

ONCOLYTIC ADENOVIRUS, IMMUNE CHECKPOINT INHIBITOR AND
CHEMOTHERAPEUTIC AGENT COMBINATION THERAPY OF CANCER

FIELD

The present invention relates generally to virology, immunology and medicine. In certain aspects, the invention relates to combination therapy with oncolytic viruses, particularly oncolytic adenoviruses, checkpoint inhibitors, and chemotherapeutic agents for the treatment of cancer.

BACKGROUND

Chemotherapy has been used for more than half a century in the treatment of cancer. These are chemical agents with diverse mechanisms of action that are cytotoxic for cancer cells and are grouped in several categories including, but not limited to, antimicrotubule agents, alkylating agents, topoisomerase inhibitors and antimetabolites. By demonstrating good anti-tumor responses in patients, chemotherapy became standard in clinical practice for several tumor types (Tilsed et al., 2022). Clinical responses, however, are often short-lived, leading to chemotherapy-resistance, prompting the cancer to return more aggressively in some cases. New therapeutic modalities are needed to optimize current cancer treatments.

Immune checkpoint inhibitors (CPIs) have revolutionized cancer therapy and validated immunotherapy as an approach. Unfortunately, responses are seen in a minority of patients. Some patients benefit only for a limited time while the majority derive no detectable benefit, especially when it comes to common types of non-melanoma solid tumors. Thus, CPI have definitely been validated as an approach, but since only a minority of patients benefit, there is still an unmet clinical need.

An example of the above is urothelial cancer (UC), which ranks as one of the most common cancers occurring in men and women, affecting either the lower urinary tract (i.e. bladder) or less commonly, the upper urinary tract (i.e. ureter, renal pelvis). While there have been advancements in surgical resection techniques and the recent approval of a number of checkpoint inhibitors to UC treatment protocol, the 5-year survival rate for muscle invasive cancer remains largely unchanged at <50% and often involves drastic surgical intervention, such as full bladder removal (Knowles and Hurst, 2015). While CPIs and chemotherapy are effective in some patients, many patients are either nonresponsive or fail to sustain their response to CPIs and chemotherapy.

Another example is breast cancer (BC), which is the most common cancer affecting women worldwide, and is divided into subtypes based on the expression of hormone receptors; estrogen receptor (ER), progesterone receptor (PgR), and human

epidermal growth factor receptor 2 (HER2). The subtype classification for BC is particularly useful for the selection of the appropriate systemic therapy. Of these subtypes, triple-negative breast cancer (TNBC), which is negative for ER, PgR and HER2, is associated with more aggressive tumor types, higher risk of metastasis and recurrence. TNBC has limited treatment options, with standard of care including chemotherapies such as anthracycline, alkylating agents, taxanes and 5-FU. Relapsed TNBC has no standard chemotherapy regimen, and responses to treatment are typically short in duration with commonly occurring visceral and brain metastases. While CPI monotherapies have demonstrated some success as a first-line treatment of advanced TNBC, multiple ongoing clinical studies are investigating the synergistic potential of chemotherapy and CPIs.

Another example is prostate cancer (PC), which is one of the most common cancers afflicting men, and cases of advanced prostate cancer are currently on the rise. Prostate cancer typically develops slowly and can be undetected for many years as abnormal cells divide in the prostate gland. Early interventions can often be curative and involve surgical removal of the prostate, or radiotherapy either alone or alongside hormone therapy. Patients with metastatic hormone-sensitive prostate cancer (mHSPC) may initially respond to hormone therapy, but all patients eventually develop castration-resistant prostate cancer. Early treatment with cytotoxic chemotherapy docetaxel has demonstrated improved overall survival in men with mHSPC and remains as the most common chemotherapy used in advanced PC. As PC is known to be an immunologically cold tumor, its transformation into an immunologically inflamed tumor with the use of oncolytic adenoviral vectors alone or in combination with other therapeutic compositions could potentially reduce tumor burden.

After years of development, the oncolytic viruses are currently starting to be used as cancer therapeutics. Although there have been some discoveries relating to the mechanisms of action and factors that influence the efficacy of the viruses, there is still a need to identify pathways that determine the overall response to virotherapy. In clinical trials, oncolytic viruses have demonstrated a favorable safety profile and promising efficacy. However, there is still room for improvement in the responses, especially in patients with a significant metastasis burden. Further characterization of pathways related to the activity of oncolytic viruses could reveal potential targets for improving the efficacy of virotherapy.

WO2014170389 relates to oncolytic adenoviral vectors alone or together with therapeutic compositions for therapeutic uses and therapeutic methods for cancer.

SUMMARY

This disclosure provides means to improve cancer therapy response by enhancing immune activation with the administration of immunogenic agents, specifically oncolytic adenovirus and checkpoint inhibitors, with chemotherapeutic agents.

- 5 Oncolytic viruses are able to selectively replicate in and lyse cancer cells, and oncolytic viruses can be armed with transgenes to enhance therapeutic efficacy.

CD40L is one such transgene, which is transiently expressed by T-cells, and binds to its receptor CD40 on antigen presenting cells. The CD40-CD40L interaction results in robust immune response involving dendritic cells and B cells. In addition to a CD40L
10 armed adenovirus (preferably TILT-234) and a checkpoint inhibitor (preferably anti-PD1), the incorporation of a chemotherapeutic agent, preferably paclitaxel (also referred as PTX), provides unexpected synergistic effects for the combination therapy.

PTX is an antimicrotubule agent drug used in the treatment of various cancer types,
15 and has additionally demonstrated the ability to induce immunogenic cell death. This disclosure shows that CD40L-armed adenovirus therapy enhances anti-PD-1 and chemotherapy efficacy in an unexpected fashion by transforming the tumor microenvironment (TME) into an immunogenic environment, thus resulting in decreased tumor burden.

20 Enhancing tumor immunogenicity by administration of TILT-234 holds promise for converting immunologically cold tumors into inflamed tumors, primed for a response to CPIs. TILT-234 induced lysis results in the release of virus and tumor associated antigens that can activate both the innate and adaptive immune systems resulting in immune cell infiltration of tumors (Kyo et al., 2008). In addition, PTX can also induce
25 the release of immunogenic cell death related damage-associated molecular patterns, and enhance infiltration of tumor infiltrating T cells. The combination of immunogenic agents TILT-234 and PTX can aid in the generation of an inflamed tumor microenvironment thus leading to enhanced immune cell infiltration for CPIs to act upon.

30 The present invention is based on a discovery that administration of an oncolytic adenoviral virus coding for CD40L and the immune checkpoint inhibitor anti-PD-L1 or PD-1, in combination with paclitaxel chemotherapy, to clinically relevant cancer models results in a significant enhancement in cytotoxicity against the treated cancer and a significant decrease in tumor growth relative to the said administration of the
35 adenoviral vector and the immune checkpoint inhibitor alone or the paclitaxel chemotherapy alone. Accordingly, in several embodiments, the present application

- provides a combination therapy for use in the treatment and/or prevention of cancer and/or the establishment of metastases in a mammal and/or for use in initiating, enhancing or prolonging an anti-tumor response in a mammal comprising administering to the mammal (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1 and (c) a chemotherapeutic agent. In a specific embodiment, said chemotherapeutic agent can be an antimicrotubule agent such as paclitaxel, a topoisomerase inhibitor such as epirubicin, an alkylating agent such as cyclophosphamide or cisplatin, or an antimetabolite such as gemcitabine.
- 10 In certain aspects, administration of an oncolytic virus and immune checkpoint inhibitor to a subject with cancer during chemotherapy provides an enhanced and even synergistic anti-tumor effects compared to any of the treatments alone.
- In other related aspects, a method for enhancing, potentiating or prolonging the effects of the checkpoint inhibitor and chemotherapy or enabling the toxicity or dose or number of treatments of the checkpoint inhibitor and chemotherapy to be reduced, comprising administering to a mammal in need thereof (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1 and (c) a chemotherapeutic agent is provided.
- 20 Furthermore, the present invention relates to the use of a serotype 3 (Ad3) oncolytic adenoviral vector comprising: a deletion in the E3 area and a tumor specific promoter for expression of a CD40L transgene in the place of the deleted area of E3.

BRIEF DESCRIPTION OF THE DRAWINGS

- Figure 1. Paclitaxel and TILT-234 combination results in highest cell killing in UM-UC-3 cells and in ex vivo tumor histocultures.** A statistically significant enhancement of cell killing in the paclitaxel plus TILT-234 combination therapy group compared to either monotherapy alone ($p < 0.05$) is observed (Figure 1a). Patient sample 1 demonstrates a trend in enhanced cell killing in the combination therapy group (Figure 1b). Patient sample 2 demonstrated decreased susceptibility to TILT-234 treatment alone, however, combination with paclitaxel resulted in the lowest cell viability in all treatment groups (Figure 1c). Data sets were analyzed for statistical significance using unpaired T-test. Presented as mean $\pm$ SEM. * p -value < 0.05 , *** p -value < 0.001 , **** p -value < 0.0001 .

- Figure 2. Summary of treatment schedule where adenovirus was administered every 3 days, followed by aPD-1 and PTX every 6 days.**

Figure 3. Triple combination therapy of anti-PD1, paclitaxel and TILT-234

results in improved tumor control in a humanized mouse model in vivo. In vivo characterization of tumor response to TILT-234 in combination with paclitaxel and aPD-1. Statistical significance analyzed by linear-mixed model. Results are presented as mean $\pm$ SEM. ***p-value < 0.001.

Figure 4. Evaluation of cytotoxic effects of TILT-123, paclitaxel and anti-PD-1 in surgically resected urothelial tumors.

Single cell suspensions from resected tumors were plated and treated with different combination of TILT-234 (250 VP), paclitaxel (0.01 μ M) and anti-PD-1 (20 μ g/ml). After 6 days of incubation, differences in cell proliferation were assessed by MTS assay. Data represent mean $\pm$ SEM. **p-value < 0.001, ***p-value < 0.0001 assessed by one-way ANOVA with Tukey's post hoc test.

Figure 5. Flow cytometry analysis of T cell activation in mouse tumors.

Median fluorescence intensity of Granzyme B and IFNG in **a)** CD4⁺ T cells and **b)** CD8⁺ T cells. Data are presented as mean $\pm$ SEM. n = 6-7 per treatment group. *p-value < 0.05, **p-value < 0.001, ***p-value < 0.0001, p-value < 0.00001 assessed by one-way ANOVA with Tukey's post hoc test.

EMBODIMENTS

In several embodiments, a combination therapy for use in the treatment of cancer and/or the establishment of metastases in a mammal is provided comprising administering to the mammal (i) an oncolytic adenoviral vector encoding CD40L in combination with (ii) an immune checkpoint inhibitor and with (iii) a first chemotherapeutic agent, such as paclitaxel and/or with (iv) a second chemotherapeutic agent, such as cisplatin, epirubicin, gemcitabine, or cyclophosphamide. In a preferred embodiment, said immune checkpoint inhibitor selectively binds to PD-L1 or PD-1. In preferred embodiments, said oncolytic adenoviral vector is administered simultaneously or sequentially with the immune checkpoint inhibitor and said first chemotherapeutic agent, such as paclitaxel. In an embodiment, said oncolytic adenoviral vector is administered sequentially with the immune checkpoint inhibitor and said first chemotherapeutic agent within 24 or 48 hours, or within 3-21 days.

Oncolytic Virus

In preferred embodiments, the oncolytic virus of the combination therapy is an oncolytic adenovirus.

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. **WO2014170389** discloses examples of oncolytic adenoviral vectors encoding cytokine CD40L as transgene(s) that can be used in this invention.

5 The vectors may be modified in any way known in the art, *e.g.* by deleting, inserting, mutating or modifying any viral areas. The vectors are made tumor specific with regard to replication.

As used herein, expression "adenovirus serotype 3 (Ad3) nucleic acid backbone" refers to the genome of Ad3.

10 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 Rb and E1A requires eight amino acids (121 to 127) of the E1A protein conserved region, which can be deleted. The vector
15 can comprise 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.

20 It is also possible to replace E1A endogenous viral promoter for example by a tumor specific promoter. hTERT (human telomerase reverse transcriptase) is regulated by multiple tumor suppressor gene products, which at least in part constitute to the cancer specific upregulation of hTERT promoter. This property can be utilized in developing oncolytic viruses that replicate specifically in cancer cells. The vectors of
25 the present invention comprise the tumor specific hTERT promoter for expression of E1A.

In one embodiment of the invention the oncolytic adenoviral vector is based on an adenovirus serotype 3 (Ad3) nucleic acid backbone, and comprises the following: a deletion in the E3 area, and a tumor specific promoter (*e.g.*, CMV or E2F) for
30 expression of a transgene (*e.g.*, CD40L) in the place of the deleted area of E3. In one embodiment of the invention, the adenoviral vector is based on a human adenovirus. (The construction of the TILT-234 type viral vectors Ad3-hTERT-E3del-CMV-CD40L and Ad3-hTERT-E3del-E2F-CD40L is disclosed in **WO2014170389**.)

In another embodiment of the invention E3 gp19kis kept in the vector but one or
35 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).

In a specific embodiment of the invention the oncolytic adenoviral vector is based on an adenovirus serotype 3 (Ad3) nucleic acid backbone, and comprises the following: a promoter (e.g. hTERT) for tumor specific expression of E1A, a deletion in the E3
5 area (e.g. a deletion affecting E3 9-kDa, E3 10.2 kDa, E3 15.2 kDa and E3 15.3 kDa), and a tumor specific promoter (e.g. CMV or E2F) for expression of a transgene (e.g. CD40L) in the place of the deleted area of E3. In one embodiment of the invention, the nucleic acid backbone of the vector is fully adenovirus serotype 3. In one embodiment of the invention in Ad3 delE3 viruses the following features have
10 been deleted: E3 9-kDa, E3 10.2-kDa, E3 15.2-kDa, E3 15.3-kDa and furthermore, CD40L with a promoter (CMV or E2F) has been inserted in their place. These viruses induce apoptosis of tumor cells and triggers several immune mechanisms, including a T-helper type 1 (TH1) response, which leads to activation of cytotoxic T cells and reduction of immunosuppression.

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

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

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

Cytokines participate in immune response by acting through various mechanisms
30 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.

35 Particular embodiments of the present invention include viral vectors coding for at

least one cytokine. Cytokines used in the present invention can be selected from any known cytokines in the art. In one embodiment of the invention the cytokine is selected from a group consisting of interferon alpha, interferon beta, interferon gamma, complement C5a, IL-2, TNFalpha, CD40L, IL12, IL-23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7 and XCL2. In a specific embodiment of the invention the cytokine is CD40L.

The viral vectors of the invention may code for one, two, three, four, five or more cytokines. In one embodiment of the invention the oncolytic adenoviral vector codes for two or more cytokines. These two cytokines may be any known cytokines, for example including but not limited to the ones listed above. The two cytokines may be different cytokines. In one embodiment of the invention the oncolytic adenoviral vector codes for any two or more cytokines selected from a cytokine group consisting of interferon alpha, interferon beta, interferon gamma, complement C5a, GMCSF, IL-2, TNFalpha, CD40L, IL12, IL-23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7 and XCL2, or the oncolytic adenoviral vector codes for CD40L and a cytokine or cytokines selected from a cytokine group consisting of interferon alpha, interferon beta, interferon gamma, complement C5a, GMCSF, TNFalpha, IL-2, IL12, IL-23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7 and XCL2. In a specific embodiment of the invention the

cytokine is CD40L. In an embodiment, the oncolytic adenoviral vector codes for only one transgene, said transgene being CD40L. Preferably, said CD40L transgene expresses a CD40L cytokine, which is secreted from the infected tumor cell. More preferably, said CD40L transgene is not expressing a trimerized CD40L (TMZ-
5 CD40L). 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.

10 In one embodiment of the invention the virus vector comprises an internal ribosomal entry site (IRES) or optionally a ribosome shunt site 2A between the two transgenes. Thus, IRES or a ribosome shunt site 2A may be between any cytokines, such as CD40L and any other cytokine, preferably selected from the above listed cytokine group. As used herein "IRES" refers to a nucleotide sequence that enables initiation of the translation in the middle of a messenger RNA sequence in protein synthesis.
15 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 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).

20 In summary, the key advantages of the present invention utilizing viral vectors comprising at least one cytokine transgene are: i) cytokines and virus *per se* cause a danger signal which recruits T cells and other immune cells to tumors, ii) cytokines induce T-cell proliferation both at the tumor and in local lymphoid organs, iii) cytokines and virus *per se* are able to induce T cells (both natural and innate anti-
25 tumor T cells, or even an adoptive T-cell graft) to propagate at the tumor, iv) 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
30 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.

35 In summary, the present invention reveals that the replication of oncolytic virus can recruit T cells and induce danger signals at the tumor, reducing immunosuppression and cellular anergy. These effects are mediated through pathogen associated

molecular pattern recognition receptors, an evolutionarily conserved mechanism for inducing immunity and not subject to tolerance. The present invention also reveals that an added benefit of the oncolytic platform, capable of replication in tumors but not normal cells, is self-amplification at the tumor. In addition, the oncolytic effect
5 *per se* may add to the overall anti-tumor effect in humans.

Checkpoint inhibitor

Immune checkpoint proteins interact with specific ligands which send a signal into T cells that inhibits T-cell function. Cancer cells exploit this by driving high level expression of checkpoint proteins on their surface thereby suppressing the anti-
10 cancer immune response.

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

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

In some embodiments, the checkpoint inhibitor of the combination is an antibody. The
25 term “antibody” as used herein encompasses naturally occurring and engineered antibodies as well as full length antibodies or functional fragments or analogs thereof that are capable of binding *e.g.* the target immune checkpoint or epitope (*e.g.* retaining the antigen-binding portion). The antibody for use according to the methods described herein may be from any origin including, without limitation, human,
30 humanized, animal or chimeric and may be of any isotype with a preference for an IgG1 or IgG4 isotype and further may be glycosylated or non-glycosylated. The term “antibody” also includes bispecific or multispecific antibodies so long as the antibody(s) exhibit the binding specificity herein described. Preferably, the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-L1, more
35 preferably selected from the group consisting of: BMS-936559, LY3300054, atezolizumab, durvalumab and avelumab. Examples of monoclonal antibodies that

bind to human PD-1, are described in US 7521051, US 8008449, and US 8354509. Specific anti-human PD-1 mAbs useful as the PD-1 antagonist in the treatment method include, without limitation: pembrolizumab (MK-3475), nivolumab (BMS-936558), cemiplimab, dostarlimab, retifanlimab, toripalimab, and the humanized
5 antibodies h409A11, h409A16 and h409A17, which are described in WO2008156712.

Humanized antibodies refer to non-human (e.g. murine, rat, etc.) antibodies whose protein sequences have been modified to increase similarity to a human antibody. Chimeric antibodies refer to antibodies comprising one or more element(s) of one
10 species and one or more element(s) of another species, for example a non-human antibody comprising at least a portion of a constant region (Fc) of a human immunoglobulin.

Many forms of antibody can be engineered for use in the combination of the invention, representative examples of which include an Fab fragment (monovalent fragment
15 consisting of the VL, VH, CL and CHI domains), an F(a'')₂ fragment (bivalent fragment comprising two Fab fragments linked by at least one disulfide bridge at the hinge region), a Fd fragment (consisting of the VH and CHI domains), a Fv fragment (consisting of the VL and VH domains of a single arm of an antibody), a dAb fragment (consisting of a single variable domain fragment (VH or VL domain), a single chain Fv
20 (scFv) comprising the two domains of a Fv fragment, VL and VH, that are fused together, eventually with a linker to make a single protein chain.

In some embodiments, checkpoint inhibitors (also referred to as CPIs) of the combination therapy are antibodies or fragments thereof that specifically bind to an immune checkpoint protein PD-L1 or PD-1. In particularly preferred embodiments, the
25 immune checkpoint inhibitor is a monoclonal antibody, a fully human antibody, a chimeric antibody, a humanized antibody or fragment thereof that capable of at least partly antagonizing PD-L1 or PD-1.

Chemotherapy agents

The term "chemotherapy", as used herein, refers to treatment with a cytostatic or
30 cytotoxic agent (i.e., a chemotherapeutic agent) to reduce or eliminate the growth or proliferation of undesirable cells, for example cancer cells. Thus, the use of chemotherapeutic agents or combinations of chemotherapeutic agents, is aimed to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing. When chemotherapy is taken by mouth or injected into a vein or muscle,
35 the drugs enter the bloodstream and can reach cancer cells throughout the body (systemic chemotherapy). When chemotherapy is placed directly into the

cerebrospinal fluid, an organ, or a body cavity such as the abdomen, the drugs mainly affect cancer cells in those areas (regional chemotherapy).

The term “chemotherapy agent”, as used herein, means any compound or agent that directly or indirectly kills tumor cells as part of the anti-tumour effect. This group of therapeutic agents is a broad one covering many chemotherapeutic agents having different mechanisms of action. Generally, chemotherapeutic agents can be classified according to the mechanism of action.

The term “antimicrotubule agent”, as used herein, includes, but not limited to, taxanes, e.g., paclitaxel and docetaxel; vinca alkaloids, e.g., vinblastine sulfate, vincristine sulfate, and vinorelbine.

The term “alkylating agent”, as used herein, includes, but is not limited to, chemotherapy agents such as cyclophosphamide, ifosfamide, melphalan, nitrosourea, cisplatin, carboplatin, oxaliplatin, heptaplatin, denaplatin, and enloplatin.

The term “antimetabolite”, as used herein, includes, but is not limited to, chemotherapy agents such as gemcitabine, 5-FU, capecitabine, and methotrexate.

The term “topoisomerase inhibitor”, as used herein, includes, but is not limited to, chemotherapy agents such as epirubicin and doxorubicin.

In one embodiment of the invention, the chemotherapeutic agent is selected from the group of antimicrotubule agents. Some examples of antimicrotubule agents are paclitaxel, docetaxel, and cabazitaxel.

In one embodiment of the invention, the chemotherapeutic agent is selected from the group consisting of: topoisomerase inhibitor such as epirubicin or doxorubicin, alkylating agents such as cyclophosphamide, or cisplatin, and antimetabolites such as gemcitabine.

In some embodiments, the combination treatment comprises the use of antimicrotubule agents, such as paclitaxel, for enhancing the effectiveness of the combination treatment. The combination treatment may further comprise the use of a first and a second chemotherapeutic agent, such as cisplatin together with antimicrotubule agents for further enhancing the effectiveness of the combination treatment.

In an embodiment of the invention, paclitaxel (PTX) can be nab-paclitaxel, wherein paclitaxel is bound to albumin nanoparticles. In a preferred embodiment, the patient is given only one type of chemotherapy agent in the present combination treatment, the chemotherapy agent being paclitaxel (PTX).

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
5 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 is mediated by the E2F promoter, which only
10 activates in the presence of free E2F, as seen in Rb/p16 pathway defective cells. In the absence of free E2F, no transcription of E1A occurs and the virus does not replicate. Inclusion of the E2F promoter is important to prevent expression of E1A in normal tissues, which can cause toxicity both directly and indirectly through allowing transgene expression from the E3 promoter.

15 In another embodiment, the presence of a human telomerase (hTERT) promoter in the viral construct allows for tumor selective viral replication in cancer cells. Telomerase is responsible for maintaining the lengths of telomeres and is not active in adult cells but is highly active in cancer cells. This telomerase activity in cancer cells allows cells to replicate and proliferate in an uncontrolled manner. The hTERT
20 promoter therefore confers neoplasm selective replication by only becoming active and driving expression of viral genes needed for replication in cancer cells with activated telomerase.

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

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

As used herein, the term “treatment” or “treating” refers to administration of at least oncolytic adenoviral vectors and checkpoint inhibitors that selectively binds to PD-L1
35 or PD-1 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. In an embodiment, the treatment may comprise the administration of a chemotherapeutic agent, such as paclitaxel to provide synergistic effects to the treatment. In a further embodiment, the treatment may comprise the administration of a second chemotherapeutic agent to provide

5 further synergistic effects to the treatment. Therapeutic effect may be assessed by monitoring the symptoms of a patient, tumor markers in blood, or for example a size of a tumor, or the length of survival of the patient, or the length of time in which the patient is free of tumor progression.

- 10 In another embodiment of the invention the cancer or tumor is selected from a group consisting of nasopharyngeal cancer, synovial cancer, hepatocellular cancer, renal cancer, cancer of connective tissues, melanoma, lung cancer, bowel cancer, colon cancer, rectal cancer, colorectal cancer, brain cancer, throat cancer, oral cancer, liver cancer, bone cancer, pancreatic cancer, choriocarcinoma, gastrinoma,
- 15 pheochromocytoma, prolactinoma, T-cell leukemia/lymphoma, neuroma, von Hippel-Lindau disease, Zollinger-Ellison syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, esophagus
- 20 cancer, gall bladder cancer, head and neck cancer, eye cancer, kidney cancer, Wilms' tumor, Kaposi's sarcoma, prostate cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, parathyroid cancer,
- 25 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
- 30 cancer, nerve cancer, palate cancer, parotid gland cancer, peritoneum cancer, pharynx cancer, pleural cancer, salivary gland cancer, tongue cancer and tonsil cancer.

Preferably, the cancer or tumor treated is selected from the group consisting of

35 urothelial cancer, bladder cancer, prostate cancer and breast 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.

In an embodiment, the present treatment is the subject's or patient's first cancer treatment. In another embodiment of the invention, the subject or patient to be treated according to the present invention has already failed at least one previous chemotherapy treatment. In another embodiment of the invention, the subject or patient to be treated according to the present invention has already failed at least one previous immunotherapy treatment such as a CPI treatment, *i.e.* the cancer of the patient is a checkpoint inhibitor (CPI) refractory tumor. In a preferred embodiment, the present invention is directed to the treatment of a CPI refractory tumor.

Pharmaceutical composition

A pharmaceutical composition of the invention comprises at least one type of viral vectors of the invention. Preferably, the present invention provides a pharmaceutical composition containing (a) an oncolytic virus in combination with (b) a checkpoint inhibitor and (c) a chemotherapeutic agent, such as paclitaxel, cyclophosphamide, epirubicin, cisplatin and gemcitabine. The present invention also provides said pharmaceutical combination for use in the treatment of cancer. Furthermore, the composition may comprise at least two, three or four different vectors. In addition to the vector and checkpoint inhibitor and said chemotherapeutic agent, a pharmaceutical composition may also comprise other therapeutically effective agents, any other agents such as pharmaceutically acceptable carriers, buffers, excipients, adjuvants, additives, preservatives, antiseptics, filling, stabilising and/or thickening agents, and/or any components normally found in corresponding products. Selection of suitable ingredients and appropriate manufacturing methods for formulating the compositions belongs to general knowledge of a man skilled in the art.

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.

A pharmaceutical kit of the present invention comprises an oncolytic adenoviral

vector encoding CD40L as a transgene, one or more immune checkpoint inhibitors that selectively binds to PD-L1 or PD-1 and, a chemotherapeutic agent, preferably paclitaxel. The oncolytic adenoviral vector encoding CD40L as a transgene is formulated in a first formulation and said one or more immune checkpoint inhibitors
5 that selectively binds to PD-L1 or PD-1 are formulated in a second formulation, the third formulation containing a chemotherapeutic agent, preferably paclitaxel, cyclophosphamide, epirubicin, cisplatin or gemcitabine. In another embodiment of the invention the first, the second and the third formulations are for simultaneous or sequential, in any order, administration to a subject. In another embodiment, said kit
10 is for use in the treatment of cancer or tumor.

Administration

The adenoviral vector, checkpoint inhibitor, chemotherapeutic agent, or pharmaceutical composition comprising any of them may be administered to any
15 mammal subject. In a specific embodiment of the invention, the subject is a human. A mammal may be selected from a group consisting of pets, domestic animals and production animals.

Any conventional method may be used for administration of the vector, checkpoint
20 inhibitor, chemotherapeutic agent, or composition to a subject. The route of administration depends on the formulation or form of the composition, the disease, location of tumors, the patient, comorbidities and other factors. Accordingly, the dose amount and dosing frequency of each therapeutic agent in the combination depends in part on the particular therapeutic agent, the severity of the cancer being treated,
25 and patient characteristics. Preferably, a dosage regimen maximizes the amount of each therapeutic agent delivered to the patient consistent with an acceptable level of side effects. In a preferred embodiment, the checkpoint inhibitor is administered in an amount from about 2 mg/kg to 50 mg/kg, more preferably about 2 mg/kg to 25 mg/kg.

30 In another preferred embodiment, the chemotherapeutic is an antimicrotubule agent (such as paclitaxel) which is administered in the dosage range of about 60-300 mg/m² (including for example about 80-260 mg/m², for example about 100 mg/m²). In an embodiment, paclitaxel is administered in the range of 100mg/m²-260mg/m². Examples of dosage schedules for paclitaxel are the following (without limitation): i)
35 breast cancer: every 3 weeks; ii) lung cancer: day 1, 8, and 15 in a 21-day cycle; and iii) pancreatic cancer: day 1, 8, and 15 in a 28-day cycle.

In another preferred embodiment, the chemotherapeutic is an antimetabolite agent (such as gemcitabine) which is administered in the dosage range of about 60-3000 mg/m² (including for example about 80-2600 mg/m², for example about 1000 mg/m²). In a specific embodiment, gemcitabine is administered in the dosage range
5 of about 1000-1250 mg/m² depending on the cancer indication.

In another preferred embodiment, the chemotherapeutic is an alkylating agent (such as cyclophosphamide, cisplatin, or epirubicin) which is administered in the dosage range of about 20-300 mg/m² (including for example about 80-260 mg/m², for
10 example about 100 mg/m²). In a specific embodiment, epirubicin is administered in the dosage range of about 45-135 mg/m² depending on the cancer indication. In another specific embodiment, cyclophosphamide is administered in the dosage range of about 10-50 mg/m² depending on the cancer indication. In another specific embodiment, cisplatin is administered in the dosage range of about 20-100 mg/m²
15 depending on the cancer indication.

In one embodiment of the invention, the separate administration(s) of (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) a
20 chemotherapeutic agent to a subject is (are) conducted simultaneously or consecutively, in any order. This means that (a), (b) and (c) may be provided in a single unit dosage form for being taken together or as separate entities (e.g. in separate containers) to be administered simultaneously or with a certain time difference. This time difference may be between 1 hour and 1 week, preferably
25 between 12 hours and 3 days, more preferably up to 24 or 48 hours. In a preferred embodiment, the first administration of the adenoviral vector is conducted before the first administration of the checkpoint inhibitor or the first administration of the chemotherapeutic agent. In addition, it is possible to administer the virus via another administration way than the checkpoint inhibitor or the chemotherapeutic agent. In
30 this regard, it may be advantageous to administer either the virus and/or checkpoint inhibitor intratumorally and the chemotherapeutic agent systemically or orally. In a particular preferred embodiment, the virus, the checkpoint inhibitor and/or the chemotherapeutic agent are administered intravenously. Preferably, the virus, the checkpoint inhibitor and the chemotherapeutic agent are administered as separate
35 compounds. Concomitant treatment with the three agents is also possible.

As used herein "separate administration" or "separate" refers to a situation, wherein (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more

immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) the chemotherapeutic agent are three different products or compositions distinct from each other.

- 5 Only one combined administration of (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) the chemotherapeutic agent may have therapeutic effects. There may be any period between the administrations depending for example on the patient and type, degree or location of cancer. In one embodiment
- 10 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, most specifically up to 24 or 48 hours between the consecutive administration of (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) the chemotherapeutic agent and/or there are
- 15 several administrations of a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) the chemotherapeutic agent. The numbers of administration times of (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds
- 20 selectively to PD-L1 or PD-1, and (c) the chemotherapeutic agent may also be different during the treatment period. Oncolytic adenoviral vectors, checkpoint inhibitors or chemotherapeutic agents 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
- 25 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. In another specific embodiment, administration of the adenoviral vector is carried out three times (in one embodiment the first dose is given intravenously, second and third dose intratumorally) and of the
- 30 checkpoint inhibitor/chemotherapeutic agent one time or two times during the first four weeks, then the viral vector and the checkpoint inhibitor/chemotherapeutic agent are administered once per month. The length of the treatment period may vary, and for example may last from two to 24 months or more.
- 35 In a specific embodiment of the invention (a) an oncolytic adenoviral vector encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) the chemotherapeutic agent 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 24 months or more.

In one embodiment of the invention, the administration of oncolytic virus is conducted through an intratumoral, intranasal, 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. Checkpoint inhibitors may be administered intravenously or intratumorally. In one embodiment the administration of the checkpoint inhibitors is conducted through an intratumoral, intranasal, intra-arterial, intravenous, intrapleural, intravesicular, intracavitary or peritoneal injection, or an oral administration. Chemotherapeutic agents may be administered intravenously or intratumorally. In one embodiment the administration of the chemotherapeutic agent is conducted through an intratumoral, intranasal, intra-arterial, intravenous, intrapleural, intravesicular, intracavitary or peritoneal injection, or an oral administration.

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

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, or other anti-cancer 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.

Other embodiments

The present invention is also directed to (a) an oncolytic adenoviral vector encoding

CD40L as a transgene, (b) one or more immune checkpoint inhibitors, and (c) a chemotherapeutic agent, preferably paclitaxel, for use in the treatment of cancer or tumor. Preferably, a human cancer or tumor.

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

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

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

- 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
20 embodiments are not limited to the examples described below but may vary within the scope of the claims.

EXPERIMENTAL SECTION

EXAMPLE 1

25 **Materials and methods**

Adenoviral vectors

- The Ad3-hTERT-CMV-hCD40L virus was generated from the pWEA-Ad3-GFP vector (Zafar et al., 2017). A 13.2 kb FseI/NotI digested fragment from pWEA-Ad3-GFP was inserted into modified pBluescript KS (-) vector. CMV-GFP-PA transgene cassette
30 and the 3' end of the Ad3 genome was removed using EcoRI/FseI digestion. A 1.1 kb fragment containing the Ad3 5'end, hTERT promoter and part of Ad3 E1 region was amplified from pKSB2-hTERT. The portion containing the first 800 bp of Ad3 genome was replaced by ligation of the PmeI/NheI digested PCR fragment. The GFP gene in the construct was then replaced by the hCD40L gene (pWEA-Ad3-3'end-CMV-
35 hCD40L). The final construct was formed by joining of the 13.4 kb fragment containing the 5'end of the Ad3 genome and hTERT, 6-5 kb fragment containing Ad3

3' end genome and CMV-hCD40L (pWEA-Ad3-3'end-CMV-hCD40L). Ligation product was packaged into phages using Gigapack III Plus Packaging Extract (Stratagene, La Jolla, CA) and propagated. The Ad3-hTERT-CMV-hCD40L viral genome was released by FseI digestion and transfected into 293 cells using a standard calcium phosphate method. HeLA cells were then infected with cell lysate containing Ad3-hTERT-CMV-hCD40L virus. Subsequent virus propagation was performed on A549 cells.

Cell Lines:

Human bladder cancer cell line UM-UC-3 was obtained from Sigma-Aldrich (Missouri, USA) and cultured in RPMI 1640 (BioWest, Nuallie, France) supplemented with 10 % fetal bovine serum, 1 % L-glutamine, 1 % penicillin, and 1% streptomycin.

Patient Sample Processing:

Single-cell tumor digests were prepared from patient tumors (bladder cancer) using a previously described protocol (Taipale et al., 2018). Briefly, upon surgical resection patient tumors were sliced into smaller fragments and placed in RPMI 1640 supplemented with 1% L-glutamine, 1% penicillin/streptomycin, collagenase type I, collagenase type IV, DNase I, and elastase (Worthington Biochemical, New Jersey, USA) at 37 °C for 2 hours. After digestion, the sample was filtered using a 70 µM filter, and ACK lysis buffer (Sigma-Aldrich, Missouri, USA) was added to remove the remaining red blood cells. Samples were then frozen in freezing media (90% FBS, 10% DMSO) and stored at -180 °C until future use.

Cell Viability Assay:

UM-UC-3 cells were seeded in RPMI 1640 (2% FBS) at a density of 1E4 cells/well in a 96-well flat bottom plate and incubated at 37 °C for 24 hours. When patient samples were used, cells were seeded at a density of 1E5 cells/well in RPMI 160 (10% FBS). Approximately 24 hours post-seeding, cells were treated with TILT-234 (250 VP), paclitaxel (Fresenius Kabi, Germany) (0.01 µM), or anti-PD-1 (20 µg/ml) (Merck & Co, New Jersey, USA) alone or in combination. 72 hours post-treatment, CellTiter 96 AQueous One Solution Proliferation Assay reagent (Promega, Wisconsin, USA) was added and incubated for 2 hours at 37°C. For human patient samples, CellTiter 96 AQueous One Solution Proliferation Assay reagent was added 144 hours (six days) post-treatment. Following the two-hour incubation, the absorbance of each well was measured at 490 nm using a Hidex Sense plate reader.

PBMC Expansion:

HLA-A 02:01 PBMCs obtained from Cellular Technology Limited (CTL, Ohio, USA) were thawed and washed using CTL-Anti-Aggregate Wash (CTL, Ohio, USA). PBMC expansion was performed according to a modified 'rapid expansion' protocol described elsewhere (Jin et al., 2012). 1E7 cells were plated into 6-well G-rex culturing plates (Wilson Wolf, Minnesota, USA) in 30 mL of TIL medium consisting of RPMI 1640 supplemented with 20% FBS, 1% L-glutamine, 1% P/S, 15 mM HEPES, 1 mM Na-pyruvate, 50 uM 2-mercaptoethanol, 100 IU/ml recombinant human IL-2 (Peprotech, New Jersey, USA), 50 ng/ml anti-human CD3 (Thermofisher, Massachusetts, USA). PBMCs were incubated at 37 °C for 3 days. Irradiated PBMCs from multiple donors were then added to the PBMCs at a ratio of 1:200 in 1:1 media (TIL:REM). Rapid expansion media (REM) consisted of RPMI 1640 supplemented with 20 % FBS, 1% L-glutamine, 1% P/S and 100 IU/ml recombinant hIL-2. After 2 days of incubation, cells were counted and a cell density of 5E6 cells/well was maintained until day 14 when cells were collected, washed in plain medium, and stored at -180 °C until use.

DC Generation:

DCs were generated from HLA-A 02:01 PBMCs using a previously established protocol (Zafar et al., 2017). Briefly, HLA-A 02:01 PBMCs (CTL, Ohio, USA) were thawed and CD14+ cells were isolated using human CD14+ Microbeads (Miltenyi Biotec, North Rhine-Westphalia, Germany) as directed by the manufacturer. CD14+ cells were then cultured in a T75 flask containing 10 mL RPMI supplemented 1000 U/ml GM-CSF (Peprotech, New Jersey, USA) and 20 ng/ml IL-4 (Peprotech, New Jersey, USA). After 5 days of incubation, 50 ug/ml of UM-UC-3 cell lysate was added. The following day, 100 ng/ml lipopolysaccharide (Sigma-Aldrich, Missouri, USA) was added for overnight culturing, mature DCs were then used the next day in the animal experiment.

Animal Experiment:

2E6 UM-UC-3 cells/animal were engrafted into the lower left flank of 56 immunodeficient female (4-5 weeks old) NOD.Cg-PrkdcscidII2rgtm1Sug/JicTac (Taconic Biosciences GmbH, Leverkusen, Germany). 1E7 partially HLA-matched PBMCs and 2E3 DCs were administered through intraperitoneal injection. The following day (Day 1), mice were treated with 1E9 VP of TILT-234 administered through the tail vein (i.v. injection) on a 3-day interval for 6 rounds of treatment. Paclitaxel (Fresenius Kabi, Germany) (2.5 mg/kg) and aPD-1 (Merck & Co, New Jersey, USA) (5 mg/kg) were administered intraperitoneally (i.p.) on a 6-day interval

for 3 rounds of treatment. Tumors were measured and weights were recorded every second day until Day 6 and every day thereafter until Day 19, when the animals were euthanized. Tumor volume was calculated using the formula (formula (length × width²)/2, and percentage of tumor growth was calculated by normalizing
5 measurements to their Day 0 tumor volumes.

Results

Paclitaxel and TILT-234 combination results in highest cell killing in UM-UC-3 cells and in ex vivo tumor cultures

To assess the ability of the combination therapy to induce cell killing, UM-UC-3 cells,
10 and ex vivo samples surgically resected from urothelial cancer patients were treated with TILT-234 and paclitaxel. In the commercial cell line (UM-UC-3), we observed statistically significant enhanced cell killing in the paclitaxel plus TILT-234 combination therapy group compared to either monotherapy alone ($p < 0.05$) (**Figure 1a**). When the cytotoxic effects of the combination therapy was assessed in patient
15 samples, some variation was observed between patients' samples, most likely due to the heterogeneity of cell populations present in each individual tumor (**Figure 1 b,c**). Despite this variation, patient sample #1 demonstrates a trend in enhanced cell killing in the combination therapy group, although not statistically significant (p -value 0.0887) (**Figure 1 b**). In contrast, patient sample #2 demonstrated decreased
20 susceptibility to TILT-234 treatment alone, however, combination with paclitaxel resulted in the lowest cell viability in all treatment groups (**Figure 1 c**).

Triple combination therapy of anti-PD1, paclitaxel and TILT-234 results in improved tumor control in a humanized mouse model in vivo.

Humanized mice were treated with TILT-234 every 3 days, and paclitaxel/aPD-1
25 every 6 days for a total of 18 days, as described in **Figure 2**. Mice treated with TILT-234, paclitaxel and aPD-1 demonstrated a significant decrease in tumor growth when compared to virus, paclitaxel or aPD-1 alone ($p < 0.01$) (**Figure 3**). When comparing dual combination therapies comprising of TILT-234 combined with chemotherapy or antibody immunotherapy, there was a statistically significant benefit in combining
30 virus with aPD-1 and paclitaxel (triple combination therapy).

EXAMPLE 2

Materials and Methods

Patient Sample Processing

Upon surgical resection patient urothelial tumors (bladder cancer) were sliced into smaller fragments and placed in RPMI 1640 supplemented with 1% L-glutamine, 1% penicillin/streptomycin, collagenase type I, collagenase type IV, DNase I, and elastase (Worthington Biochemical, NJ, USA) at 37 °C for 2 hours. After digestion, the sample was filtered using a 70 uM filter and ACK lysis buffer (Sigma-Aldrich, MI, USA) was added to remove remaining red blood cells resulting in a single cell suspension. Samples were then frozen in freezing media (90% FBS, 10% DMSO) and stored at -180 °C until future use.

Cell Viability Assay

- Single cell suspensions isolated from patient urothelial tumor (bladder cancer) samples were seeded at a density of 1E5 cells/well in RPMI 160 (10% FBS). Approximately 24 hours post-seeding, cells were treated with alone or in combinations of TILT-234 (250 VP), paclitaxel (0.01 uM), and anti-PD1 (20 ug/ml). 144 hours (six days) post-treatment, Cell Titer 96 AQueous One Solution Proliferation Assay reagent was added and incubated for two hours at 37 °C. Absorbance of each well was measured at 490 nm using Hidex Sense plate reader (Hidex, Turku, Finland).

Flow Cytometry – Mouse Tumors

- Tumors were harvested from mice euthanized on Day 19 of the treatment schedule (see example 1, material and methods, animal experiment section), mechanically processed into a single cell suspension, and frozen down at -80°C. 1E6 cells/well were seeded per well for subsequent staining for flow cytometry. For intracellular staining, cells were permeabilized using BD Cytofix/Cytoperm Plus Fixation/Permeabilization kit (BD Biosciences, NJ, USA), according to manufacturer instructions and stained using the antibodies listed below. Cells were acquired with Novocyte Quanteon Flow Cytometer (Agilent, CA, USA) upon acquisition of 100k events per well. Cell gating and data processing were performed in FlowJo v10.7.1 (FlowJo LLC, OR, USA). A list of all antibodies used in this analysis can be found in Table 1.
- Table 1.

Antigen	Fluorochrome	Clone	Catalog No.	Provider
CD3	AF700	SK7	344822	BioLegend
CD4	FITC	RPA-T4	300506	BioLegend
CD8	V450	RPA-T8	560347	BD Biosciences

Granzyme B	PE	GB11	561142	BD Biosciences
IFN gamma	APC	4S.B3	502512	BioLegend
HLA-DR	BV711	G46-6	563696	BD Biosciences

Results

Triple combination results in enhanced tumor cell killing in ex vivo tumor histocultures

When comparing the cytotoxic effects of the triple combination therapy to other
 5 treatment regimens, treatment with the triple combination therapy resulted in the
 lowest cell viability, highlighting the cytotoxic capabilities of this combination in this
 patient sample (Figure 4).

Enhanced T cell granzyme B and IFNG expression in tumors of mice treated with triple combination therapy

10 Flow cytometry analysis of tumors isolated from humanized mice treated with
 different therapeutic combinations revealed significant increases in both CD4+ and
 CD8+ T cell activation with the triple combination therapy group. Specifically, the
 median fluorescence intensity (MFI) of granzyme B and IFNG was significantly
 enhanced in both CD4+ and CD8+ T cells compared to other treatment groups
 15 (Figure 5). Statistical analysis revealed significant differences between the triple
 combination therapy and both TILT-234 monotherapy and TILT-234 + PTX
 combination therapy in CD4+ and CD8+ granzyme B intensity. Additionally, CD4+
 IFNG intensity was significantly higher in the triple combination group compared to
 TILT-234 monotherapy. However, no significant differences were observed in CD8+
 20 IFNG intensity across the treatment groups

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CLAIMS

1. A method for treating cancer in a subject in need of such treatment comprising administering to the subject an effective amount of (a) an oncolytic adenoviral vector
5 encoding CD40L as a transgene, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1, and (c) a chemotherapeutic agent.
2. The method of claim 1, wherein said one or more immune checkpoint inhibitors selectively binds to PD-L1 or PD-1.
3. The method of claim 1, wherein said chemotherapeutic agent is an antimicrotubule
10 agent.
4. The method of claim 3, wherein said antimicrotubule agent is paclitaxel.
5. The method of claim 1, wherein the oncolytic adenoviral vector is administered intratumorally, intravenously, intra-arterially, or intraperitoneally.
6. The method of claim 5, wherein the oncolytic adenoviral vector is administered
15 intravenously.
7. The method of claim 2, wherein the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-L1.
8. The method of claim 7, wherein said monoclonal antibody that selectively binds to PD-L1 is selected from the group consisting of: BMS-936559, LY3300054,
20 atezolizumab, durvalumab, and avelumab.
9. The method of claim 2, wherein the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-1.
10. The method of claim 9, wherein said monoclonal antibody that selectively binds to PD-1 is selected from the group consisting of: pembrolizumab (MK-3475),
25 nivolumab (BMS-936558), cemiplimab, dostarlimab, toripalimab, and retifanlimab.
11. The method of claim 1, wherein the virus is administered in an amount from about 10^6 - 10^{14} VP, 10^6 - 10^{12} VP, 10^8 - 10^{14} VP, 10^8 - 10^{12} VP, 10^{10} - 10^{12} VP or 10^8 - 10^{10} VP.
12. The method of claim 1, wherein the checkpoint inhibitor is administered in an
30 amount from about 2 mg/kg to 25 mg/kg.

13. The method of claim 1, wherein the chemotherapeutic agent is an antimicrotubule agent selected from the group consisting of paclitaxel and docetaxel, and is preferably administered in the dosage range of about 60-300 mg/m².

14. The method of claim 1, wherein the subject has a cancer selected from
5 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,
10 Zollinger-Ellison syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, esophagus cancer, gall bladder cancer,
15 head and neck cancer, eye cancer, kidney cancer, Wilms' tumor, Kaposi's sarcoma, prostate cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, parathyroid cancer, penis cancer, pituitary cancer, soft tissue sarcoma, retinoblastoma, small intestine cancer, stomach cancer, thymus cancer,
20 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,
25 tongue cancer and tonsil cancer.

15. The method of claim 14, wherein the subject has urothelial cancer, breast cancer, prostate cancer or bladder cancer.

16. The method of claim 1, wherein said treatment is the subject's first cancer treatment.

30 17. The method of claim 1, wherein the subject has failed at least one previous chemotherapy or immunotherapy treatment such as a treatment with a checkpoint inhibitor.

18. The method of claim 1, further comprising a step of treating the subject with one or more additional therapy selected from radiotherapy, chemotherapy, antiangiogenic
35 agents or targeted therapies, such as alkylating agents, nucleoside analogs, cytoskeleton modifiers, cytostatic agents, monoclonal antibodies, kinase inhibitors.

19. The method of claim 18, wherein said chemotherapeutic agent is cisplatin, epirubicin, cyclophosphamide, or gemcitabine.

20. The method of claim 1, wherein the subject is a human.

21. The method of claim 1, wherein a first dose of the oncolytic adenoviral vector and
5 a first dose of the immune checkpoint inhibitor are simultaneously administered to the subject.

22. The method of claim 1, wherein a first dose of the oncolytic adenoviral vector and a first dose of the chemotherapy are simultaneously administered to the subject.

23. The method of claim 1, wherein a first dose of the checkpoint inhibitor and a first
10 dose of the chemotherapy are simultaneously administered to the subject.

24. The method of claim 1, wherein a first dose of the oncolytic adenoviral vector, a first dose of the immune checkpoint inhibitor, and a first dose of the chemotherapy are administered sequentially.

25. The method of claim 1, wherein the oncolytic adenoviral vector encoding CD40L
15 as a transgene encodes one or more further cytokines selected from a group consisting of interferon alpha, interferon beta, interferon gamma, complement C5a, IL-2, TNFalpha, IL12, IL-23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2,
20 CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7 and XCL2.

25 26. The method of claim 1, wherein the oncolytic adenoviral vector encoding CD40L as a transgene comprises

- a deletion in the E3 area and a tumor specific promoter for expression of the transgene in the place of the deleted area of E3.

- hTERT promoter for tumor specific expression of E1A,

30 - a nucleic acid sequence encoding CD40L in the E3 region resulting in replication-associated control of transgene expression under the viral E3 promoter.

27. The method of claim 26, wherein the E3 area comprises deletion of one or more areas selected from E3 9-kDa, E3 10.2 kDa, E3 15.2 kDa and E3 15.3 kDa

28. The method of claim 26 or 27, wherein the tumor specific promoter for expression of the transgene is CMV or E2F

5 29. The method of any of the claims 24-27, wherein the oncolytic adenoviral vector is serotype 3 (Ad3) oncolytic adenoviral vector.

30. The method of any of the claims 1-29, wherein the oncolytic adenoviral vector is coding for only one transgene, said transgene being CD40L.

31. Pharmaceutical composition comprising (a) an oncolytic adenoviral vector
10 encoding CD40L as a transgene, preferably as the only transgene in the vector, (b) one or more immune checkpoint inhibitors that preferably binds selectively to PD-L1 or PD-1 and (c) a chemotherapeutic agent.

32. The composition according to claim 31, wherein said one or more immune checkpoint inhibitors selectively binds to PD-L1 or PD-1.

15 33. The pharmaceutical composition of claim 31, wherein said oncolytic adenoviral vector encoding CD40L as a transgene comprises

- a deletion in the E3 area and a tumor specific promoter for expression of CD40L in the place of the deleted area of E3,

- hTERT promoter for tumor specific expression of E1A,

20 - a nucleic acid sequence encoding at least CD40L as transgene(s) in the E3 region resulting in replication-associated control of transgene expression under the viral E3 promoter, and

- CMV or E2F promoter for tumor specific expression of CD40L.

34. The pharmaceutical composition of claim 31, wherein said chemotherapeutic
25 agent is a taxane preferably selected from the group consisting of paclitaxel and docetaxel.

35. A kit which comprises a first container, a second container, a third container and a package insert, wherein the first container comprises at least one dose of a pharmaceutical composition containing an oncolytic adenoviral vector encoding
30 CD40L as a transgene, preferably as the only transgene in the vector, the second container comprises at least one dose of a pharmaceutical composition comprising a checkpoint inhibitor, the third container containing a chemotherapeutic agent,

preferably a taxane, and the package insert comprises instructions for treating an individual having cancer using the pharmaceutical composition(s).

36. The kit according to claim 35, wherein said oncolytic adenoviral vector encoding CD40L as a transgene comprises

5 - a deletion in the E3 area and a tumor specific promoter for expression of CD40L in the place of the deleted area of E3

- hTERT promoter for tumor specific expression of E1A,

- a nucleic acid sequence encoding CD40L in the E3 region, and

- CMV or E2F promoter for tumor specific expression of CD40L.

10 37. An oncolytic adenoviral vector encoding CD40L as a transgene together with an immune checkpoint inhibitor and a chemotherapeutic agent for use in the treatment of cancer or tumor in a subject.

38. The oncolytic adenoviral vector for use according to claim 37, wherein said immune checkpoint inhibitor selectively binds to PD-L1 or PD-1.

15 39. The oncolytic adenoviral vector for use according to claim 37 or 38, wherein said chemotherapeutic agent is an antimicrotubule agent.

40. The oncolytic adenoviral vector for use according to any of claims 37-39, wherein said antimicrotubule agent is paclitaxel.

20 41. The oncolytic adenoviral vector for use according to any of claims 37-40, wherein the oncolytic adenoviral vector is administered intranasally, intratumorally, intravenously, intra-arterially, or intraperitoneally.

42. The oncolytic adenoviral vector for use according to any one of claims 37-41, wherein the oncolytic adenoviral vector is administered intravenously.

25 43. The oncolytic adenoviral for use according to any one of claims 37-42, wherein the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-L1.

44. The oncolytic adenoviral for use according to claim 43, wherein said monoclonal antibody that selectively binds to PD-L1 is selected from the group consisting of: BMS-936559, LY3300054, atezolizumab, durvalumab, and avelumab.

45. The oncolytic adenoviral vector for use according to any one of claims 37-42, wherein the immune checkpoint inhibitor is a monoclonal antibody that selectively binds to PD-1.

46. The oncolytic adenoviral for use according to claim 45, wherein said monoclonal antibody that selectively binds to PD-1 is selected from the group consisting of:
5 pembrolizumab (MK-3475), nivolumab (BMS-936558), cemiplimab, dostarlimab, and retanflimab.

47. The oncolytic adenoviral vector for use according to any one of claims 37-46, wherein the virus is administered in an amount from about 10^6 - 10^{14} VP, 10^6 - 10^{12} VP,
10 10^8 - 10^{14} VP, 10^8 - 10^{12} VP, 10^{10} - 10^{12} VP or 10^8 - 10^{10} VP.

48. The oncolytic adenoviral vector for use according to any one of claims 37-47, wherein the checkpoint inhibitor is administered in an amount from about 2 mg/kg to 25 mg/kg.

49. The oncolytic adenoviral vector for use according to any one of claims 37-48,
15 wherein the chemotherapeutic agent is a taxane selected from the group consisting of paclitaxel and docetaxel, and is preferably administered in the dosage range of about 60-300 mg/m².

50. The oncolytic adenoviral vector for use according to any one of claims 37-49, wherein the subject has a cancer selected from the group consisting of
20 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,
25 Zollinger-Ellison syndrome, adrenal cancer, anal cancer, bile duct cancer, bladder cancer, ureter cancer, oligodendroglioma, neuroblastoma, meningioma, spinal cord tumor, osteochondroma, chondrosarcoma, Ewing's sarcoma, cancer of unknown primary site, carcinoid, carcinoid of gastrointestinal tract, fibrosarcoma, breast cancer, Paget's disease, cervical cancer, esophagus cancer, gall bladder cancer,
30 head and neck cancer, eye cancer, kidney cancer, Wilms' tumor, Kaposi's sarcoma, prostate cancer, testicular cancer, Hodgkin's disease, non-Hodgkin's lymphoma, skin cancer, mesothelioma, multiple myeloma, ovarian cancer, endocrine pancreatic cancer, glucagonoma, parathyroid cancer, penis cancer, pituitary cancer, soft tissue sarcoma, retinoblastoma, small intestine cancer, stomach cancer, thymus cancer,
35 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.

5 51. The oncolytic adenoviral vector for use according to claim 50, wherein the subject has urothelial cancer, breast cancer, prostate cancer or bladder cancer.

52. The oncolytic adenoviral vector for use according to any one of claims 37-51, wherein said treatment is the subject's first cancer treatment.

10 53. The oncolytic adenoviral vector for use according to any one of claims 37-51, wherein the subject has failed at least one previous chemotherapy or immunotherapy treatment such as a treatment with a checkpoint inhibitor.

54. The oncolytic adenoviral vector for use according to claim 53, wherein the subject has an anti-PD-1 refractory cancer, said cancer preferably being urothelial cancer, breast cancer, prostate cancer or bladder cancer.

15 55. The oncolytic adenoviral vector for use according to any one of claims 37-54, comprising administering to the subject one or more additional therapy selected from radiotherapy, chemotherapy, antiangiogenic agents or targeted therapies, such as alkylating agents, nucleoside analogs, cytoskeleton modifiers, cytostatic agents, monoclonal antibodies, kinase inhibitors.

20 56. The oncolytic adenoviral vector for use according to claim 55, wherein the chemotherapeutic agent in the additional chemotherapy is cisplatin, cyclophosphamide, epirubicin or gemcitabine, preferably together with an antimicrotubule agent.

25 57. The oncolytic adenoviral vector for use according to any one of claims 37-56, wherein the subject is a human.

58. The oncolytic adenoviral vector for use according to any one of claims 37-57, wherein a first dose of the oncolytic adenoviral vector and a first dose of the immune checkpoint inhibitor are simultaneously administered to the subject.

30 59. The oncolytic adenoviral vector for use according to any one of claims 37-57, wherein a first dose of the oncolytic adenoviral vector and a first dose of the chemotherapy are simultaneously administered to the subject.

60. The oncolytic adenoviral vector for use according to any one of claims 37-57, wherein a first dose of the checkpoint inhibitor and a first dose of the chemotherapy are simultaneously administered to the subject.

61. The oncolytic adenoviral vector for use according to any one of claims 37-57, wherein a first dose of the oncolytic adenoviral vector, a first dose of the immune checkpoint inhibitor, and a first dose of the chemotherapy are administered sequentially.

62. The oncolytic adenoviral vector for use according to any one of claims 37-61, wherein the oncolytic adenoviral vector encoding CD40L as a transgene encodes one or more further cytokines selected from a group consisting of interferon alpha, interferon beta, interferon gamma, complement C5a, IL-2, TNFalpha, IL12, IL-23, IL15, IL17, CCL1, CCL11, CCL12, CCL13, CCL14-1, CCL14-2, CCL14-3, CCL15-1, CCL15-2, CCL16, CCL17, CCL18, CCL19, CCL19, CCL2, CCL20, CCL21, CCL22, CCL23-1, CCL23-2, CCL24, CCL25-1, CCL25-2, CCL26, CCL27, CCL28, CCL3, CCL3L1, CCL4, CCL4L1, CCL5, CCL6, CCL7, CCL8, CCL9, CCR10, CCR2, CCR5, CCR6, CCR7, CCR8, CCRL1, CCRL2, CX3CL1, CX3CR, CXCL1, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL9, CXCR1, CXCR2, CXCR4, CXCR5, CXCR6, CXCR7 and XCL2.

63. The oncolytic adenoviral vector for use according to any one of claims 37-62, wherein said oncolytic adenoviral vector encoding CD40L as a transgene comprises

- a deletion in the E3 area and a tumor specific promoter for expression of CD40L in the place of the deleted area of E3,

- hTERT promoter for tumor specific expression of E1A,

- a nucleic acid sequence encoding CD40L in the E3 region, and

- CMV or E2F promoter for tumor specific expression of CD40L.

64. The oncolytic adenoviral vector for use according to claim 63, wherein the E3 area comprises deletion of one or more areas selected from E3 9-kDa, E3 10.2 kDa, E3 15.2 kDa and E3 15.3 kDa.

65. The oncolytic adenoviral vector for use according claims 63 or 64, wherein wherein the tumor specific promoter for expression of the transgene is CMV or E2F.

66. The oncolytic adenoviral vector for use according to any one of claims 37-65, wherein the oncolytic adenoviral vector is serotype 3 (Ad3) oncolytic adenoviral vector.

5 67. The oncolytic adenoviral vector for use according to any one of claims 37-66, wherein the oncolytic adenoviral vector is coding for only one transgene, said transgene being CD40L.