-OV-Ab virus), and day 50 (Ad5/3-OV-Ab virus). **A**) Endpoint tumors and blood samples were measured by human IgG ELISA to assess the antibody concentration: Ad5/3-OV-Ab treated mice sacrificed on days 46 and 50 post-treatment showed still significantly higher antibody concentrations in tumors ($P < 0.001$, left), while presenting much lower circulating levels ($P < 0.001$, right), as compared to systemic Ab treated mice that were sacrificed earlier on days 32, 40 and 46. **B**) Antibody levels in tumor and blood samples of each individual animal were compared to assess the antibody distribution. The average ratio of antibody in tumor *versus* blood was above 1.0 in mice treated with Ad5/3-OV-Ab virus, whereas systemic Ab treatment resulted in very low ratio of less than 0.01. Thus, treatment with antibody expressing oncolytic virus can achieve improved intratumoral antibody concentration, while significantly reducing systemic exposure in animals. Notably, most of the virus-treated mice survived longer (up to 50 days) and therefore showed evidence of sustained local antibody production. Error bars represent the mean + SEM. **, $P < 0.01$, Student's T test.

Figure 10 shows that expression of T-cell exhaustion marker and immunosuppressive receptor TIM3 decreases after oncolytic adenovirus treatment and correlates with improved survival. 15 patients with advanced solid tumors were treated with oncolytic adenoviruses in the context of an Advanced Therapy Access Program. Baseline and post-treatment tumor biopsies were analyzed by RNA microarray (HumanHT-12 v4 Expression BeadChips array, Illumina), and gene expression levels were compared to identify differentially expressed genes. T-cell immunoglobulin mucin-3 (TIM3), which is an exhaustion marker and negative regulator of both innate and adaptive immune responses in tumors, was among the top differentially expressed genes: TIM3 showed major downregulation in 5 patients (change over 1.0, Δ[log2]) and minor decrease in 4 patients (average change of 0.38, Δ[log2]). Meanwhile, 6 patients failed to show downregulation of TIM3, out of which two patients showed upregulation post-treatment. When overall survival was compared between these groups, the patients with TIM3 downregulation ($n = 9$) showed significantly improved survival ($P = 0.004$, Log-rank test) over the patients with “TIM3 no change / upregulation” ($n = 6$). Median survival was 204 days and 64 days in TIM3 down- and upregulation groups, respectively. Thus, two-thirds of oncolytic adenovirus treatments

seemed to result in decrease of immunosuppressive receptor and exhaustion marker TIM3, which strongly correlated with prolonged overall survival.

Figure 11 shows improved in vitro cell killing with TIL and oncolytic adenovirus combination. HapT1 cells were infected with oncolytic adenovirus (100 VP/cell) for 3 days before adding HapT1 TIL. Target cell viability was determined 24 hours after TIL addition. Error bars, SE. ****p<0.0001. The best killing was seen when T-cells were stimulated with an oncolytic adenovirus.

Figure 12 show that in the absence of BiTe molecules, TILs extracted from HapT1 tumors don't have an additive effect on target cell killing when combined with oncolytic adenoviruses. HapT1 cells were plated on 96 well plate and incubated five days with oncolytic adenovirus Ad5/3-E2F-d24 only or armed with human IL-2. TILs extracted from established HapT1 tumors were added to cells 10:1 24 h before measuring the viability of the cells with MTS assay. Synergy was not observed between viruses and TILs.

Figures 13 (A and B) reveal in vitro lytic activity of Ad 5/3-E2F-d24-E3 virus in combination with human CD3 specific EpCAM targeted BiTE (Anti-human EpCam, Cat#CABT-33295MH) and PBMCs against colon carcinoma cell line SW480. Figure 13A: a) SW480 tumor cells were infected with increasing VPs (0,01, 0,1 , 1 , 10, 100, 1000 VP) of Ad 5/3-E2F-d24-E3 virus and with 10ng of BiTE. Effector cells (PBMCs) were added at an effector to target ratio of 5:1. MTS assay was used to determine the cell viability at 48 hours post infection. Error bars indicate SEM of triplicate measurements. Virus + Cells Vs Virus + PBMCs *P = 0.0184, Virus + Cells Vs Virus + PBMCs + BiTE *** P = 0.001. Figure 13B: a) SW480 tumor cells were infected with 1000 VP of Ad 5/3-E2F-d24-E3 virus and with 10ng of BiTE. Effector cells (PBMCs) were added at an effector to target ratio of 5:1. MTS assay was used to determine the cell viability at 48 hours post infection. Error bars indicate SEM of triplicate measurements. Virus + Cells Vs Virus + PBMCs *P =0.0184, Virus + Cells Vs Virus + BiTE + PBMCs ***P = 0.001.

Figure 14 shows that adenovirus or adenovirus armed with IL2 is not enough to accumulate T-cells at tumors. Adenovirus treatment combined with adoptive T-cell transfer results in suboptimal T-cell infiltration into B16.OVA melanoma tumors. Tumors collected 18 days after treatment start were flow cytometrically analyzed for ovalbumin-specific CD8+ T-cells (OVA) and gp100-specific CD8+ T-cells. OVA and gp100 are epitopes expressed on melanoma

cells. Differences between different treatment groups were not statistically significant, and not different from T-cell therapy alone (no virus). Horizontal lines, mean values.

Figure 15 reveals cytotoxic T cells in hamster pancreatic tumors. Oncolytic adenoviruses are unable to recruit cytotoxic CD8+ T cells to tumors. Subcutaneous hamster pancreatic tumors (HapT1) were treated with oncolytic adenoviruses Ad5/3-E2F-d24 alone or armed with human IL-2 five times in total during 19 days. On day 25 the animals were sacrificed and tumor cells labeled with cross-reactive anti-rat CD8b PE antibody. (Sample numbers: mock and unarmed n=5, IL2 n=1). Oncolytic adenovirus alone was not able to recruit Cd8 cells to the tumor. IL2 seemed more promising but the increase was not significant.

Figure 16 shows results of rechallenge in immunocompetent hamsters. Hamsters previously cured with an unarmed oncolytic adenovirus Ad5/3-E2F-d24 or with adenovirus armed with a cytokine (TNFα, IL-2 or both) treatment resisted same tumor type (HapT1) but not different one (DDT1-MF2). Naïve animals which had not encountered either of the cell lines previously were used as a control. Arming the virus with a molecule able to induce anti-tumor immunity (for example BITe) is necessary for inducing protective immunity (=a sign of memory response against tumor epitopes).

Figure 17 shows in vivo efficacy of armed or unarmed oncolytic adenovirus, with or without T-cell therapy. Established HapT1 tumors were injected intratumorally with oncolytic adenovirus Ad5/3-E2F-d24 (1×10^7 VP/tumor) on Days 1 and 8. On Day 2, HapT1 tumor infiltrating lymphocytes grown ex vivo (1.5×10^6 TIL/tumor) were administered intratumorally. Error bars, SE. *$p<0.05$, **$p<0.01$. The best anti-tumor efficacy was seen when tumors were treated with an oncolytic virus and TILs were also given.

Figure 18 shows hypothetical results from *in vivo* antitumor efficacy experiment combining Ad-BiTE and OT1 T-cell transfer in immunocompetent mice bearing B16-OVA tumors. Subcutaneously implanted B16-OVA tumors (0.25 x 10e6 cells/tumor) will be treated with a single intraperitoneal injection of CD8-enriched OT1 T-cells, intratumoral injection of Ad-BiTE (1 x 10e9 VP/tumor) or both. Virus injections will be repeated every 7 days.

Figure 19 shows that adenoviral delivery of cytokines IL2 and TNFa enhance efficacy of adoptive cell therapy, providing the rationale for including cytokines in oncolytic adenovirus coding for BiTE. B16-OVA tumor-bearing C57

mice were treated intratumorally with 1 x 10e9 viral particles of armed adenoviruses and intraperitoneally with 1.5 x 10e6 CD8-enriched OT-1 T-cells on Day 1. Virus treatments continued every 7 days.

Figure 20 shows construct design of the present invention.

Figure 21 shows a construct map of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

Viral vectors

The oncolytic adenoviral vectors used in the present invention can be any adenoviral vectors suitable for treating a human or animal. 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.

In one embodiment of the invention, the adenoviral vectors are vectors of human viruses. In one embodiment the adenoviral vectors are selected from the group consisting of Ad5, Ad3 and Ad5/3 vectors. As used herein, expression "adenovirus serotype 5 (Ad5) nucleic acid backbone" refers to the genome of Ad5. Similarly "adenovirus serotype 3 (Ad3) nucleic acid backbone" refers to the genome of Ad3. "Ad5/3 vector" refers to a chimeric vector comprising or having parts of both Ad5 and Ad3 vectors. In a specific embodiment a backbone of the adenoviral vector is an adenovirus serotype 5 (Ad5) or serotype 3 (Ad3) nucleic acid backbone with specific mutations. E.g. fiber areas of the vector can be modified. In one embodiment the backbone is Ad5 nucleic acid backbone further comprising an Ad3 fiber knob. In other words the construct has the fiber knob from Ad3 while the remainder or the most of the remainder of the genome is from Ad5. (See e.g. figure 20)

The adenoviral 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, 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.

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. 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 pRb and E1A requires amino acids 121 to 127 of the E1A protein conserved region, which are deleted in the present invention. The vector of the present invention comprises a deletion of nucleotides corresponding to amino acids 122-129 of the vector according to Heise C. et al. (2000, Nature Med 6, 1134-1139). 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. In one embodiment of the invention the vector comprises a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1 (See figure 20)

It is also possible to replace E1A endogenous viral promoter for example by a tumor specific promoter. In a specific embodiment of the invention e.g. E2F1 (e.g. in Ad5 based vector) or hTERT (e.g. in Ad3 based vector) promoter is utilized in the place of E1A endogenous viral promoter. On one embodiment the vector comprises E2F1 promoter for tumor specific expression of E1A.

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. In one embodiment of the invention the deletion of a nucleic acid sequence in the E3 region of the oncolytic adenoviral vector is a deletion of viral *gp19k* and *6.7k* reading frames. 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, Gene Therapy 12, 87-94). 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. (See figure 20)

Both of deletions gp19k and 6.7K provide a surprising advantage with regard to a specific embodiment of the invention. Since we are attempting to regain expression of HLA/MHC for presentation of tumor epitopes to the 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 one specific 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.

In one embodiment of the invention, one or more 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. In a specific embodiment a nucleic acid sequence encoding a bipartite molecule comprising a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen is inserted into the place of the deleted nucleic acid sequence of viral *gp19k* and *6.7k* reading frames. In another embodiment of the invention E3 gp19k/6.7k is kept in the vector but one or many other E3 areas have been deleted (e.g. E3 9-kDa, E3 10.2 kDa, E3 15.2 kDa and/or E3 15.3 kDa).

E3 promoter may be any exogenous (e.g. CMV or E2F promoter) 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 of critical importance to regulate also E1 expression to restrict E3 promoter mediated transgene expression to tumor cells.

Specific embodiments of the invention include oncolytic adenoviral vectors (e.g. Ad5 or Ad3 vectors) whose replication is restricted to the p16/Rb pathway by dual selectivity devices: an E2F (e.g. E2F1) tumor specific promoter placed in front of the adenoviral E1A gene which has been mutated in constant region 2, so that the resulting E1A protein is unable to bind Rb in cells. Furthermore, the fiber is modified by 5/3 chimerism to allow efficient entry into tumor cell. And still, the BiTE transgene, optionally with other transgenes, is placed into the E3 region, which has been deleted for gp19k and 6.7k open reading frames. This arming approach links transgene expression to virus replication without the need for heterologous promoters. L(left)- and/or R(right)-ITR sequences may also be comprised in the vector in specific embodiments. The inverted terminal repeat (ITR) sequences enable efficient multiplication of the viral genome and give ability to form a hairpin among other properties.

In a specific embodiment of the invention the oncolytic adenoviral vector comprises:

1) E2F1 promoter for tumor specific expression of E1A

2) a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1;

3) a nucleic acid sequence deletion of viral *gp19k* and *6.7k* reading frames; and

4) a nucleic acid sequence encoding a bipartite molecule comprising a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen in the place of the deleted nucleic acid sequence as defined in point 3). (See figure 20)

A bispecific monoclonal antibody (BsMAb, BsAb) is an artificial protein that is composed of fragments of two different monoclonal antibodies and consequently is able to bind two different types of antigens. In other words, bispecific antibodies combine two or more antigen-recognizing elements into a single construct, which is able to bind to two or more targets.

Examples of bispecific monoclonal antibodies include BsMAbs, which are engineered to simultaneously bind to a cytotoxic cell (using a receptor such as CD3) and a target like a tumor cell to be destroyed. First-generation BsMAb, called trifunctional antibody, has been developed. It consists of two heavy and two light chains, one each from two different antibodies. The two Fab regions (the arms) are directed against two antigens. The Fc region (the foot) is made up from the two heavy chains and forms the third binding site; hence the name. Other types of bispecific antibodies include chemically linked Fabs, consisting only of the Fab regions, and various types of bivalent and trivalent single-chain variable fragments (scFvs) (i.e. fusion proteins mimicking the variable domains of two antibodies). In a specific embodiment of the invention, the bispecific monoclonal antibody is selected from the group consisting of trifunctional antibodies and bivalent and trivalent single-chain variable fragments (scFvs). In one embodiment of the invention the bispecific monoclonal antibody is a bivalent single-chain variable fragment. The group of bivalent single-chain variable fragments comprises bi-specific T-cell engagers (BiTEs) and mAb2's (i.e. antibodies engineered to contain an Fcab antigen-binding fragment instead of the Fc constant region).

Bi-specific T-cell engagers (BiTEs) are a class of artificial bispecific monoclonal antibodies. They direct a host's immune system, more specifically

the T cells' cytotoxic activity, against cancer cells. BiTEs are fusion proteins consisting of two single-chain variable fragments (scFvs) of different antibodies, or amino acid sequences from four different genes, on a single peptide chain of about 55 kilodaltons. One of the scFvs binds to T cells via a cell surface molecule (e.g. the CD3 receptor), and the other to a tumor cell via a tumor specific molecule.

In a specific embodiment the bispecific monoclonal antibody is a bipartite molecule comprising a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen. As used herein "specific for a cell surface molecule" refers to an ability to bind a specific type cell surface molecule. Also as used herein "specific for a tumor antigen" refers to an ability to bind a specific type tumor antigen.

In one embodiment of the invention the cell surface molecule is on immunological effector cells. As used herein "an immunological effector cell" refers to a cell selected from the group consisting of T-cells, CD8+ cells, CD4+ cells, NK-cells, delta-gamma T-cells, regulatory T-cells, and peripheral blood mononuclear cells. In a specific embodiment, the effector cells are T-cells i.e. T lymphocytes. In one embodiment the cell surface molecule may be selected from CD3, CD8 and CD4.

In one emboidment the tumor antigen is selected from Table 1 or from the group consisting of mesothelin, EpCAM1 and MUC1.

In one embodiment the cell surface molecule is CD3 and the tumor antigen is selected from Table 1 or from mesothelin, EpCAM1 or MUC1. In another embodiment the cell surface molecule is CD8 and the tumor antigen is selected from Table 1 or from mesothelin, EpCAM1 or MUC1. In further embodiment the cell surface molecule is CD4 and the tumor antigen is selected from Table 1 or from mesothelin, EpCAM1 or MUC1. In a very specific embodiment, the tumor antigen is mesothelin and the cell surface molecule is CD3; 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. Indeed, regarding the BiTe transgenes, specific examples include anti-mesothelin-linker-anti-CD3, anti-EpCAM1-linker-anti-CD3 and anti-MUC1-linker-anti-CD3.

Table 1. Examples of tumor antigens suitable for the present invention (http://cvc.dfci.harvard.edu/cvccgi/tadb/nomenclature.pl).

Antigen Name

ERBB2	SSX2	KRAS	TERT
BIRC5	SSX4	PRAME	MGAT5
CEACAM5	KRAS	NRAS	CEL
WDR46	PRAME	ACTN4	F4.2
BAGE	NRAS	CTNNB1	CAN
CSAG2	ACTN4	CASP8	ETV6
DCT	CTNNB1	CDC27	BIRC7
MAGED4	CASP8	CDK4	CSF1
GAGE1	CDC27	EEF2	OGT
GAGE2	CDK4	FN1	MUC1
GAGE3	EEF2	HSPA1B	MUC2
GAGE4	FN1	LPGAT1	MUM1
GAGE5	HSPA1B	ME1	CTAG1
GAGE6	LPGAT1	HHAT	CTAG2
GAGE7	ME1	TRAPPC1	CAMEL
GAGE8	HHAT	MUM3	MRPL28
IL13RA2	TRAPPC1	MYO1B	FOLH1
MAGEA1	MUM3	PAPOLG	RAGE
MAGEA2	MYO1B	OS9	SFMBT1
MAGEA3	PAPOLG	PTPRK	KAAG1
MAGEA4	OS9	TPI1	SART1
MAGEA6	PTPRK	ADFP	TSPYL1
MAGEA9	TPI1	AFP	SART3
MAGEA10	ADFP	AIM2	SOX10
MAGEA12	AFP	ANXA2	TRG
MAGEB1	AIM2	ART4	WT1
MAGEB2	ANXA2	CLCA2	TACSTD1 (EPCAM)
MAGEC2	ART4	CPSF1	SILV
TP53	CLCA2	PPIB	SCGB2A2
TYR	CPSF1	EPHA2	MC1R
TYRP1	PPIB	EPHA3	MLANA
SAGE1	SSX2	FGF5	GPR143
SYCP1	SSX4	CA9	OCA2
KLK3	UBXD5	SIRT2	SPA17
SUPT7L	EFTUD2	SNRPD1	KLK4
ARTC1	GPNMB	HERV-K-MEL	ANKRD30A
BRAF	NFYC	CXorf61	RAB38
CASP5	PRDX5	CCDC110	CCND1
CDKN2A	ZUBR1	VENTXP1	CYP1B1
MDM2	NPM1	LRP1	CCNB1

MMP2	ALK	ADAM17	PAX3-FKHR
ZNF395	PML1	JUP	PAX3
RNF43	RARA	DDR1	FOXO1
SCRN1	SYT	ITPR2	XBP1
STEAP1	SSX1	HMOX1	SYND1
707-AP	MSLN	TPM4	ETV5
TGFBR2	UBE2V1	BAAT	HSPA1A
PXDNL	HNRPL	DNAJC8	HMHA1
AKAP13	WHSC2	TAPBP	TRIM68
PRTN3	EIF4EBP1	LGALS3BP	
PSCA	WNK2	PAGE4	
RHAMM	OAS3	PAK2	
ACPP	BCL-2	CDKN1A	
ACRBP	MCL1	PTHLH	
LCK	CTSH	SOX2	
RCVRN	ABCC3	SOX11	
RPS2	BST2	TRPM8	
RPL10A	MFGE8	TYMS	
SLC45A3	TPBG	ATIC	
BCL2L1	FMOD	PGK1	
DKK1	XAGE1	SOX4	
ENAH	RPSA	TOR3A	
CSPG4	COTL1	TRGC2	
RGS5	CALR3	BTBD2	
BCR	PA2G4	SLBP	
BCR-ABL	EZH2	EGFR	
ABL-BCR	FMNL1	IER3	
DEK	HPSE	TTK	
DEK-CAN	APC	LY6K	
ETV6-AML1	UBE2A	IGF2BP3	
LDLR-FUT	BCAP31	GPC3	
NPM1-ALK1	TOP2A	SLC35A4	
PML-RARA	TOP2B	HSMD	
SYT-SSX1	ITGB8	H3F3A	
SYT-SSX2	RPA1	ALDH1A1	
FLT3	ABI2	MFI2	
ABL1	CCNI	MMP14	
AML1	CDC2	SDCBP	
LDLR	SEPT2	PARP12	
FUT1	STAT1	MET	

In one embodiment the vector of the invention encodes a bispecific monoclonal antibody but also may comprise other transgenes. In a specific embodiment the oncolytic adenoviral vector codes for two or more transgenes. Particular embodiments of the present invention include adenoviral vectors encoding bispecific T-cell engager and at least one cytokine. Cytokines used in the present invention can be selected from any known cytokines in the art. In a specific embodiment of the invention the cytokine is IL-2, TNFalpha or CD40L. Indeed, in addition to a bispecific monoclonal antibody the oncolytic adenoviral vector may further comprise e.g. IL-2, TNFalpha and/or CD40L transgene(s).

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.

Furthermore, the combination of adenoviral vectors encoding both a BiTE and at least one cytokine, with adoptive cell therapeutics provides more effective results on wider targets than could have been assumed.

The other cytokines function by attracting and activating the T cells and reducing tumor immunosuppression, while IL-2 induces the propagation of the T-cell graft. Thus, IL-2 is produced locally at the tumor where it is needed, instead of injected systemically as is typically done in T-cell therapy, which can cause side effects, and therefore a major problem of the prior art therapies (i.e. toxicity of systemic IL-2) can be prevented by this embodiment. Indeed, severe adverse events, even deaths are prevented, because separate addition of IL2 used in the prior art methods to propagate and sustain transferred cells after transferring them into a patient is not needed if the virus produces it while replicating in the tumor. Local production at the tumor can also enhance the sought-after effects of IL-2 (stimulation and propagation of the graft) while reducing systemic exposure which is the cause of adverse events. The present invention provides selective treatments, with less toxicity or damage to healthy tissues.

The danger signaling provided by replication of the oncolytic virus, and activation of pathogen associated molecular pattern recognition receptors by viral DNA, together with the action of the transgene(s) may reduce tumor

immunosuppression to such degree that preconditioning therapy can be omitted. Consequently, and major issue in prior art, toxicity due to preconditioning chemotherapy and radiation can be avoided.

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 transgenes, such as a bispecific monoclonal antibody and any cytokine. 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). IRES may be used for example in the following places in adenoviral constructs (Figure 20): aMesothelin-aCD3-IRES-IL2 (see SEQ ID NOs: 1, 2, 3, 5, 6, 9); aMesothelin-aCD3-IRES-TNFa (see SEQ ID NOs: 1, 2, 3, 5, 6, 7); aEpCAM-aCD3-IRES-IL2 (see SEQ ID NOs: 1, 2, 3, 4, 5, 6); aEpCAM-aCD3-IRES-TNFa (see SEQ ID NOs: 1, 2, 3, 4, 5, 7); aMUC1-aCD3-IRES-IL2 (see SEQ ID NOs: 1, 2, 3, 5, 6, 8); aMUC1-aCD3-IRES-TNFa (see SEQ ID NOs: 1, 2, 3, 5, 7, 8). Nucleotide sequences are from the adenoviral constructs of the invention and are presented in Table 2.

Schematics of the general layouts of the virus genomes, which may be used, for example, in the present invention, are shown in Figure 20 (Ad5/3-E2F-D24-transgene). Nucleotide sequences of the viral vectors comprising transgenes aMesothelin-aCD3 (e.g. aMesothelin-aCD3-IRES-IL2 see SEQ ID NOs: 1, 2, 3, 5, 6, 9; aMesothelin-aCD3-IRES-TNFa see SEQ ID NOs: 1, 2, 3, 5, 6, 7), aEpCAM-aCD3 (e.g. aEpCAM-aCD3-IRES-IL2 see SEQ ID NOs: 1, 2, 3, 4, 5, 6; aEpCAM-aCD3-IRES-TNFa see SEQ ID NOs: 1, 2, 3, 4, 5, 7), aMUC1-aCD3 (e.g. aMUC1-aCD3-IRES-IL2 see SEQ ID NOs: 1, 2, 3, 5, 6, 8; aMUC1-aCD3-IRES-TNFa see SEQ ID NOs: 1, 2, 3, 5, 7, 8) were constructed according to the sequences listed in Table 2. General methods for constructing adenoviral vectors are well known to a person skilled in the art and are described e.g. in Koski et al. 2010, Hemminki et al. 2015. These methods may also be utilized for constructing adenoviral vetors of the present invention.

In addition to other advantages described above, further 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 (both the adoptive T-cell graft and 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.

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.

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 and they can also be utilized in the present invention.

Adoptive cell therapy

One approach of the present invention is the development of a treatment for patients with cancer using the transfer of immune lymphocytes that are capable of reacting with and destroying the cancer. Isolated tumor infiltrating lymphocytes are grown in culture to large numbers and infused into the patient. In the present invention adenoviral vectors encoding at least a bispecific monoclonal antibody may be utilized for increasing the effect of lymphocytes. As used herein “increasing the efficacy of adoptive cell therapy” refers to a situation, wherein the adenoviral vector of the invention is able to cause a stronger therapeutic effect in a subject when used together with an adoptive cell therapeutic composition compared to the therapeutic effect of the adoptive cell therapeutic composition alone. Figure 1 refers to the mechanism of increasing the efficacy by illustrating T-cell therapy with oncolytic adenovirus coding for bi-specific T-cell engager BiTE. A specific embodiment of the invention is a method of treating cancer in a subject, wherein the method comprises administration of an oncolytic adenoviral vector of the invention to a subject, said method further comprising administration of adoptive cell therapeutic composition to the subject. Adoptive cell therapeutic composition and the vectors of the invention are administered

separately. Separate administrations of an adoptive cell therapeutic composition and adenoviral vectors may be preceded by myeloablating or non-myeloablating preconditioning chemotherapy and/or radiation. The adoptive cell therapy treatment is intended to reduce or eliminate cancer in the patient.

A specific embodiment of the invention relates to therapies with adenoviral vectors and an adoptive cell therapeutic composition, e.g. tumor infiltrating lymphocytes, TCR modified lymphocytes or CAR modified lymphocytes. T-cell therapies in particular, but also any other adoptive therapies such as NK cell therapies or other cell therapies may be utilized in the present invention. Indeed, according to the present invention the adoptive cell therapeutic composition may comprise unmodified cells such as in TIL therapy or genetically modified cells. There are two common ways to achieve genetic targeting of T-cells to tumor specific targets. One is transfer of a T-cell receptor with known specificity (TCR therapy) and with matched human leukocyte antigen (HLA, known as major histocompatibility complex in rodents) type. The other is modification of cells with artificial molecules such as chimeric antigen receptors (CAR). This approach is not dependent on HLA and is more flexible with regard to targeting molecules. For example, single chain antibodies can be used and CARs can also incorporate costimulatory domains. However, the targets of CAR cells need to be on the membrane of target cells, while TCR modifications can utilize intracellular targets.

As used herein “adoptive cell therapeutic composition” refers to any composition comprising cells suitable for adoptive cell transfer. In one embodiment of the invention 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 lymphocytes. In another embodiment of the invention, 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. In another embodiment, 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 embodiment of the invention the adoptive cell therapeutic composition comprises T cells. As used herein “tumor-infiltrating lymphocytes” or TILs refer to white blood cells that have left the bloodstream and migrated into a tumor. Lymphocytes can be divided into

three groups including B cells, T cells and natural killer cells. In another specific embodiment of the invention the adoptive cell therapeutic composition comprises T-cells which have been modified with target-specific chimeric antigen receptors or specifically selected T-cell receptors. As used herein "T-cells" refers to CD3+ cells, including CD4+ helper cells, CD8+ cytotoxic T-cells and γδ T cells.

In addition to suitable cells, adoptive cell therapeutic composition used in the present invention may comprise any other agents such as pharmaceutically acceptable carriers, buffers, excipients, adjuvants, additives, 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.

The adoptive cell therapeutic 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, emulsions, suspensions, tablets, pellets and capsules. The compositions 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.

A combination of an oncolytic adenoviral vector of the invention and an adoptive cell therapeutic composition refers to use of an oncolytic adenoviral vector and an adoptive cell therapeutic composition together but as separate compositions. It is clear to a person skilled in the art that an oncolytic adenoviral vector of the present invention and an adoptive cell therapeutic composition are not used as one composition. Indeed, adenoviral vectors are not used for modifying the adoptive cells but for modifying the target tumor, so that the tumor is more amenable to the desired effects of the cellular transplant. In particular, the present invention enhances recruitment of the adoptive transplant to the tumor, and increases its activity there. In a specific embodiment of the invention oncolytic adenoviral vectors and an adoptive cell therapeutic composition of a combination are for simultaneous or sequential, in any order, administration to a subject.

Cancer

The recombinant vectors of the present invention are replication competent in 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 CR2, that is normally needed for effective replication, is unnecessary. Further selectivity of the adenoviral vector of the present invention 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 E2F1 promoter is important to prevent expression of E1A in normal tissues, which can cause toxicity both directly and indirectly through allowing transgene expression from the E3 promoter.

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

The approach of the present invention 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 particulary appealing for treatment of metastatic solid tumors featuring tumor infiltrating lymphocytes. In another embodiment the T-cell graft has been modified by a tumor or tissue specific T-cell receptor of chimeric antigen receptor.

As used herein, the term “treatment” or “treating” refers to administration of at least oncolytic adenoviral vectors or at least oncolytic adenoviral vectors and adoptive cell therapeutic composition 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 e.g. in blood or for example a size of a tumor or the length of survival of the patient

In one embodiment of the invention 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.

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.

Pharmaceutical composition

A pharmaceutical composition of the invention comprises at least one type of viral vectors of the invention. In one embodiment a pharmaceutical composition of the invention comprises 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, wherein the bispecific monoclonal antibody comprises a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen. 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, 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.

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, 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.

In one embodiment of the invention, the viral vector or pharmaceutical composition acts as an *in situ* vehicle for recruitment of T-cells, enhancing their therapeutic effect and allowing their propagation at the tumor.

A pharmaceutical kit of the present invention may comprises oncolytic adenoviral vectors encoding bispecific monoclonal antibodies or an adoptive cell therapeutic composition and oncolytic adenoviral vectors coding for bispecific monoclonal antibodies. In a specific embodiment the adoptive cell therapeutic composition is formulated in a first formulation and the oncolytic adenoviral vectors are 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.

Administration

The adenoviral vector or pharmaceutical composition of the invention may be administered to any eukaryotic subject selected from a group consisting of plants, animals and human beings. In a specific embodiment of the invention, the subject is a human or an animal. An animal may be selected from a group consisting of pets, domestic animals and production animals.

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.

In one embodiment of the invention both adenoviral vectors and adoptive cell therapeutic composition are administered to a subject. The administration(s) of adoptive cell therapeutic composition and oncolytic adenoviral vectors coding for at least one bispecific monoclonal antibody to a subject may be conducted simultaneously or consecutively, in any order. In one embodiment of the invention the oncolytic viral vectors and an adoptive cell therapeutic composition are administered separately. As used herein "separate administration" or "separate" refers to a situation, wherein adoptive cell therapeutic composition and oncolytic adenoviral vectors are two different products or compositions distinct from each other.

Only one administration of adenoviral vectors of the invention or single administrations of an adoptive cell therapeutic composition and oncolytic adenoviral vectors may have therapeutic effects. There may be any period between the administrations of oncolytic adenoviruses or between the administrations of oncolytic adenoviruses and adoptive cell therapeutic composition depending for example on the patient and type, degree or location of cancer. In one embodiment of the invention there is a time period of one minute to four weeks, specifically 1 to 10 days, more specifically 1 to five days, between the consecutive administration of adoptive cell therapeutic composition and oncolytic adenoviral vectors coding for a bispecific monoclonal antibody. Several administrations of adoptive cell therapeutic composition and oncolytic adenoviral vectors are also possible. The numbers of administration times of adoptive cell therapeutic composition and oncolytic adenoviral vectors may also be different during the treatment period. Oncolytic adenoviral vectors or pharmaceutical or adoptive cell compositions may be administered for example from 1 to 10 times in the first 2 weeks, 4 weeks, monthly or during the treatment period. In one embodiment of the invention, administration of vectors or any compositions is done three to seven times in the first 2 weeks, then at 4 weeks and then monthly. In a specific embodiment of the invention, administration is done four times in the first 2 weeks, then at 4 weeks and then monthly. The length of the treatment period may vary, and for example may last from two to 12 months or more.

In a specific embodiment of the invention an adoptive cell therapeutic composition and oncolytic adenoviral vectors are administered on the same day and thereafter oncolytic adenoviral vectors are administered every week, two weeks, three weeks or every month during a treatment period which may last for example from one to 6 or 12 months or more.

In one embodiment of the invention, the administration of oncolytic virus is conducted through an intratumoral, intra-arterial, intravenous, intrapleural, intravesicular, intracavitary or peritoneal injection, or an oral administration. Any combination of administrations is also possible. The approach can give systemic efficacy despite local injection. Adoptive cell therapeutic composition may be administered intravenously or intratumorally. In one embodiment the administration of the adoptive cell therapeutic composition and/or oncolytic viral vectors coding for at least one bispecific monoclonal antibody is conducted through an intratumoral, intra-arterial, intravenous, intrapleural, intravesicular, intracavitary or peritoneal injection, or an oral administration. In a specific embodiment of the invention TILs or T cells are administered intravenously and viral vectors intratumorally and/or intravenously. Of note, virus is delivered to the tumor separately from administration of T-cells; virus is not used to modify the T-cell graft ex vivo. In essence, the virus modifies the tumor in such a way that the T-cell graft can work better.

The effective dose of vectors depends on at least the subject in need of the treatment, tumor type, location of the tumor and stage of the tumor. The dose may vary for example from about $1x10^8$ viral particles (VP) to about $1x10^{14}$ VP, specifically from about $5x10^9$ VP to about $1x10^{13}$ VP and more specifically from about $8x10^9$ VP to about $1x10^{12}$ VP. In one embodiment oncolytic adenoviral vectors coding for a bispecific monoclonal antibody are administered in an amount of $1x10^{10}$- $1x10^{14}$ virus particles. In another embodiment of the invention the dose is in the range of about $5x10^{10}$ - $5x10^{11}$ VP.

The amount of cells transferred will also depend on the patient, but typical amounts range from $1x10^9$- $1x10^{12}$ cells per injection. The number of injections also varies but typical embodiments include 1 or 2 rounds of treatment several (e.g. 2-4) weeks apart.

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, monoclonal antibodies, chemotherapy or other anticancer drugs or interventions (including surgery) to a subject.

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. In this respect,

the present inventive methods can provide any amount of increase in the efficacy of T-cell therapy or any degree of treatment or prevention of a disease.

Figures 1 and 7 illustrate the methods and mechanisms of the present invention.

It will be obvious to a person skilled in the art that, as the technology advances, the inventive concept can be implemented in various ways. The invention and its embodiments are not limited to the examples described above but may vary within the scope of the claims.

EXAMPLES

Materials & methods

B16-OVA animal model: ovalbumin-expressing B16 cells (B16-OVA) were maintained in RPMI, 10% FBS, 5 mg/ml G418, 20 mM L-Glutamine, 1x Pen/Strep solution (GIBCO). 4-7-week-old C57BL/6 immunocompetent female mice were implanted subcutaneously with 2.5 x 10^5 B16-OVA cells in 50 ul RPMI, 0% FBS, in the right flank, one tumor per mouse. Roughly ten days post tumor implantation (when tumors became injectable, ~3 mm minimum diameter), mice were divided into groups and treated in some experiments on six consecutive days with intratumoral injections of either 50 ul PBS or 1 x 10^9viral particles (VPs) of oncolytic adenovirus in 50 ul PBS. In other experiments, three injections were given on days 0, 2 and 4. As murine cells are non-permissive to human adenovirus, multiple intratumoral virus injections were used to mimic virus replication-induced inflammation, (Blair et al., 1989).

Adoptive transfer: On the first day of the i.t. treatment, the mice also received by adoptive transfer in the intraperitoneal cavity 5 x 10^5 to 2 x 10^6 overnight-rested CD8a-enriched and expanded splenocytes from 4-8-week-old C57BL/6-Tg(TcraTcrb)1100Mjb/J (OT-1) mice, genetically engineered to have only ovalbumin (OVA)-specific CD8 T-cell receptors, in 100 ul RPMI, 0% FBS. CD8a-enrichment was performed by mouse CD8a (Ly-2) MicroBeads 5 days prior to transfer, per manufacturer's instructions (Miltenyi Biotech, USA, cat. no 130-049-401). Enriched cells were expanded in numbers for five days in lymphocyte medium (RPMI, 10 % FBS, 20 mM L-Glutamine, 1x Pen/Strep solution, 15 mM HEPES, 50 µM 2-mercaptoethanol, 1 mM Na pyruvate) in the presence of recombinant murine IL-2 (160 ng/ml) and soluble anti-mouse CD3ε antibody (0,3 ug/ml, Abcam, clone 145-2C11).

Tissue processing for flow cytometry: Mice were euthanized and spleens, draining lymph nodes and tumors were harvested in 1 to 10 ml RPMI, 10% FBS, and blood was collected by terminal heart bleed into the pleural cavity and transferred by disposable syringe into EDTA-containing microcentrifuge tubes, and processed for analysis: solid tissues were roughly dissociated by scalpel and triturated in a 10 ml disposable sterile pipette tip in 5 to 10 ml ACK lysing buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2) and incubated at room temperature (RT) for ~ 20 minutes, upon which cells were pelleted at 1200 rpm 5 min +4ºC, following which cells were re-suspended in 1 to 10 ml RPMI, 10% FBS, depending on the estimated amount of cells, and passed through a 40 µm sterile filter to create a single-cell solution. In some experiments, tumor tissue was instead processed directly after scalpel cutting (before addition of ACK) in 1 ml total volume of protease-coctail (RPMI supplemented with collagenase type A, H or P, Roche, at 1 mg/ml and benzonase, 125 units/ml final conc., Sigma, E1014-25KU) for 1-2 hours at 37°C, 5% CO_2, after which 10 ml ACK lysing buffer was added and cells were treated as above. 200 µl whole blood was pipetted into 5 ml ACK lysing buffer and treated as above. Cells were either incubated overnight at 37°C, 5% CO_2, or analyzed directly by immunostaining and flow cytometry.

Tissue processing for cytokine analysis: Mice were euthanized and ~2-10 mm^3 tumor pieces were frozen in 2 ml microcentrifuge tubes on dry ice and stored at -80°C. Tumor pieces were weighed and 200 µl ice-cold PBS added. Pieces were homogenized by Tissue Master 125 rotor, 1x protease inhibitor cocktail (Sigma) and 0.1 % BSA final conc. was added and tubes were kept on ice. Tumor homogenate was spun at 2000 rpm 10 min +4°C and the supernatant was analyzed with CBA Flex Set cytokine beads (BD, USA) on BD FACSArray, per manufacturer's instructions.

Experiments supporting the invention

The experiments were carried out according to the materials and methods chapter in this disclosure and according to the experimental section described in the publication WO2014170389 (A1) and in the previously published articles (Parviainen et al. 2014, Tahtinen et al. 2015, Tahtinen et al. 2015).

Experiment 1 (Treatment with adenovirus induces danger signals in tumors):

Treatment with Ad5/3-d24-GMCSF 5/3 chimeric adenovirus induced danger signals in B16.OVA tumors. Binding of adenoviral pathogen-associated molecular patterns (PAMP) to toll-like receptors (TLR) on host cells induce secretion of interferon-γ, associated with immune cell activation and T-cell stimulation leading 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 2)

Experiment 2 (Adenovirus has anti-immunosuppressive effects in the tumor microenvironment):

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

Experiment 3 (Lifting of immunosuppression alone is not sufficient to induce trafficking of T-cells to tumors: BiTE are needed):

Lifting of immunosuppression was not sufficient to induce trafficking of T-cells to B16.OVA tumors. Intratumoral injection of 5/3 chimeric adenovirus induced CD8+ T-cells in peripheral blood but these cells did not infiltrate the tumors efficiently. This poor tumor-trafficking of T-cells highlights the shortcomings of oncolytic adenovirus and adoptive T-cell therapies used as single agents, supporting the present invention to enhance the trafficking of adoptively transferred T-cells by BiTe-expressing oncolytic adenovirus. (Figure 4)

Experiment 4 (Adenovirus is superior to vaccinia in inducing cellular anti-tumor immunity; a critical feature for enhancing adoptive cell therapy)

Comparison between adenovirus (Ad) and vaccinia virus (VV) immunogenicity. Levels of splenic and B16.OVA tumor-infiltrating CD8+ T-cells were higher in 5/3 chimeric adenovirus treated mice compared to mice treated with double-deleted oncolytic Western reserve vaccinia virus (this strain was used by Yu et al Mol Ther 2014). Thus, oncolytic adenovirus appears to be an ideal expression platform for BiTe due to its inherent immunogenicity, especially in context of adoptive T-cell therapy. (Figure 5)

Experiment 5 (Adenovirus is more effective than vaccinia in inducing anti-tumor immunity)

Mice bearing syngeneic B16.OVA tumors were injected intratumorally with PBS, adenovirus or vaccinia virus. Tumors cell samples were stained with pentamer-APC detecting T-cell receptors specific for SIINFEKL residues of ovalbumin and assessed by flow cytometry (n=3). Data indicated change in anti-tumor T-cells following adenovirus or vaccinia virus injection; adenovirus was much more effective in inducing anti-tumor immunity. (Figure 6)

Experiment 6 (BiTE delivered by oncolytic adenovirus targets all classes of T-cells against tumors, including anti-viral T-cells (which are generally considered counterproductive for tumor therapy))

In addition, the present invention utilizes the extensive pre-existing Ad5 T-cell immunity in human populations that usually limits the clinical utility of adenoviral vectors. As TILs of adenovirus-treated tumors contain both anti-tumor and anti-viral T-cells, CD3-scFV of BiTe will activate these T-cells regardless of their endogenous specificity (MHC I-independently). Consequently, tumor-specific killing by these T-cells is achieved by scFV specific for tumor cell surface antigen (such as mesothelin, EpCAM1, MUC1) and no off-tumor/off-target reactivity is expected to be seen. Thus, this approach re-directs all CD8+ TILs (=anti-tumoral and anti-viral) into anti-tumor T-cells via binding of virus-produced BiTe. (Figure 7)

Experiment 7 (Oncolytic adenovirus, but not non-replicating adenovirus, coding for functional antibody results in efficient antibody production and release from cancer cells)

SKOV-3, BT-474 and 293 cells were infected with indicated adenoviruses at 100 virus particles (VP)/cell, and several days later analyzed for antibody expression by human IgG ELISA (A) or Western blot (B). At each indicated time point after infection, (A) oncolytic virus Ad5/3-d24-Trastuzumab (grey and black bars) showed high production of functional antibody from ovarian cancer SKOV-3 cells: Antibody levels decreased in cell lysate (LYS) during progressive infection and cancer cell killing, and showed significant accumulation in the supernatant (SN). (OV refers to Ad5/3-d24 and Ab refers to antibody Trastuzumab) In contrast, non-replicating virus Ad5/3-Ab failed to produce detectable antibody

in the supernatant, even though cell lysate showed evidence of antibody at day 7 post-infection (white bars). Of note, non-replicating Ad5/3-Ab virus treated cells were viable throughout the experiment, indicating the lack of active antibody secretion by cancer cells. (B) Supernatant of breast cancer BT-474 cells (left) and human embryonic 293 cells (right) was analyzed by Western blot 6 days after infection with indicated viruses. Under reducing conditions, heavy-chain (HC), light-chain (LC), and the full-length antibody produced by the oncolytic virus Ad5/3-OV-Ab were visualized in supernatant of both cell lines, whereas non-replicating Ad5-Ab and Ad5/3-Ab viruses failed to show antibody release from BTB-474 cells that do not allow their replication. To confirm antibody expression by the non-replicating viruses, we used human embryonic 293 cells (right), which allow replication of also E1A-deleted adenoviruses, followed by cell lysis and release of the antibody, readily detected by Western blot. A non-replicating control virus Ad5/3-Luc coding for luciferase was used as a negative control. HC and LC were detected using polyclonal goat anti-human IgG and donkey anti-goat IgG-HRP antibodies, respectively. The antibody affinity was lower to the LC than to the HC resulting in a weaker signal. Bars represent the mean ± SEM. **, $P < 0.01$; *, $P < 0.05$; all Student's T tests. (Figure 8)

Experiment 8 (Oncolytic adenovirus coding for antibody shows higher intratumoral while lower systemic antibody levels than after systemic antibody treatment)

Subcutaneous N87 gastric cancer (Park et al. 1990) xenograft bearing nude/NMRI mice (n = 5 per group) were treated with intratumoral injections of oncolytic Ad5/3-OV-Ab virus (2×10^8 VP/tumor) or intraperitoneal injections of commercial antibody (Ab; 0.3 µg/g) on days 0, 4, 8, and 15. Health of the animals was monitored and tumors and blood samples were collected from mice sacrificed on days 32 and 40 (systemic Ab), day 46 (systemic Ab and Ad5/3-OV-Ab virus), and day 50 (Ad5/3-OV-Ab virus). **A**) Endpoint tumors and blood samples were measured by human IgG ELISA to assess the antibody concentration: Ad5/3-OV-Ab treated mice sacrificed on days 46 and 50 post-treatment showed still significantly higher antibody concentrations in tumors ($P < 0.001$, left), while presenting much lower circulating levels ($P < 0.001$, right), as compared to systemic Ab treated mice that were sacrificed earlier on days 32, 40 and 46. **B**) Antibody levels in tumor and blood samples of each individual animal

were compared to assess the antibody distribution. The average ratio of antibody in tumor *versus* blood was above 1.0 in mice treated with Ad5/3-OV-Ab virus, whereas systemic Ab treatment resulted in very low ratio of less than 0.01. Thus, treatment with antibody expressing oncolytic virus can achieve improved intratumoral antibody concentration, while significantly reducing systemic exposure in animals. Notably, most of the virus-treated mice survived longer (up to 50 days) and therefore showed evidence of sustained local antibody production. Error bars represent the mean + SEM. **, $P < 0.01$, Student's T test. (Figure 9)

Experiment 9 (Expression of T-cell exhaustion marker and immunosuppressive receptor TIM3 decreases after oncolytic adenovirus treatment and correlates with improved survival)

15 patients with advanced solid tumors were treated with oncolytic adenoviruses in the context of an Advanced Therapy Access Program (Taipale et al. 2016). Baseline and post-treatment tumor biopsies were analyzed by RNA microarray (HumanHT-12 v4 Expression BeadChips array, Illumina), and gene expression levels were compared to identify differentially expressed genes. T-cell immunoglobulin mucin-3 (TIM3), which is an exhaustion marker and negative regulator of both innate and adaptive immune responses in tumors, was among the top differentially expressed genes: TIM3 showed major downregulation in 5 patients (change over 1.0, Δ[log2]) and minor decrease in 4 patients (average change of 0.38, Δ[log2]). Meanwhile, 6 patients failed to show downregulation of TIM3, out of which two patients showed upregulation post-treatment. When overall survival was compared between these groups, the patients with TIM3 downregulation ($n = 9$) showed significantly improved survival ($P = 0.004$, Log-rank test) over the patients with “TIM3 no change / upregulation” ($n = 6$). Median survival was 204 days and 64 days in TIM3 down- and upregulation groups, respectively. Thus, two-thirds of oncolytic adenovirus treatments seemed to result in decrease of immunosuppressive receptor and exhaustion marker TIM3, which strongly correlated with prolonged overall survival. (Figure 10)

Experiment 10 (Improved in vitro cell killing with TIL and oncolytic adenovirus combination)

HapT1 cells were infected with oncolytic adenovirus Ad5/3-d24 (100 VP/cell) for 3 days before adding HapT1 TIL. Target cell viability was determined

24 hours after TIL addition. Error bars, SE. ****p<0.0001. The best killing was seen when T-cells were stimulated with an oncolytic adenovirus. (Figure 11)

Experiment 11 (In the absence of BiTe molecules, TILs extracted from HapT1 tumors don't have an additive effect on target cell killing when combined with oncolytic adenoviruses)

HapT1 cells were plated on 96 well plate and incubated five days with oncolytic adenovirus Ad5/3-E2F-d24 only or armed with human IL-2. TILs extracted from established HapT1 tumors were added to cells 10:1 24 h before measuring the viability of the cells with MTS assay. Synergy was not observed between viruses and TILs. (Figure 12)

Experiment 12 (Excellent lytic activity of a combination virus + BiTE + PBMCs)

SW480 tumor cells were seeded on 96 well plate, 10 000 cells/well, and incubated for 24 h. The cells are infected with Ad 5/3-E2F-d24-E3 virus, 0.01, 0.1, 1, 10, 100 and 1000 viral particles per cell and 10ng of human CD3 specific EpCAM targeted BiTE (Antihuman EpCam, Cat#CABT-33295MH) at least in three replicates, 50 ul/well in assay media (L-15, 2% FBS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin). Effector cells (PBMCs) were added at an effector to target ratio of 5:1. Next day, 50 ul 10% L-15 was added to cells. 48 h after infection the infection media was replaced with 100 ul growth media containing 10% CellTiter 96 AQueous One Solution (Promega, Madison, WI, USA) and incubated for two hours. The absorbance was read at 490 nm. Error bars indicate SEM of triplicate measurements. Virus + Cells Vs Virus + PBMCs *P = 0.0184, Virus + Cells Vs Virus + PBMCs + BiTE *** P = 0.001 (Figure 13A). Figure 13B: SW480 tumor cells were infected with 1000 VP of Ad 5/3-E2F-d24-E3 virus and with 10 ng of BiTE. Effector cells (PBMCs) were added at an effector to target ratio of 5:1. MTS assay was used to determine the cell viability at 48 hours post infection. Error bars indicate SEM of triplicate measurements. Virus + Cells Vs Virus + PBMCs *P =0.0184, Virus + Cells Vs Virus + BiTE + PBMCs ***P = 0.001.

Fractional Product Method:

Fractional Product Method was used to assess synergy, this method is derived from a method originally developed by Webb (Webb J, 1963).

Formula = Expected Values (Product of Monotherapies) = (Virus+Cells) (Virus+PBMCs+Cells)
Observed Values (Virus+PBMCs+BiTE)

0,1 VP = 1.245 = synergistic
1 VP = 1.32 = synergistic.
10 VP = 1.2 = synergistic
100 VP = 1.1 = synergistic
1000 VP = 1.1 = synergistic

Key:

Synergistic = Ratio greater than 1
Additive Effect = equal to 1
Antagonism = less than 1

Results:

These findings indicate that BiTE are synergistic with TILT's oncolytic adenovirus.

Experiment 13 (*In vitro* cell viability experiment combining Ad-BiTE and OT1 T-cells on B16-OVA target cells)

B16-OVA cells are plated on 96-well plates at 1 x 10e4 cells/well and infected with 100 VP/cell of Ad-BiTE, T-cells (2:1 effector to target ratio) or both. Cell viability is determined 24 hours later by MTS assay.

Experiment 14 (Adenovirus or adenovirus armed with IL2 is not enough to accumulate T-cells at tumors)

Adenovirus treatment was combined with adoptive T-cell transfer and resulted in suboptimal T-cell infiltration into B16.OVA melanoma tumors. Tumors collected 18 days after treatment start were flow cytometrically analyzed for ovalbumin-specific CD8+ T-cells (OVA) and gp100-specific CD8+ T-cells. OVA and gp100 are epitopes expressed on melanoma cells. Differences between different treatment groups were not statistically significant, and not different from T-cell therapy alone (no virus). Horizontal lines, mean values. (Figure 14)

Experiment 15 (Oncolytic adenoviruses are unable to recruit cytotoxic CD8+ T cells to tumors)

Subcutaneous hamster pancreatic tumors (HapT1) were treated with oncolytic adenoviruses Ad5/3-E2F-d24 alone or armed with human IL-2 five times in total during 19 days. On day 25 the animals were sacrificed and tumor cells labeled with cross-reactive anti-rat CD8b PE antibody. (Sample numbers: mock and unarmed n=5, IL2 n=1). Oncolytic adenovirus alone was not able to recruit Cd8 cells to the tumor. IL2 seemed more promising but the increase was not significant. (Figure 15)

Experiment 16 (Rechallenge in immunocompetent hamsters)

Hamsters previously cured with an unarmed oncolytic adenovirus Ad5/3-E2F-d24 or with adenovirus armed with a cytokine (TNFα, IL-2 or both) treatment resisted same tumor type (HapT1) but not different one (DDT1-MF2). Naïve animals which had not encountered either of the cell lines previously were used as a control. Arming the virus with a molecule able to induce anti-tumor immunity (for example BITe) is necessary for inducing protective immunity (=a sign of memory response against tumor epitopes). (Figure 16)

Experiment 17 (In vivo efficacy of armed or unarmed oncolytic adenovirus, with or without T-cell therapy)

Established HapT1 tumors were injected intratumorally with oncolytic adenovirus Ad5/3-d25 (1 x 10^7 VP/tumor) on Days 1 and 8. On Day 2, HapT1 tumor infiltrating lymphocytes grown ex vivo (1.5 x 10^6 TIL/tumor) were administered intratumorally. Error bars, SE. *p<0.05, **p<0.01. The best anti-tumor efficacy was seen when tumors were treated with an oncolytic virus (such as a BiTe coding virus) and TILs were also given. (Figure 17)

Experiment 18 (Hypothetical results from *in vivo* antitumor efficacy experiment combining Ad-BiTE and OT1 T-cell transfer in immunocompetent mice bearing B16-OVA tumors)

Subcutaneously implanted B16-OVA tumors (0.25 x 10e6 cells/tumor) will be treated with a single intraperitoneal injection of CD8-enriched OT1 T-cells, intratumoral injection of Ad-BiTE (1 x 10e9 VP/tumor) or both. Virus injections will be repeated every 7 days. (Figure 18)

Experiment 19 (Adenoviral delivery of cytokines IL2 and TNFa enhance efficacy of adoptive cell therapy: rationale for including cytokines in oncolytic adenovirus coding for BiTE)

B16-OVA tumor-bearing C57 mice were treated intratumorally with 1 x 10e9 viral particles of armed adenoviruses and intraperitoneally with 1.5 x 10e6 CD8-enriched OT-1 T-cells on Day 1. Virus treatments continued every 7 days. (Figure 19)

Experiment 20 (Novel virus constructs)

We generated new oncolytic Ad5/3 adenoviruses carrying the following backbone: Ad5/3-E2F-D24-transgene. Transgenes were in the area of deleted E3 gp19k/6.7k. Following transgenes were utilized in the vectors:

aMesothelin-aCD3
aEpCAM-aCD3
aMUC1-aCD3
aMesothelin-aCD3-IRES-IL2
aMesothelin-aCD3-IRES-TNFa
aEpCAM-aCD3-IRES-IL2
aEpCAM-aCD3-IRES-TNFa
aMUC1-aCD3-IRES-IL2
aMUC1-aCD3-IRES-TNFa

(Figures 20 and 21)

The adenoviral vectors of Figure 20 or construct maps of Figure 21 comprise nucleotide sequences comprising e.g. transgenes aMesothelin-aCD3 (SEQ ID NO: 9), aEpCAM-aCD3 (SEQ ID NO: 4) or aMUC1-aCD3 (SEQ ID NO: 8), listed in Table 2. Nucleotide sequence of the viral vector of the present invention comprises or consists of e.g. SEQ ID NOs: 1, 2, 3, 5, 6, 9 (aMesothelin-aCD3-IRES-IL2); SEQ ID NOs: 1, 2, 3, 5, 6, 7 (aMesothelin-aCD3-IRES-TNFa); SEQ ID NOs: 1, 2, 3, 4, 5, 6 (aEpCAM-aCD3-IRES-IL2); SEQ ID NOs: 1, 2, 3, 4, 5, 7 (aEpCAM-aCD3-IRES-TNFa); SEQ ID NOs: 1, 2, 3, 5, 6, 8 (aMUC1-aCD3-IRES-IL2); SEQ ID NOs: 1, 2, 3, 5, 7, 8 (aMUC1-aCD3-IRES-TNFa). The adenoviral vectors were constructed according to the sequences listed in Table 2. General methods for constructing adenoviral vectors, also utilized for the present invention, are well known to a person skilled in the art and are described e.g. in Koski et al. 2010, Hemminki et al. 2015.

Table 2. Sequence listing.

SEQ ID NO:	Name:
1	LITR
2	E2F
3	D24
4	Transgene EpCAM_CD3linker
5	5/3 knob modification
6	Transgene IRES-IL2
7	Transgene IRES-TNFa
8	Transgene MUC1_CD3linker
9	Transgene AntiMesothelin_CD3linker

5

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CLAIMS

1. 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, wherein the bispecific monoclonal antibody comprises a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen.

2. The oncolytic adenoviral vector according to claim 1, wherein a backbone of the adenoviral vector is an adenovirus serotype 5 (Ad5) or serotype 3 (Ad3) nucleic acid backbone.

3. The oncolytic adenoviral vector according to any one of the previous claims, wherein the vector further comprises E2F1 promoter for tumor specific expression of E1A.

4. The oncolytic adenoviral vector according to any one of the previous claims, wherein the vector further comprises a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1.

5. The oncolytic adenoviral vector according to any one of the previous claims, wherein the deletion of a nucleic acid sequence in the E3 region is a deletion of viral *gp19k* and *6.7k* reading frames.

6. The oncolytic adenoviral vector according to any one of the previous claims, wherein the vector comprises:

1) E2F1 promoter for tumor specific expression of E1A

2) a 24 bp deletion (D24) in the Rb binding constant region 2 of adenoviral E1;

3) a nucleic acid sequence deletion of viral *gp19k* and *6.7k* reading frames; and

4) a nucleic acid sequence encoding a bipartite molecule comprising a single chain variable fragment (scFv) specific for a cell surface molecule and a scFv specific for a tumor antigen in the place of the deleted nucleic acid sequence as defined in point 3).

7. The oncolytic adenoviral vector according to any one of the previous claims, wherein the backbone is Ad5 nucleic acid backbone and the vector further comprises an Ad3 fiber knob.

8. The oncolytic adenoviral vector according to any one of the previous claims, wherein the cell surface molecule is on immunological effector cells.

9. The oncolytic adenoviral vector according to claim 8, wherein the effector cells are T lymphocytes.

10. The oncolytic adenoviral vector according to any one of the previous claims, wherein the tumor antigen is selected from Table 1 or from the group consisting of mesothelin, EpCAM1 and MUC1.

11. The oncolytic adenoviral vector according to any one of the previous claims, wherein the cell surface molecule is selected from CD3, CD8 and CD4.

12. The oncolytic adenoviral vector according to any one of the previous claims, wherein the tumor antigen is mesothelin and the cell surface molecule is CD3; 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.

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

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

15. A pharmaceutical composition comprising an oncolytic adenoviral vector according to any one of claims 1-14.

16. An oncolytic adenoviral vector according to any one of claims 1-14 for use in treatment of cancer.

17. An oncolytic adenoviral vector for use according to claim 16 together with an adoptive cell therapeutic composition.

18. A method of treating cancer in a subject, wherein the method comprises administration of an oncolytic adenoviral vector according to any one of claims 1-14 to a subject.

19. The method according to claim 18, wherein the method further comprises administration of adoptive cell therapeutic composition to the subject.

20. The method or the oncolytic adenoviral vector for use according to claim 17 or 19, 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.

21. The method or the oncolytic adenoviral vector for use according to any one of claims 17 or 19-20, 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.

22. The method or the oncolytic adenoviral vector for use according to any one of claims 17 or 19-21, wherein the adoptive cell therapeutic composition comprises T-cells.

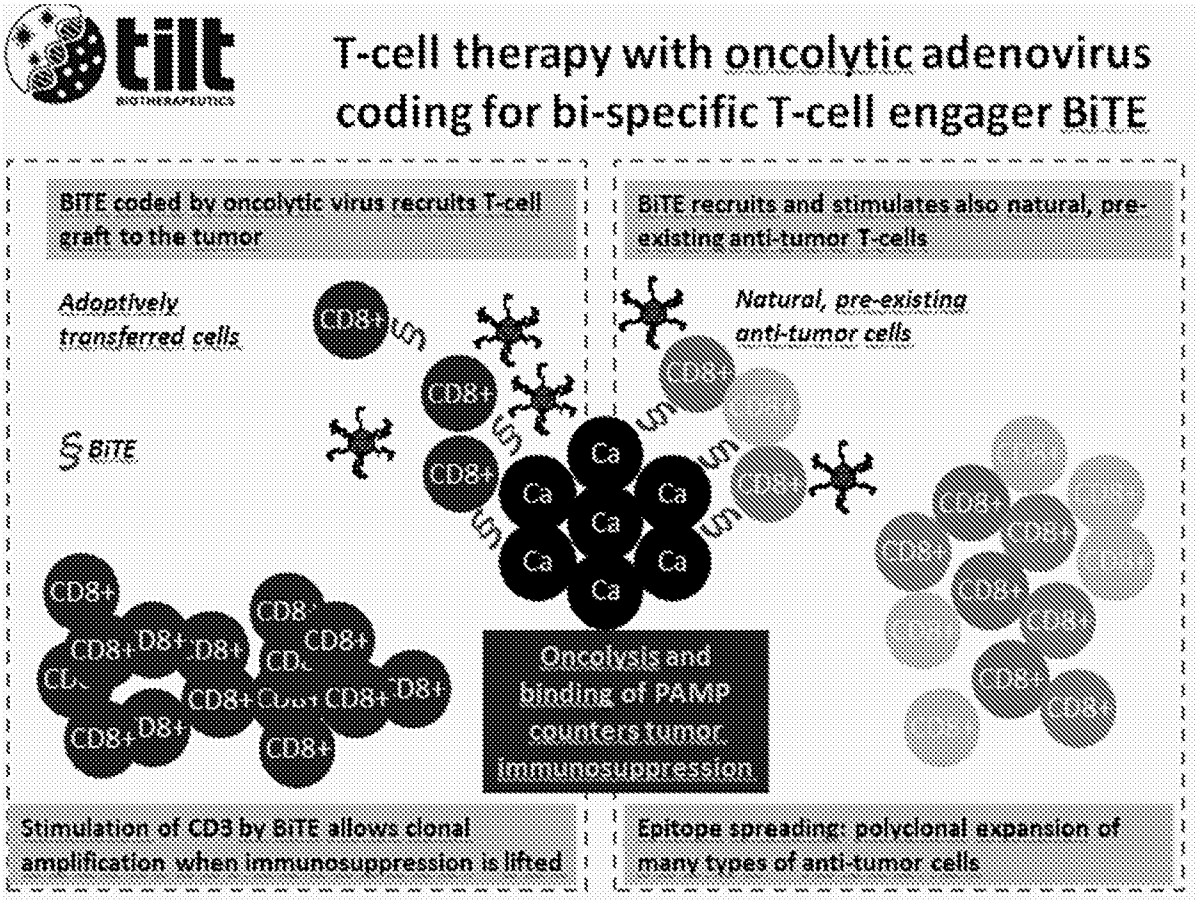
23. The method or the oncolytic adenoviral vector for use according to any one of claims 16-22, wherein the cancer is selected from a group consisting of nasopharyngeal cancer, synovial cancer, hepatocellular cancer, renal cancer, cancer of connective tissues, melanoma, lung cancer, bowel cancer, colon 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.

24. The method or the oncolytic adenoviral vector for use according to any one of claims 16-23, wherein the administration(s) of oncolytic viral vectors and an adoptive cell therapeutic composition to a subject is(are) conducted simultaneously or consecutively, in any order.

25. The method or the oncolytic adenoviral vector for use according to any one of claims 16-24, further comprising administration of concurrent or sequential radiotherapy, monoclonal antibodies, chemotherapy or other anti-cancer drugs or interventions to a subject.

26. The oncolytic adenoviral vector according to any one of claims 1-14 for use in increasing the efficacy of adoptive cell therapy in a subject.

27. A method of increasing the efficacy of adoptive cell therapy in a subject by administering an oncolytic adenoviral vector according to any one of claims 1-14 to a subject in need thereof, wherein the subject has been administered or is to be administered with adoptive cell therapy.



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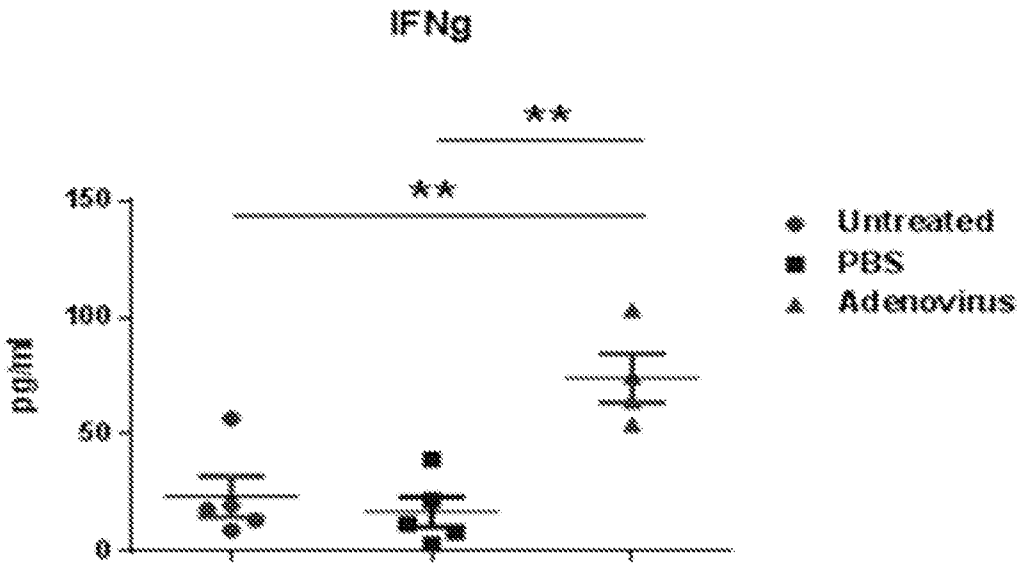

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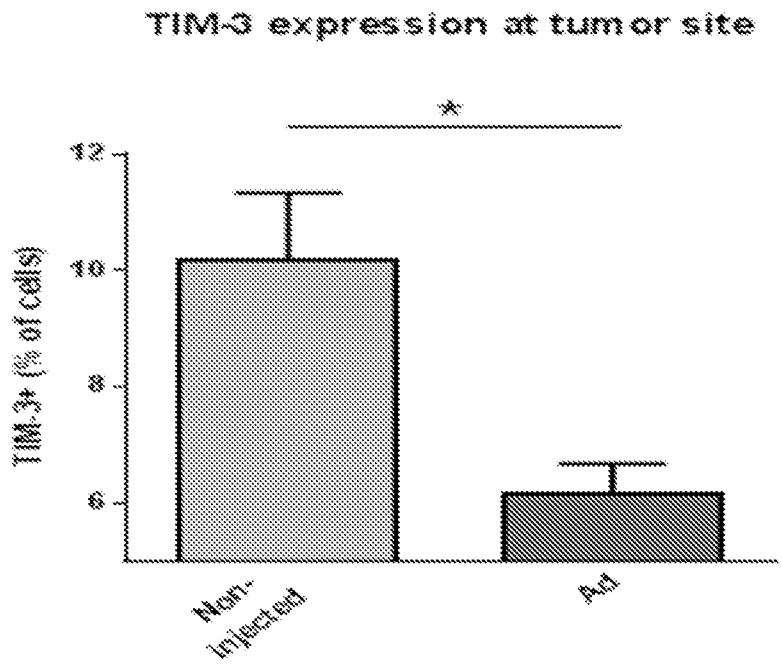

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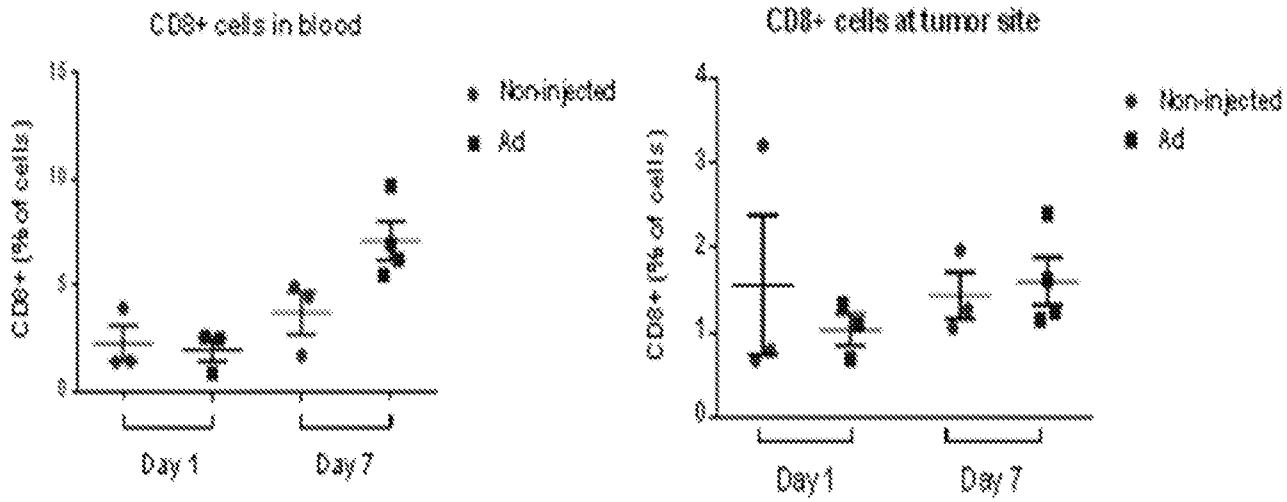

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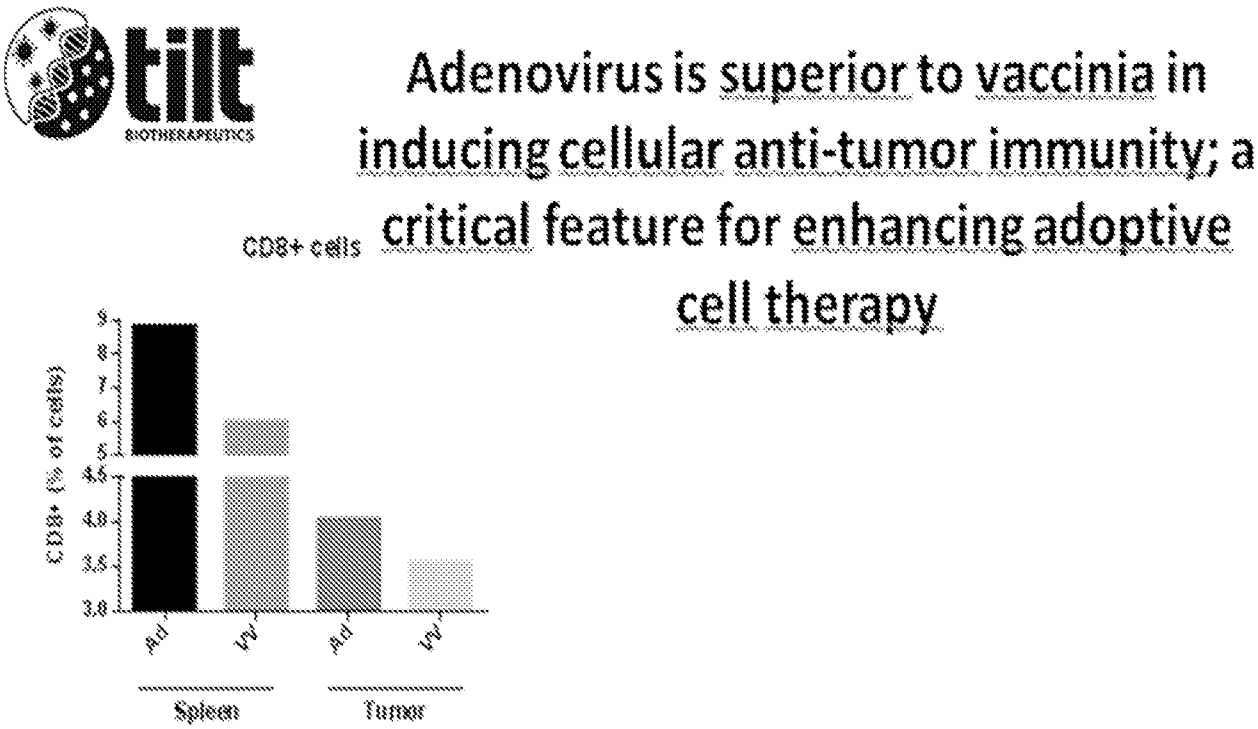

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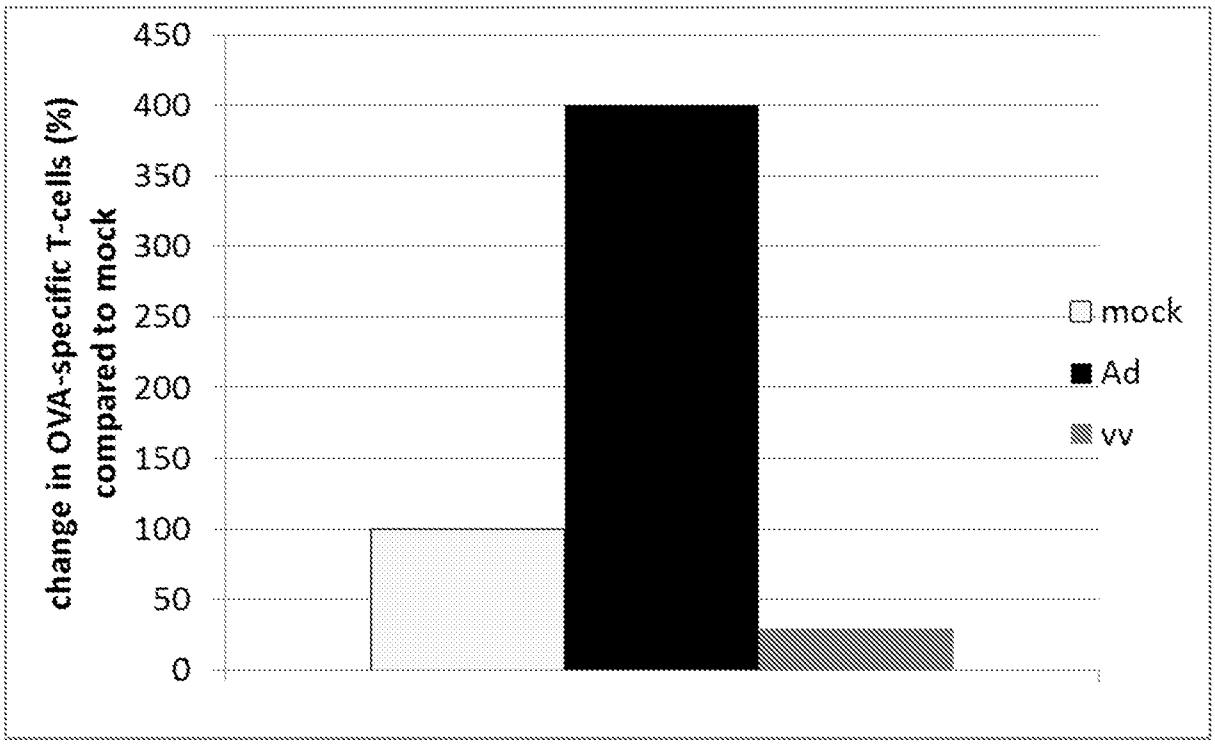

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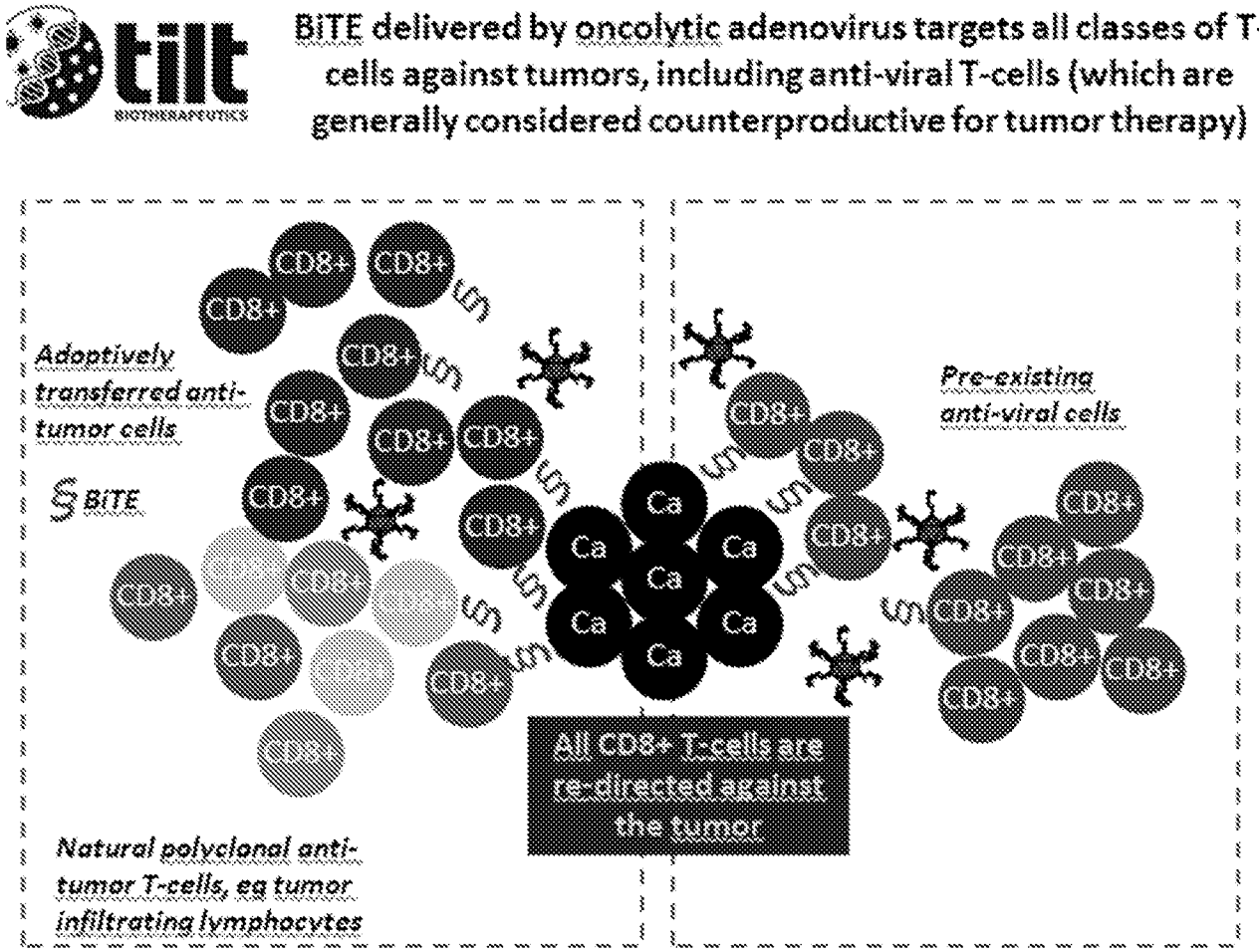

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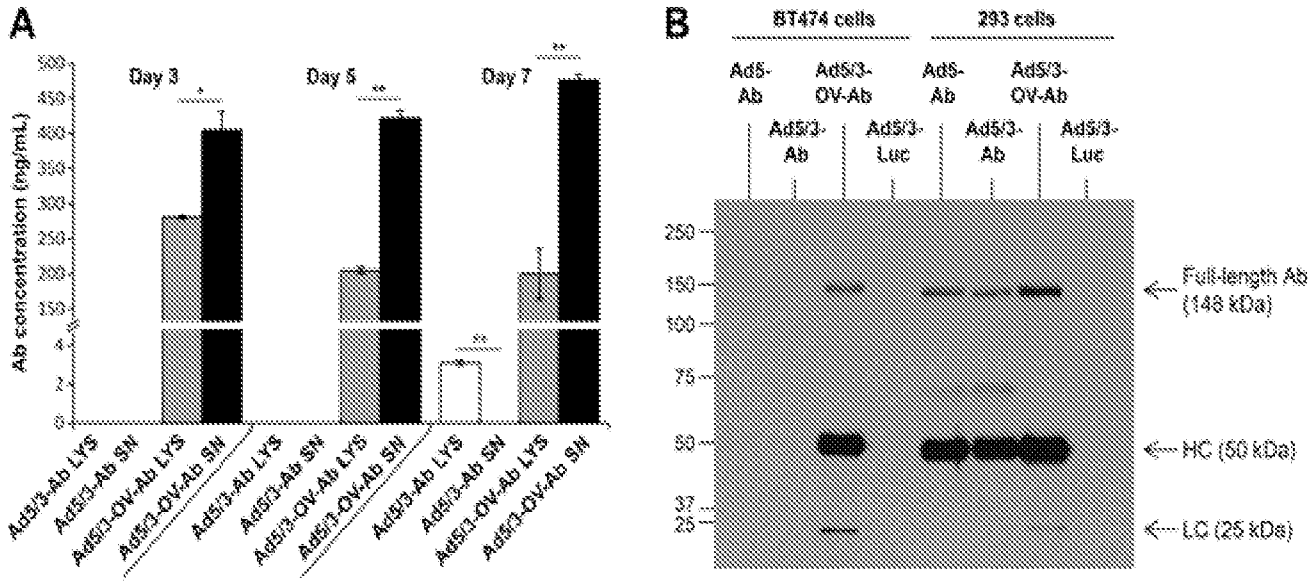

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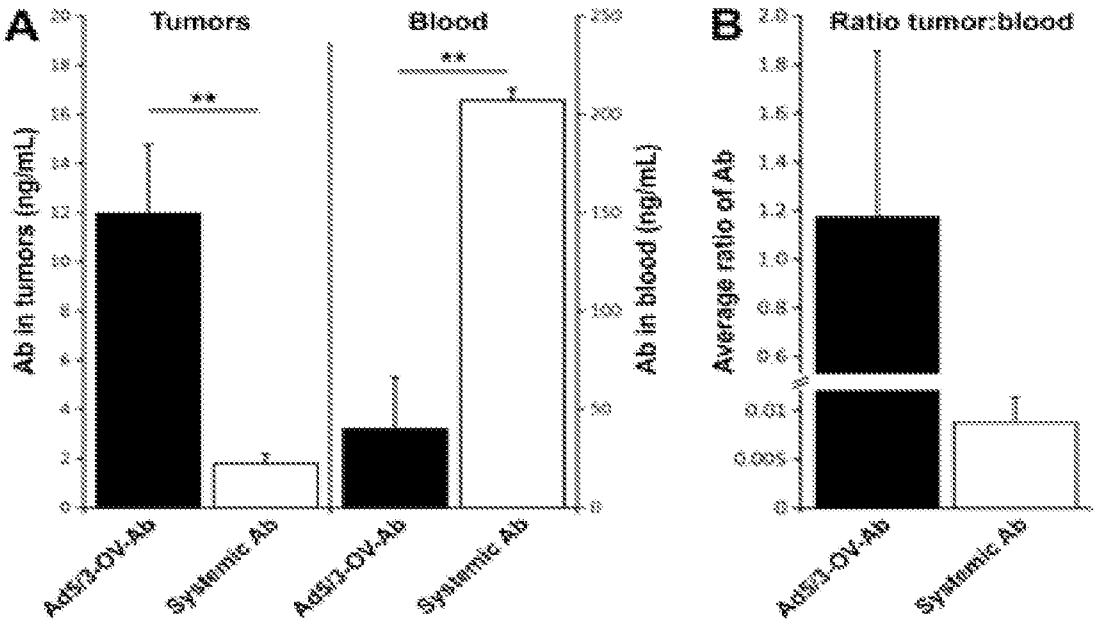

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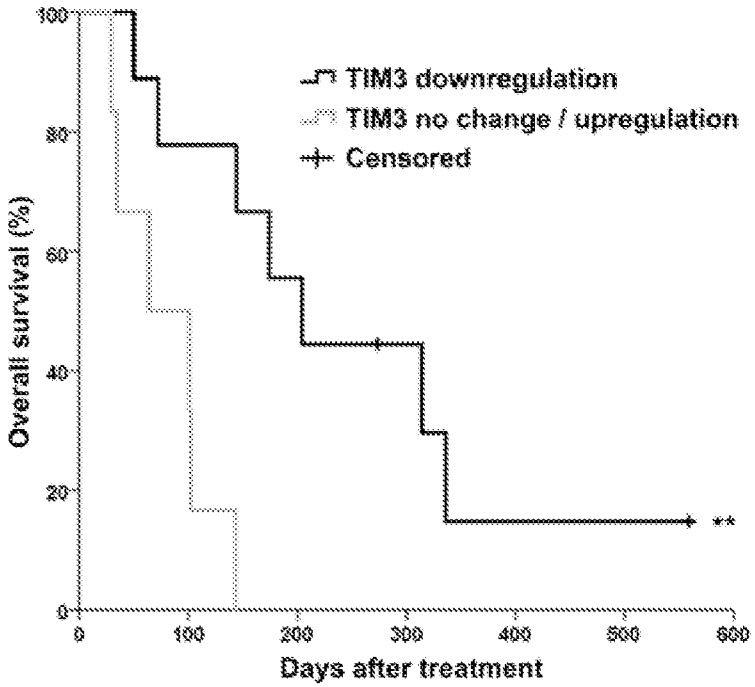

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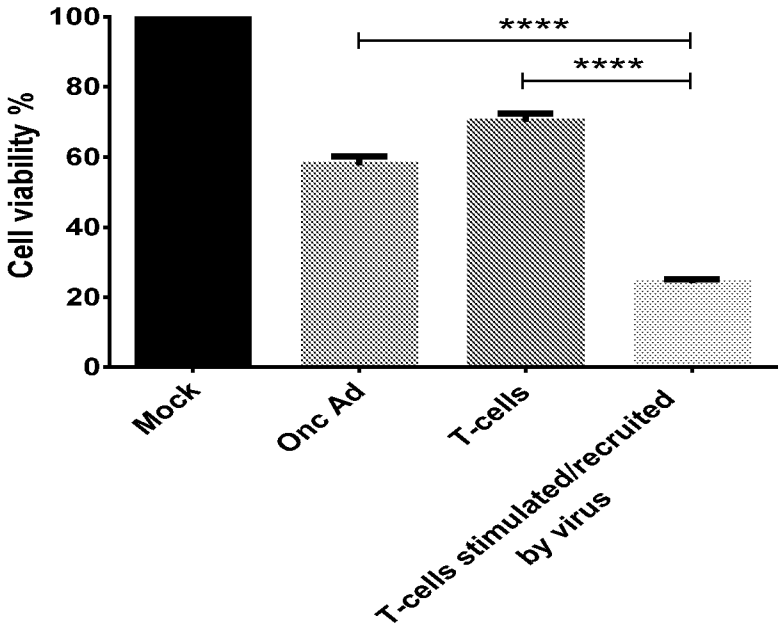

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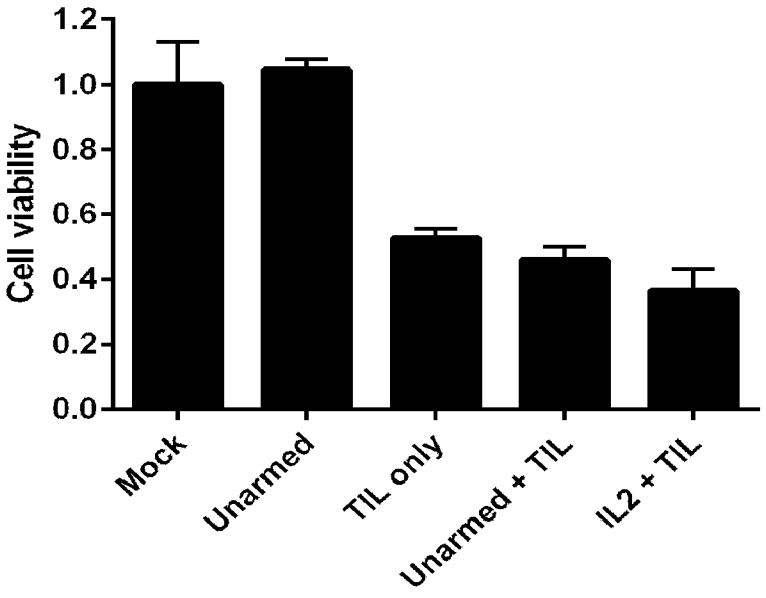

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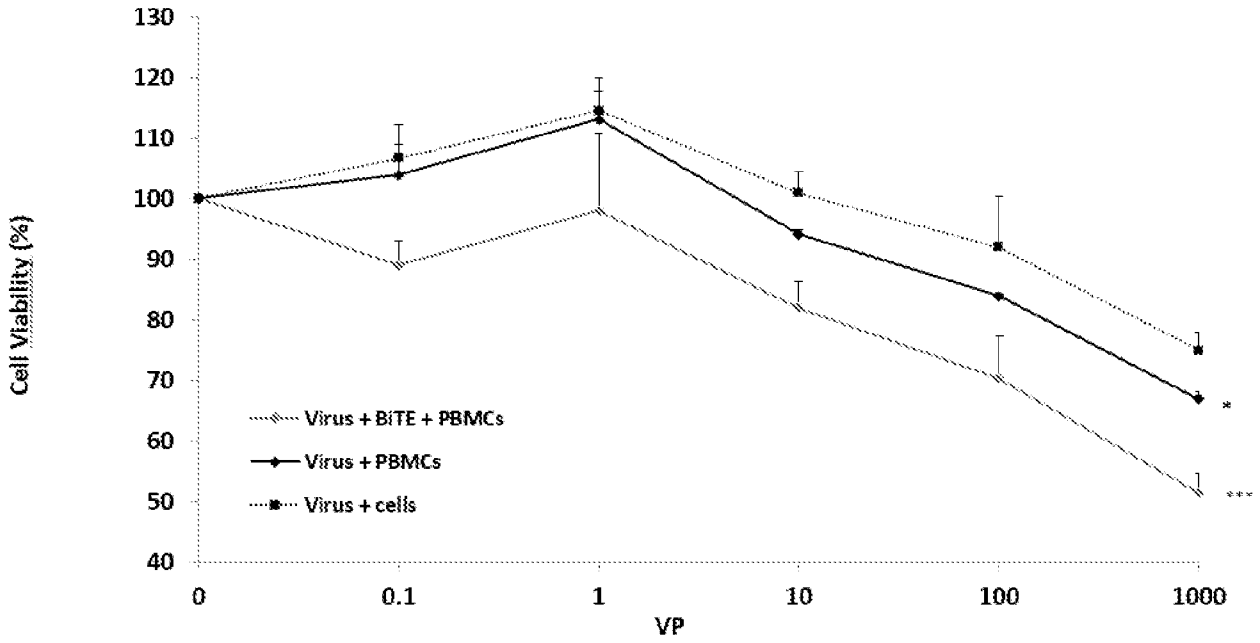


Figure 13A.

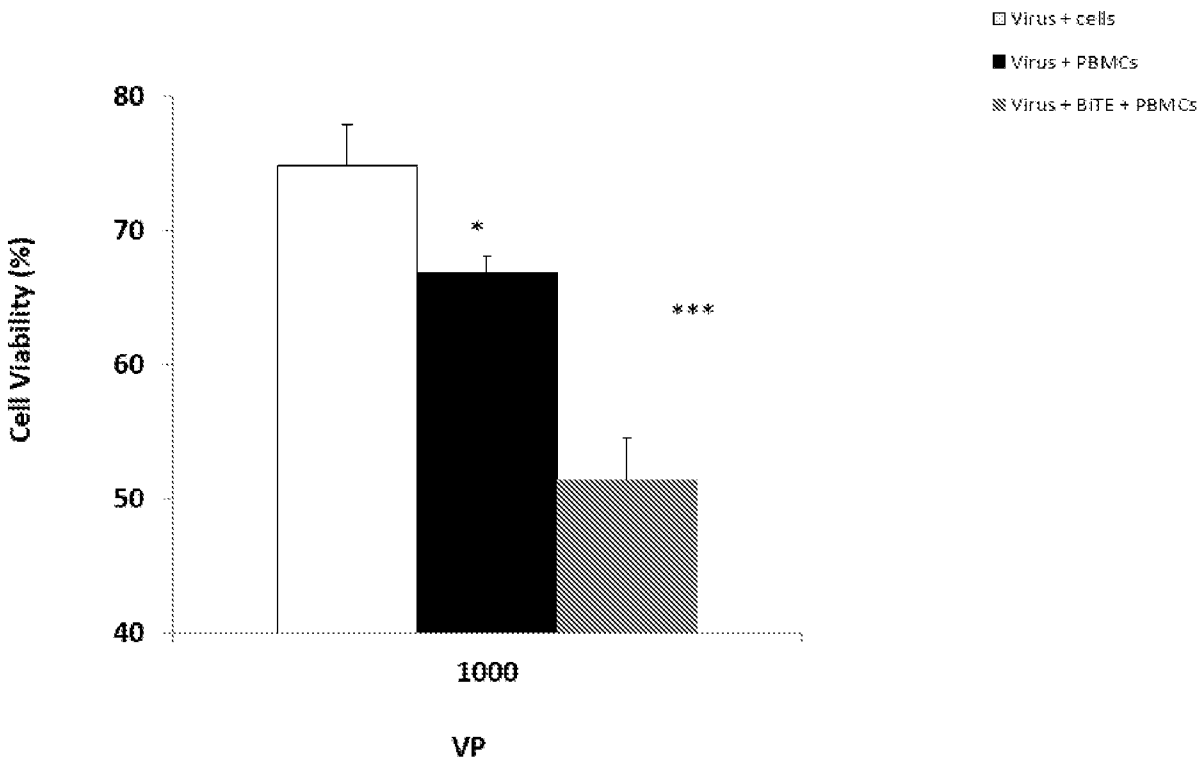
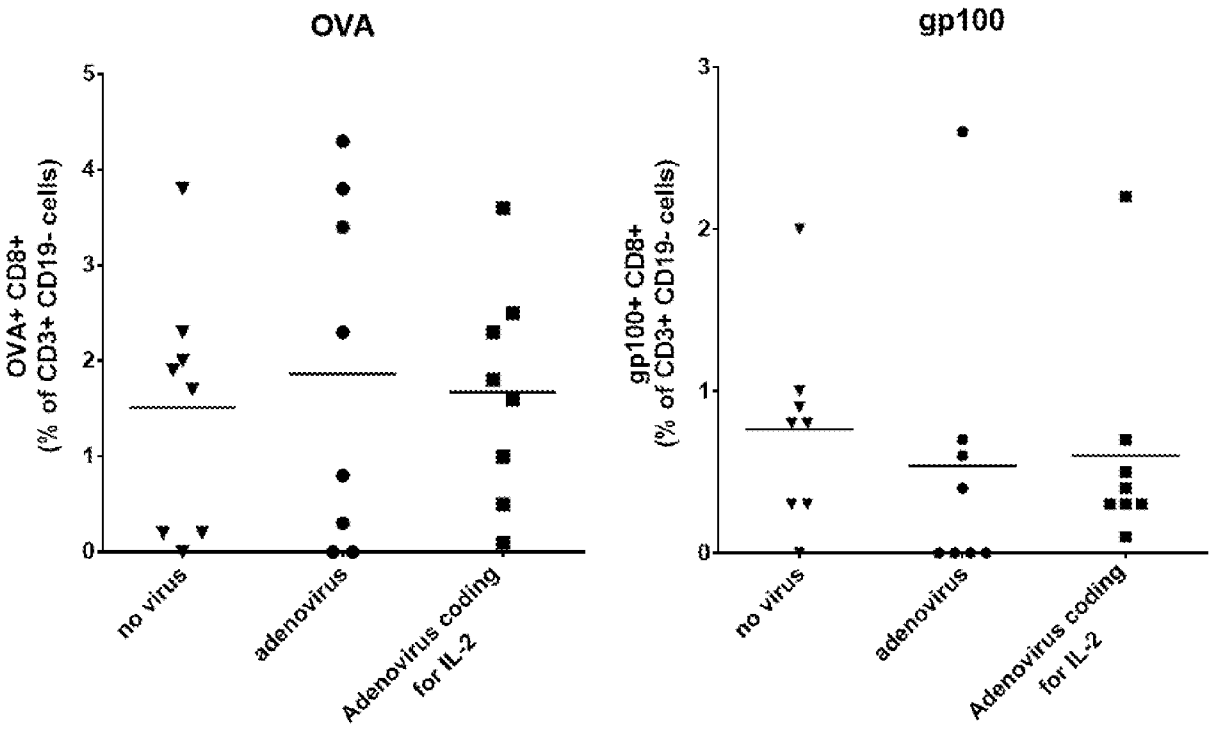


Figure 13B.



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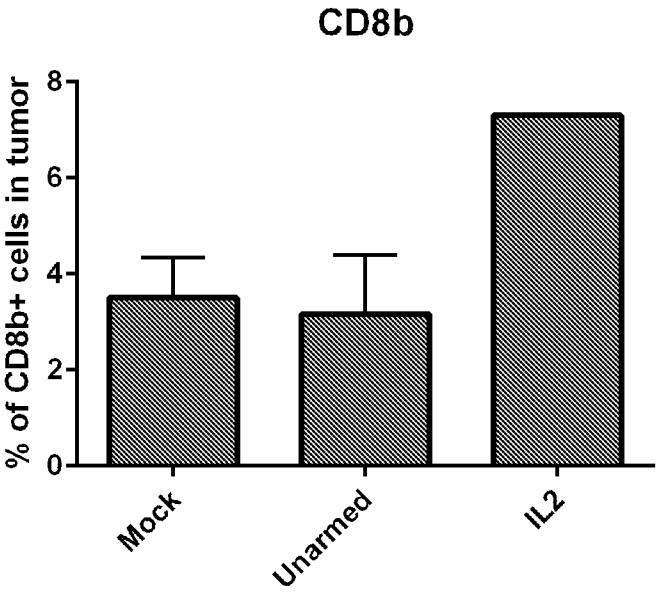

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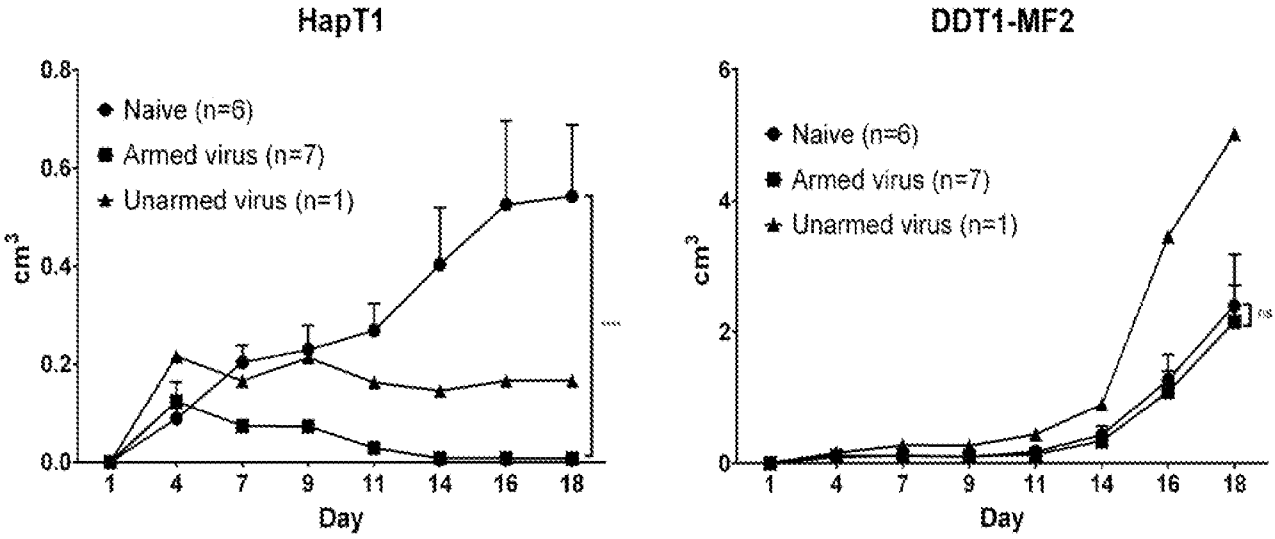

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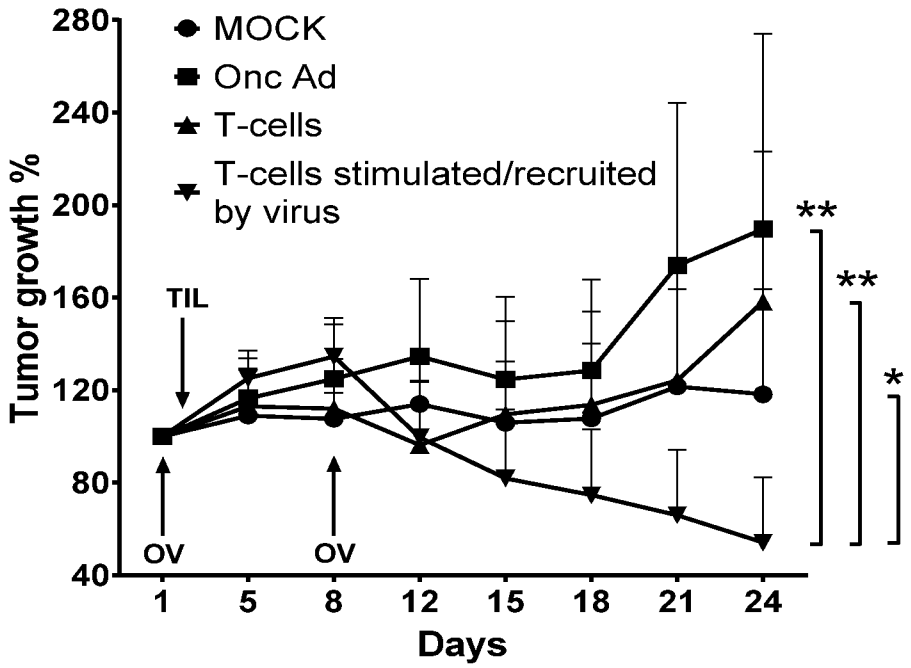

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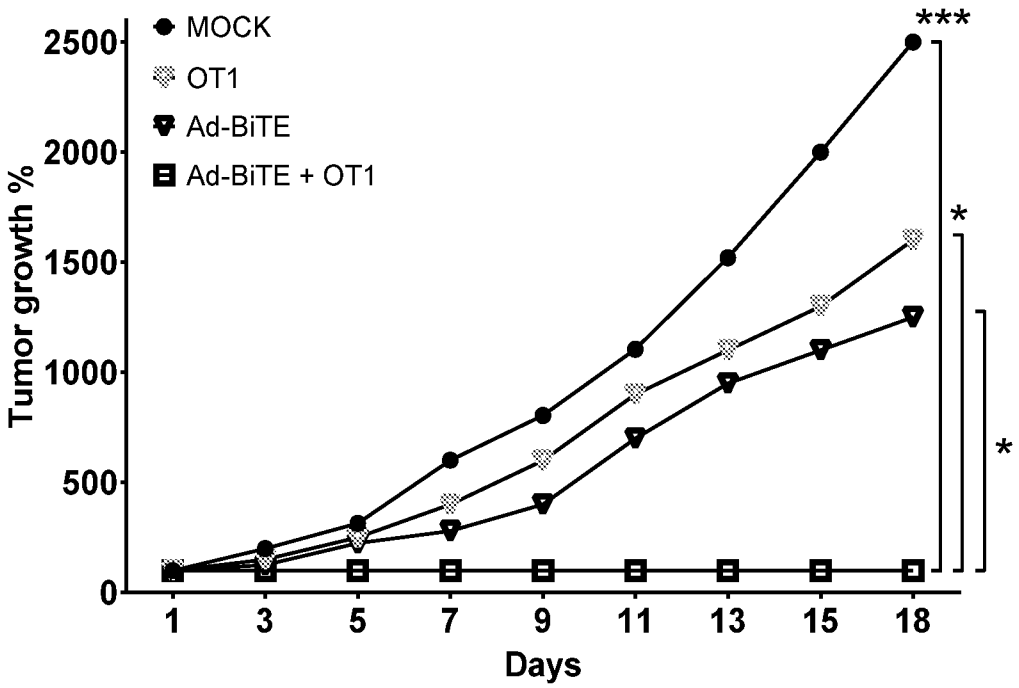

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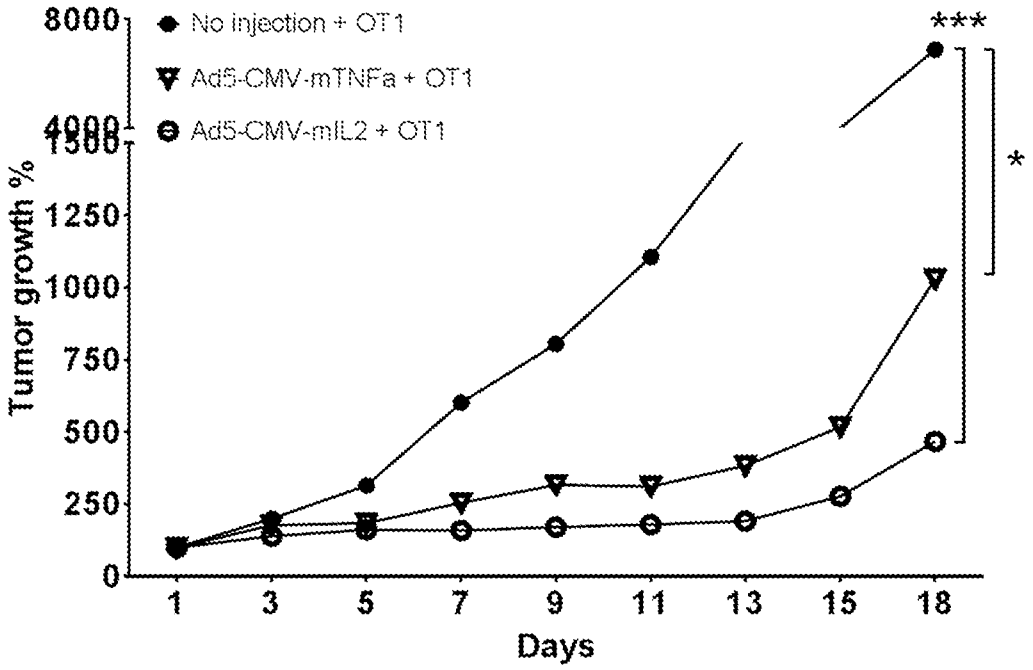

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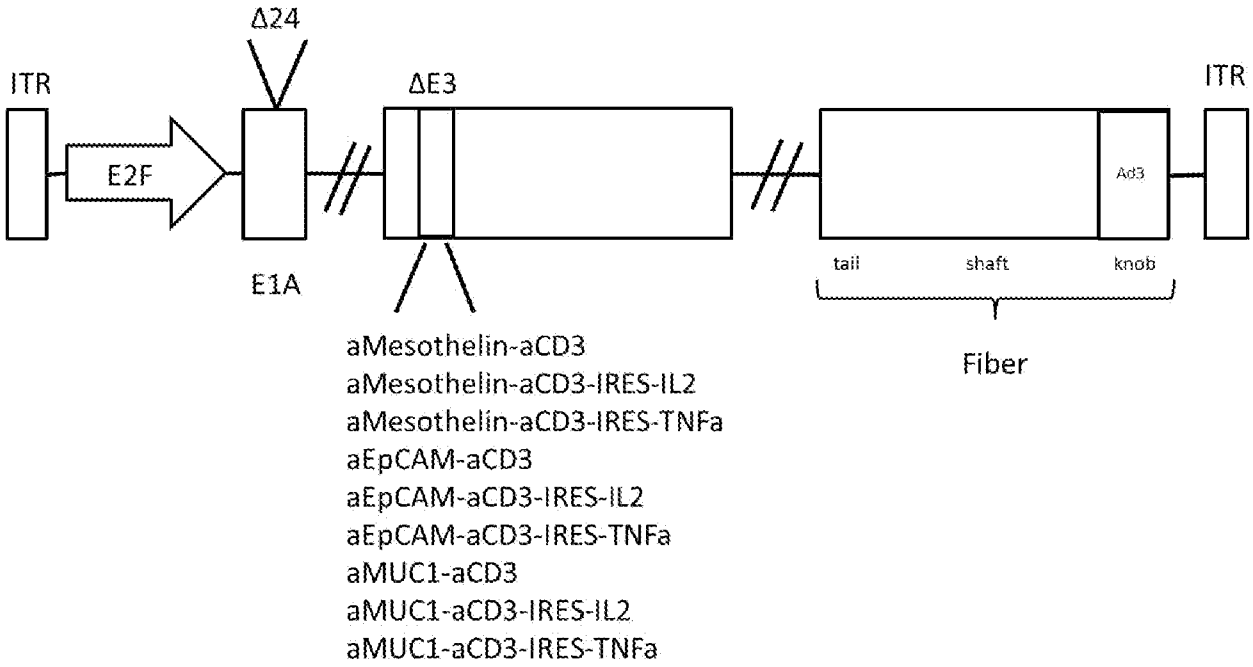

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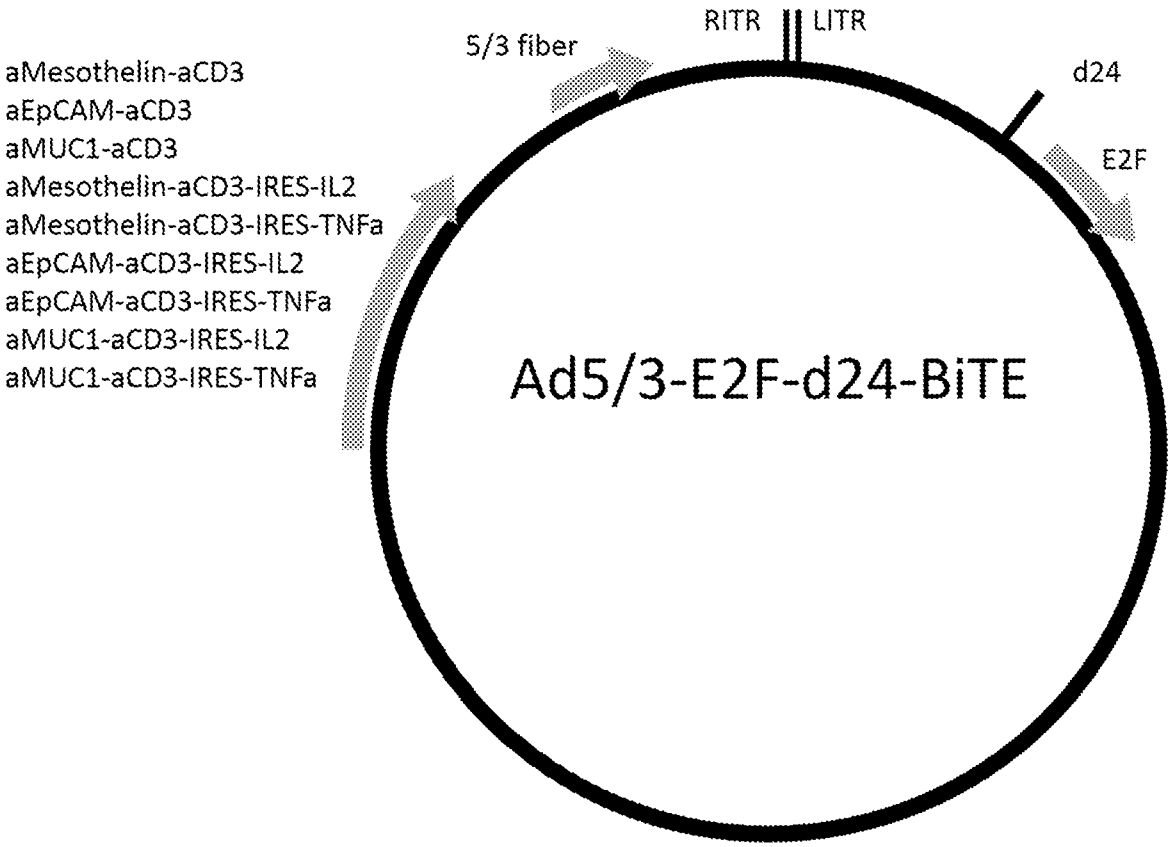

Figure 21.

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2016/050164

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K35/761 C07K16/28 C07K16/30 C12N15/861
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/138314 A1 (BAYLOR COLLEGE MEDICINE [US]) 12 September 2014 (2014-09-12) cited in the application paragraphs [0012], [0016], [0147]; claims 1-7	1-27
Y	FENG YU ET AL: "T-cell Engager-armed Oncolytic Vaccinia Virus Significantly Enhances Antitumor Therapy", MOLECULAR THERAPY, vol. 22, no. 1, 17 October 2013 (2013-10-17), pages 102-111, XP055122707, ISSN: 1525-0016, DOI: 10.1038/mt.2013.240 page 109, column 1, paragraph 3; figures 1,6,7	1-27

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 May 2016	14/06/2016

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Lonnoy, Olivier

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/FI2016/050164

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2014/170389 A1 (TILT BIOTHERAPEUTICS OY [FI]) 23 October 2014 (2014-10-23) cited in the application claim 23	1-27
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; May 2013 (2013-05), KANERVA ANNA ET AL: "Antiviral and Antitumor T-cell Immunity in Patients Treated with GM-CSF-Coding Oncolytic Adenovirus", XP002758197, Database accession no. PREV201300449851 abstract	1-27
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; February 2015 (2015-02), HEMMINKI OTTO ET AL: "Immunological data from cancer patients treated with Ad5/3-E2F-Delta 24-GMCSF suggests utility for tumor immunotherapy", XP002758198, Database accession no. PREV201500393984 abstract	1-27
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; November 2014 (2014-11), HEMMINKI AKSELI ET AL: "Armed oncolytic adenovirus can overcome critical obstacles in adoptive T-cell therapy of solid tumors", XP002758199, Database accession no. PREV201500074278 abstract	1-27
A,P	ALBERTO FAJARDO CARLOS ET AL: "Bi-specific T-cell engager-armed oncolytic adenoviruses as a strategy to improve antitumor efficacy", HUMAN GENE THERAPY, vol. 26, no. 9, September 2015 (2015-09), pages A13-A14, XP002758200, ANNUAL CONFERENCE OF THE BRITISH-SOCIETY-FOR-GENE-AND-CELL-THERAPY; GLASGOW, UK; JUNE 09 -11, 2015 the whole document	1-27

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/FI2016/050164

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014138314 A1	12-09-2014	CA 2903096 A1	12-09-2014
		CN 105407902 A	16-03-2016
		EP 2964241 A1	13-01-2016
		JP 2016512199 A	25-04-2016
		US 2016000842 A1	07-01-2016
		WO 2014138314 A1	12-09-2014
WO 2014170389 A1	23-10-2014	AU 2014255733 A1	26-11-2015
		CA 2909432 A1	23-10-2014
		CN 105307671 A	03-02-2016
		EP 2986311 A1	24-02-2016
		KR 20160002971 A	08-01-2016
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