

## Role of Adenoviruses in Cancer Therapy

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### Abstract

Cancer is one of the leading causes of death in the world, which is the second after heart diseases. Adenoviruses (Ads) have become the promise of new therapeutic strategy for cancer treatment. The objective of this review is to discuss current advances in the applications of adenoviral vectors in cancer therapy. Adenoviral vectors can be engineered in different ways so as to change the tumor microenvironment from cold tumor to hot tumor, including; 1. by modifying Ads to deliver transgenes that codes for tumor suppressor gene (p53) and other proteins whose expression result in cell cycle arrest 2. Ads can also be modified to express tumor specific antigens, cytokines, and other immune-modulatory molecules. The other strategy to use Ads in cancer therapy is to use oncolytic adenoviruses, which directly kills tumor cells. Gendicine and Advexin are replication-defective recombinant human p53 adenoviral vectors that have been shown to be effective against several types of cancer. Gendicine was approved for treatment of squamous cell carcinoma of the head and neck by the Chinese Food and Drug Administration (FDA) agency in 2003 as a first-ever gene therapy product. Oncorine and ONYX-015 are oncolytic adenoviral vectors that have been shown to be effective against some types of cancer. The Chinese FDA agency has also approved Oncorin for the treatment of head and neck cancer. Ads that were engineered to express immune-stimulatory cytokines and other immune-modulatory molecules such as TNF- $\alpha$ , IL-2, BiTE, CD40L, 4-1BBL, GM-CSF, and IFN have shown promising outcome in treatment of cancer. Ads can also improve therapeutic efficacy of immune checkpoint inhibitors and adoptive cell therapy (Chimeric Antigen Receptor T Cells). In addition, different replication-deficient adenoviral vectors (Ad5-CEA, Ad5-PSA, Ad-E6E7, ChAdOx1-MVA and Ad-transduced Dendritic cells) that were tested as anticancer vaccines have been demonstrated to induce strong antitumor immune response. However, the use of adenoviral vectors in gene therapy is limited by several factors such as pre-existing immunity to adenoviral vectors and high immunogenicity of the viruses. Thus, innovative strategies must be continually developed so as to overcome the obstacles of using adenoviral vectors in gene therapy.

**Keywords:** Adenoviruses; Cancer; Cancer Therapy; Gene Therapy

## Background

In the past few decades, gene therapy for diseases such as cancer using adenoviral vectors has been significantly advanced. Adenoviruses (Ads) can be used either as replication-competent Ads or replication-defective adenoviral vectors for gene therapy [1]. The following are the characteristics that made Ads one of the most favorable viruses for gene therapy:- 1. Ads have unique ability to infect wide range of cell types (broad cell tropism) and have the capacity to induce strong cell mediated immunity and humoral response [2, 3]; 2. The genetics of Ads has been well known and Ads have stable genome [4]; 3. Ads have lytic replication cycle. This lytic replication cycle causes lysis of tumor cells [5]; 4. Adenoviral vectors have low pathogenicity and relatively safe and well-tolerated [4, 6]; 5. Ads have large transgene carrying capacity about and can transduce both in dividing and non- dividing cells [4, 7, 8] and 6. Ads can infect professional antigen-presenting cells that are very effective in presenting antigens to T-cells [9].

There are three generations of adenoviral vectors that have been developed for gene therapy. In the first generation, two genes are deleted (E1 and E3) so as to make the adenoviral vector replication defective, but keeping them to transduce host cells without killing them and liberating nearly 8 kb of space in the genome for the transgene and regulatory sequences [10, 11]. In the second-generation adenoviral vectors, in addition to the E1/E3 genes, E2 or E4 regions are also deleted, providing additional space for cargo sequences (nearly 10.5 kb). The third-generation adenoviral vectors is also called gutless or high capacity adenoviral vectors (HCAds) because they can accept cargo sequences up to 36 Kb, which were generated after deletion of almost all viral sequences except the sequences required for genome replication and encapsulation during vector production [12, 13]. The advantages of the third-generation adenoviral vectors are that HCAds can simultaneously encode multiple transgene cassettes. The other benefits of the third-generation adenoviral vectors is that the HCAds have less cellular toxicity and reduced immunogenicity, that efficiently transduces host cells because of the reduced stimulation of anti-adenoviral neutralizing antibodies [14, 15]. The objective of this review is to discuss current advances in applications of Ads in cancer therapy.

### General Information on Adenoviruses

Ads are non-enveloped viruses with double stranded

deoxy ribonucleic acid (DNA) genome. The genome of Ads ranges in size from 26 kb to 45 kb that is encompassed within icosahedral capsid [16, 17]. The Ads virion size ranges from 90-100 nm in diameter. There are six kinds of proteins that constitute the adenoviral capsid: penton, fiber, hexon, IX, VIII, and IIIa. The IIIa involves in the assembly of the viral structure. The fiber and penton proteins involve in the attachment and entry of the Ads into host cells and the hexon constitutes most of the viral capsid [18]. The proteins IIIa, VIII, and IX make up the virion core, which are associated with the DNA genome. The VIII is important for the stability of the viral capsid [19].

The genes of Ads can be categorized into two classes: as early genes (five early genes) and late genes (five late genes). The Ads bind to host cell through its receptors, which includes scavenger receptors, CD46, integrin  $\alpha\beta 5$  heparin sulfate proteoglycans, sialic acid etc) and gains entry into the cytoplasm [20, 21].

After entry into the target host cell by micropinocytosis [22], Ads first express the five early proteins that are coded by the five early genes (E1A, E1B, E2, E3 and E4 which are involved in protein synthesis and DNA replication. Structural proteins (L1-L5) are coded by the five late genes [23]. Following replication, the adenoviral virion leaves the host cell by killing the host cell (lytic cycle). Newly produced Ads can infect wide range of host cells including antigen presenting cells and quiescent cells.

Ads belong to the family adenoviridae that is consisted of five genera: Mastadenoviruses, Avidadenoviruses, Siadenoviruses, Atadenovirus and Ichtadenovirus. The genus Mastadenovirus is consisted of Ads that infects humans (human adenoviruses) and non-human primates [24]. The genus Aviadenovirus is consisted of Ads that are isolated from birds. Viruses that are isolated from birds, ovine, bovine, deer and possum belong to the genus Atadenoviruses. Ads that are isolated from fish belong to the genus Ichtadenoviruses. The genus Siadenovirus includes viruses that are isolated from invertebrates [24].

Human adenoviruses (HAds) are categorized into seven species (A-G) that are further divided into 57 serotypes (Ad1-Ad57) [25]. Adenoviral serotyping is based on capsid proteins (VIII, hexon), phylogenetic distance ( $\geq 10\%$ ) in adenoviral genes that codes for protease, viral surface antigen neutralizing antibodies, and DNA polymerase [26].

HAds have worldwide distribution. Humans can be infected with more than one serotype or species of Ads that are usually acquired in early childhood, which leads to lifelong immunity [27]. Ads account for 5% of common cold cases. Wild type Ads often cause mild illness in immunocompetent individuals, which mainly affects the respiratory tract, eyes and digestive system [28]. However, HAds causes severe illness in immunosuppressed individuals [29].

Ads induce diverse innate immune signaling pathways that result in the secretion of a number of proinflammatory cytokines. These proinflammatory cytokines result in the induction of robust adaptive humoral and cellular immune responses. The adaptive immune responses that develop against Ads include both T cells and neutralizing antibodies against the viral surface antigens such as hexon, penton, and fiber proteins [30].

Humans can be infected with different kinds of non-human Ads because of their broad tissue tropism and the structural similarity that they have with that of HAds. These characteristics subjected to use the non-human Ads as a vector for gene therapy and recombinant vaccine development so as to overcome the pre-existing antibodies that exists against human adenoviral vectors. There are numerous non-human adenoviral vectors that have used for recombinant vaccine development and gene therapy such as chimpanzee adenoviral vectors (ChAdOx1 nCoV-19, ChAd1, ChAd2, ChAd3, ChAd5, ChAd6, ChAd7, and ChAd68); bovine adenoviral vectors; fowl adenoviral vectors; canine adenoviral vectors, ovine adenoviral vectors; porcine adenoviral vectors [31, 32].

### **Application of adenoviruses in cancer therapy**

Cancer is one of the leading causes of death in the world, which is the second after heart diseases. Globally, each year cancer causes about 10 million deaths and about 1 in 6 deaths are due to cancer [33]. Adenoviruses have become the promise of new therapeutic strategy for cancer treatment. Adenoviral vectors can be engineered in different ways so as to change the tumor microenvironment (TME) from cold tumor to hot tumor, including; 1. By modifying Ads to express cytokines, and other immune-modulatory molecules; 2. By modifying Ads to deliver tumor suppressor gene and code for tumor specific antigen. The other way to use Ads in cancer therapy is to use oncolytic adenoviruses, which directly kill tumor cells after replication [34-36].

### **Adenoviral vectors coding for tumor suppressor protein (p53)**

One of the strategies that have been developed to use Ads in cancer therapy is to use replication-deficient adenoviral vectors to carry transgenes that codes for a tumor suppressor protein (p53) or proteins that induce apoptosis or cell cycle arrest [36]. Wild-type p53 prevents development of cancer by inhibiting the activation of oncogenes and inducing programmed cell death (apoptosis) when the cell's DNA repair functions are insufficient to repair DNA damage [37]. Suppression of p53 function is common in human cancers and 50% of cancers have mutations in the gene that codes for p53 protein [38]. For this reason, the p53 gene has become one of the target genes for transformation research of cancer gene therapy.

Advexin is a replication-defective recombinant human p53 adenoviral vector with a deletion on E3 and E1 genes that expresses a functional p53 protein from a Cytomegalovirus promoter [38, 39]. Advexin was proved efficacious against bladder cancer, ovarian cancer, prostate cancer, breast cancer, squamous cell carcinoma of the head and neck, hepatocellular carcinoma, colorectal cancer, squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, and larynx and non-small cell lung cancer (NSCLC) [39, 40].

Likewise, Gendicine is also a replication- defective recombinant human p53 Ads vector (rAd-p53) expressing p53 proteins which inhibits the uncontrolled division of cancer cells and induces apoptosis of cancerous cells [25]. Gendicine is very similar to advexin except that the p53 in gendicine is expressed from Rous Sarcoma Virus promoter [25]. Gendicine was approved for treatment of squamous cell carcinoma of the head and neck by the Chinese Food and Drug Administration agency in 2003 as a first-ever gene therapy product to be used in combination with chemotherapy and have been in use for more than 15 years [41, 42]. Gendicine has been also shown to be effective for treatment of different kinds of cancer in China including malignant glioma, epithelial ovarian carcinoma, HCC, and NSCLC [41, 42].

### **Oncolytic adenoviruses as anticancer virotherapy**

Oncolytic viruses are viruses that specifically infect and replicate in a tumor cells and kill the cancer cells by their lytic replication [35, 43]. Oncolytic Ads, particularly oncolytic HAds are one of the leading candidate viruses for cancer vi-

rotherapy because of their good safety profile and high immunogenicity [3]. Oncolytic Ads are genetically engineered Ads which acquired traits that enables them to infect and preferentially replicate in tumor cells [44]. Oncolytic adenoviral vector technologies have been approved in some countries for treatment of cancer in humans [25, 35]. As compared with normal and quiescent cells, generally, tumor cells are more permissive to Ads [45], because of different reasons. The first reason is that the entire pattern of gene expression in cancer cells is conducive for Ad replication [46]. The second reason is the fact that specific viral entry receptor is highly expressed in tumor cells. The other reason is the higher cell division and metabolic rate that take place in cancerous cells than that of normal and quiescent cells [47, 48]. The advantage of an oncolytic Ads is not only to specifically replicate in and lyse tumor cells, but oncolytic adenoviruses can also stimulate potent anti-viral and anti-tumor immune responses for tumor-specific antigens that are released following lysis of Ads infected tumor cells [49, 50].

Adenoviral vectors have been engineered to efficiently undergo oncolytic replication in cancer cells without replicating in healthy cells [51, 52]. For example, ONYX-015 with a partial E1B gene deficiency is oncolytic adenoviral vector that infects and replicate in tumor cells that lacks p53 but unable to replicate in healthy cells expressing p53 [51]. ONYX-015 has been demonstrated to be effective and well-tolerated oncolytic adenoviral vector that is reported to be more effective when given in combination with different cancer chemotherapies [52]. Oncorine (H101) is also a genetically modified oncolytic adenoviral vector expressing p53 gene. The Chinese food and drug administration agency has approved Oncorin for the treatment of head and neck cancer in combination with chemotherapy [39, 53].

### **Adenoviruses expressing Immunomodulatory Molecules**

Adenoviruses can be used in cancer therapy by modifying the viruses to stimulate antitumor immune response in different ways including by expressing cytokines, and other immune-modulatory molecules [34, 54].

#### **Interferon armed adenoviruses**

Interferon (IFN) has a strong antitumor effect and has been used in the treatment of pancreatic cancer. Some studies showed that IFN- $\alpha$  significantly prolongs survival rate (by 2 to

5 years) [55, 56]. However, there are limitations in using IFN-based therapies, including dose-limiting systemic toxicities and low intratumoral concentration of IFN because of its short half-life in the bloodstream [57]. In response to this, oncolytic adenoviruses have been engineered to express IFN, which showed positive outcomes in treatment of cancer.

Armstrong *et al.* (2012) has reported oncolytic adenoviruses expressing human IFN- $\alpha$  as a promising platform for selective, long-term expression of IFN in human pancreatic cancer tissues [58, 59]. They used the oncolytic adenovirus Ad5/Ad3-Cox2- $\Delta$ E3-ADP-IFN in their study, which was developed to selectively replicate within cancer cells expressing cyclooxygenase 2 (Cox2). In order to improve the infectivity and oncolysis of the oncolytic adenoviruses, they made genetic modification in the virus to include an Ad5/Ad3 chimeric fiber and overexpress the adenovirus death protein.

Similarly, other researchers have also reported as oncolytic adenoviruses expressing IFN- $\alpha$  have promising outcomes in treatment of cancer. The researchers reported that an oncolytic adenovirus (OAd-hamIFN) that was investigated in an immunocompetent Syrian hamster model of pancreatic ductal adenocarcinoma showed efficient viral replication in tumor, significant inhibition of tumor growth, and enhanced survival when used in combination with chemo and radiation therapies [60]. Likewise, studies conducted by Tao *et al.* (2006) have shown that significant tumor regression of bladder cancers occurred following administration of an adenovirus expressing human interferon  $\alpha$  (Ad-IFN $\alpha$ ) using a mouse superficial bladder cancer model in which human bladder tumors are growing [61].

#### **GM-CSF expressing adenoviruses (ONCOS-102)**

Granulocyte macrophage colony-stimulating factor (GM-CSF) promotes activation of T cells and maturation of dendritic cells [62, 63]. ONCOS-102 is an oncolytic adenovirus that contains GM-CSF transgene [64]. A clinical study (phase-I) in patients with advanced solid tumors including colon, lung, and ovarian cancers demonstrated a strong immune cell infiltrate into tumors without dose-limiting toxicities [64].

#### **LOAd703 expressing 4-1BBL and trimerized CD40L**

The other oncolytic adenovirus that expresses immunostimulatory cytokine is LOAd703. LOAd703 is armed with

4-1BBL and trimerized CD40L that was shown to replicate and kill pancreatic cancer cells via oncolysis in both in vitro and in vivo assays [65].

### **TILT-123 (Expressing TNF- $\alpha$ )**

TILT-123 is an oncolytic adenovirus that incorporates transgenes for human tumor necrosis factor alpha (TNF- $\alpha$ ) and interleukin-2 (IL-2). TNF- $\alpha$  and IL-2 were shown to be promising T cell stimulating factors when used in combination with adoptive cell therapy [66].

### **IL-12 and other cytokines armed adenoviruses**

IL-12 represents the ideal candidate for tumor immunotherapy because of the fact that IL-12 induces a potent antitumor effect by promoting natural killer cells and cytotoxic T cell activities [67]. Several preclinical studies demonstrated promising antitumor effects of IL-12 in mice having solid tumor and hematologic malignancies [68, 69]. However, findings from clinical trials indicated severe side effects of systemic administration of IL-12 that markedly dampened hopes of the successful use of this cytokine in cancer patients [70]. But, the adverse effects associated with systemic administration of IL-12 can be reduced by using adenoviruses that express IL-12.

Ads that were engineered to express IL-12 have been shown to enhance immune stimulation and antitumor effect in a clinical trial [71] and pre-clinical studies [72]. Likewise, a study by Wang et al showed that oncolytic adenovirus (Ad-TD-nsIL-12) expressing IL-12 induces strong antitumor immune response against pancreatic cancer in Syrian hamster models without toxicity [73]. Similarly, a replication-deficient adenoviral vector encoding human IL-12 p70 transgene (Ad-RTS-hIL-12) was also shown to have no toxic effect in phase one clinical trial [74]. A recent trial found that intratumoral injection of Ad-RTS-hIL-12 was safe in patients with recurrent glioblastoma [74].

In addition to IL-12, there are also other cytokines, such as IL-24 and IL-13 that have been used to arm adenoviruses and have shown promising immune-activating properties in multiple preclinical cancer models [75, 76]. RANTES is another cytokine engineered in Ad that has been shown to enhance anticancer effect. In murine models of mammary adenocarcinoma and lymphoma, Ad-RANTES-E1A eradicated established tumors and

inhibited metastases by recruiting DCs, macrophages, NK cells, and CD8+ T cells into the immunologically cold tumors [77].

### **Adenoviruses armed with Bispecific T cell engager (BiTE)**

Bispecific T cell engager (BiTE) is a kind of artificial antibody that represents an innovative immunotherapy approach which enhances patients' immune response to tumors. BiTE has dual antigen specificity, allowing them to bind to two unique antigens at the same time, i.e. the BiTE bind simultaneously to both tumor associated antigen and T cell (usually CD3), ultimately stimulating T-cell activation, tumor killing and cytokine production [78]. BiTE has been shown to be promising immunotherapy for the treatment of cancer in preclinical and clinical studies [79, 80]. The therapeutic efficacy of BiTE can be improved by using BiTE in conjunction with adenoviruses.

Aleman group engineered an oncolytic adenovirus expressing an EGFR-targeting BiTE that showed improved T cell-mediated killing of cancer cells both in vivo and in vitro [81]. They also demonstrated that anti-EGFR BiTE-armed OAd in combination with adoptive CAR-T cell therapy results in improved antitumor efficacy and prolonged survival of mice as a result of intratumoral T cell activation by BiTE [82].

Similarly, another research group (Fisher group) that developed BiTE armed oncolytic adenovirus (EnAd-SA-EpCAM) reported promising results in use of BiTE expressing adenoviruses against cancer [83]. The BiTE of EnAd-SA-EpCAM binds to epithelial cell adhesion molecule (EpCAM) in cancer cells. The Fisher group reported that the EnAd-SA-EpCAM effectively activate endogenous T cells within the immune-suppressive microenvironment and exhibited killing of endogenous tumor cells without the addition of exogenous T cells [83].

Recently, the Fisher group engineered another BiTE armed oncolytic adenovirus (EnAd-FAP-BiTE), which is targeted fibroblast activation protein (FAP) in cancer-associated fibroblasts (CAFs). CAFs are the main cellular component of solid tumor TME. The EnAd-FAP-BiTE induced the activation of tumor-infiltrating T cells that target and kill CAFs [84]. Likewise, another BiTE armed adenovirus (ICO15K-FBiTE) that was developed by the Fisher research group was shown to enhance overall antitumor efficacy without increasing the toxicity in

mouse model [84].

### ADV/HSV-TK

ADV/HSV-TK is an adenoviral vector expressing the herpes simplex virus (HSV) thymidine kinase (TK) gene. The HSV-TK protein has two principal functions, including 1. TK is a superantigen that stimulates a potent immune reaction and 2. A nucleotide analog product of prodrug phosphorylation lead to the death of dividing cancer cells [85]. Herman et al. studied ADV/HSV-TK in combination with ganciclovir for the treatment of human prostate cancer [86]. They reported that injection of ADV/HSV-TK into the prostate gland in the region with the greatest concentration of tumor cells resulted in significant reduction in tumor burden. Herman et al also showed that ADV/HSV-TK was proven safe, with minimal toxicity [86]. Likewise, other researchers also made similar observation on the efficacy and toxicology of ADV/HSV-TK against glioma, retinoblastoma, and mesothelioma [87, 88].

### Combination therapy using adenoviruses and Chimeric Antigen Receptor (CAR) T Cells

One of the strategies that have been used for the immunotherapy of cancer is adoptive cell therapy, that includes chimeric antigen receptor (CAR) T cells, tumor-infiltrating lymphocytes, and T cell receptor modified T cells [89]. TCR-T cells are designed to encode receptors that specifically recognize cancer-specific antigens, and function through Major Histocompatibility Complex (MHC)-dependent mechanism, that limits their use [90]. Whereas CAR-T cell therapy functions through MHC-independent mechanism. CAR-T cell has been effective in the treatment of different types of cancer, which includes chronic lymphocytic leukemia and non-Hodgkin's lymphoma [91, 92]. However, the use of CAR-T cells as a monotherapy has not demonstrated much success in solid tumors because of immunosuppressive tumor microenvironment (TME) and poor tumor infiltration of CAR-T cells [93]. Combination therapy with adenoviruses viruses provides one potential strategy for improvement of CAR-T therapy in solid tumors.

A study conducted by Watanabe and his colleagues demonstrated that the combination of oncolytic adenoviruses (expressing TNF- $\alpha$  and/or IL-2) and mesothelin-redirected CAR-T cells (meso-CAR-T) overcomes the immunosuppressive nature of the pancreatic cancer TME [94] Watanabe et al

demonstrated that tumors treated with the combination of the virus expressing TNF- $\alpha$  and IL-2 (Ad5/3-E2F-d24-TNF- $\alpha$ -IRES-IL-2 (OAd-TNF $\alpha$ -IL2) and meso-CAR-T cells were infiltrated with significantly more CD4+ and CD8+ T cells compared to monotherapy with meso-CAR-T cells, or a combination with meso-CAR-T cells and the parent adenovirus lacking cytokine expression [94]. They also showed that meso-CAR-T cells in combination with OAd-TNF $\alpha$ -IL2 resulted in significantly higher accumulation of CAR-T cells at the tumor site when compared to meso-CAR-T monotherapy.

Likewise, a study that was done by a Suzuki group demonstrated better efficacy of CAR-T cell in treatment of prostate cancer when used in combination with oncolytic adenoviruses. In order to improve the efficacy of the CAR-T cell therapy, this research group (Suzuki group) used a combinatorial adenovirus vector (oncolytic adenovirus (Ad5 $\Delta$ 24) and helper-dependent adenovirus expressing a mini anti-PD-L1 antibody (HDAd-PD-L1) collectively termed CAd-VECPDL1) in combination with human epidermal growth factor receptor 2 (HER2)-specific CAR-T cells [95]. The findings showed that using this combination in an NSG mouse model was more effective in reducing tumor size and prolong survival of mice with prostate cancer [95].

The Suzuki group further modified the CAd-VECPDL1 vector by incorporating IL-12 (CAdVECIL12\_PDL1) and tested it in a head and neck squamous cell carcinoma (HNSCC) model [96, 97]. In a xenograft model of NSG mice (NOD scid gamma mouse), the combination of HER2-CAR-T cells and CAdVECIL12\_PDL1 virus significantly prolonged survival of treated animals to more than 100 days as compared to 21–24 days in the control groups, and HER2-CAR-T cells were detected in the tumors of surviving mice over 100 days after initial therapy [97]. The research group also used an orthotopic HNSCC model, establishing both primary tumors and lymphatic metastases, to test the aforementioned combination therapy. Mice that received both HER2-CAR-T cells and CAdVECIL12\_PDL1 had improved tumor growth control at both primary and metastatic sites, maintained body weight, and had prolonged survival when compared to untreated and monotherapy groups [97]. Taken together, combination therapy with oncolytic viruses provides one potential strategy for improvement of CAR-T therapy in solid tumors.

### Combination therapy using adenoviruses and antibodies against Immune checkpoint proteins

Immune checkpoint inhibitors therapy is a kind of immunotherapy that work by blocking the binding of checkpoint proteins (PD-1, PD-L and CTLA-4) with partner proteins so that T-cell became free and active to attack cancer cells. Ipilimumab is an anti-CTLA-4 antibody (that blocks CTLA-4 ligand and prevents inhibition T-cells) that was approved by the United States food and drug administrations (U.S. FDA) in 2010 for the treatment of advanced melanoma [98]. However, the systemic administrations of immune checkpoint inhibitors have been shown to cause severe immune-related adverse events [99, 100]. One of the strategies that can be employed to overcome these obstacles of using immune checkpoint inhibitors (such as anti-PD-L1, anti-PD-1 and anti-CTLA-4) in cancer therapy is to utilize immune checkpoint inhibitors in combination with oncolytic adenoviruses.

#### **Ad5/3-Δ24: adenovirus expressing anti-CTLA-4 antibodies**

Ad5/3-Δ24 is an oncolytic adenovirus that was engineered to code for anti-CTLA4 antibody [101]. Promising results have been achieved with the oncolytic adenovirus armed with anti-CTLA-4 antibodies (Ad5/3-Δ24) in mouse model. The local expression of anti-CTLA-4 antibody following administration of Ad5/3-Δ24-CTLA4 resulted in activation of T cells [101]. In addition, a significantly higher concentration of antitumor antibody was produced, while plasma levels remained at safe concentrations. The anti-CTLA-4 antibodies also showed direct proapoptotic effect both in vivo and in vitro [101].

#### **Ad5-CMV-mIL2 and Ad5-CMV-mTNF- α in combination with anti-PD-1 antibodies**

Cervera-Carrascon and his colleagues that tested nonreplicating vectors expressing IL-2 (Ad5-CMV-mIL2) and TNF-α (Ad5-CMV-mTNF- α) in combination with programmed cell-death protein 1 (PD-1) blocking antibodies in a mouse model demonstrated complete regression of murine melanoma tumors, and prolonged survival of mice [102]. Furthermore, they showed that the viral infection shifted the cytokine profile of the tumor microenvironment towards T-helper type 1, indicating that nonreplicating adenoviral vectors significantly improve antitumor immunity [102].

#### **DNX-2401 (Tasadenoturev) in combination with pembrolizumab**

DNX-2401 is a replication-competent oncolytic adenovirus that selectively infects cancer cells lacking the normal

retinoblastoma protein signaling pathway [103]. Aiken et al tested DNX-2401 in combination with intravenous pembrolizumab (PD-1 immune checkpoint inhibitor) in patients with recurrent glioma and the findings showed that treatment of glioma with combination of DNX-2401 and pembrolizumab significantly improves the disease burden [104].

#### **ONCOS102 in combination with pembrolizumab**

Ternyila (2020) investigated the recombinant oncolytic adenovirus ONCOS-102 in combination with the antibody pembrolizumab (PD-1 immune checkpoint inhibitor) in patients with malignant melanoma [105]. They reported that combination therapy with ONCOS-102 and pembrolizumab resulted in regression of the disease and increases in circulating proinflammatory cytokines, and tumor specific T cells without dose limiting toxicities [105].

#### **Adenoviral vectors as recombinant anticancer vaccines**

Genetic vaccine is a third-generation vaccine platform that is designed to induce an immune response against an antigen that is encoded by a gene delivered into a vector or nucleic acid instead of the antigen itself. There are three main classes of genetic vaccines, which include viral or bacterial vectored vaccines, ribonucleic acid (RNA) vaccines, and DNA vaccine. The most important advantage of genetic vaccine over the traditional vaccine strategies like killed or inactivated microorganisms and subunit vaccines is the fact that genetic vaccines have capacity to induce T-cell responses; especially cytotoxic CD8 T cells responses, in addition to the humoral responses [3]. Viral vectored vaccines are one of the important forms of genetic vaccines that have been shown to induce robust cell mediated immunity (transgene-specific T cell) and humoral (antibody) response [3]. Adenoviral vectors are one of the most extensively investigated vaccine vectors. Since the 1980s, adenoviral vectors have shown great promise as vaccine vectors [106-108].

Adenoviral vectors can also be used as a platform for anticancer vaccine development. This is based on the fact that adenoviral vectors can be engineered to stimulate antitumor immune response by expressing tumor-antigens. Replication-deficient adenoviral vectors are one of the viral vectors that have been extensively used as recombinant cancer vaccines as they cause potent cell mediated and humoral immune response against transgenes expressed by the adenoviral vectors [25].

#### **ETBX-011(Ad5-CEA)**

ETBX-011(also called Ad5-CEA) is an adenoviral vector-based cancer vaccine that is engineered to express a modified carcinoembryonic antigen (CEA) which contains the highly immunogenic epitope CAPI-6D. CEA is found in different kinds of cancer cells. ETBX-011 induces potent CEA-specific cell-mediated immune responses with antitumor activity [109]. ETBX-011 is well-tolerated in metastatic colorectal cancer patients and has potential survival benefit [110].

In order to overcome the challenge posed by tumor heterogeneity, such as the diversity of tumor associated antigens (TAA), a Tri-Ad vaccine (a combination of ETBX-011 with three different human TAA-expressing Ad vector vaccines (ETBX-011, ETBX-061, and ETBX-051) have been developed and tested in phase I clinical trial. The Tri-Ad vaccine regimen induces anti-tumor cytotoxic T cell responses and was proven safe and well tolerated in treatment of advanced cancer [111].

### Ad5-PSA

Ad5-PSA is replication-deficient adenoviral vector that is engineered to express human prostate specific antigen (PSA). Ad5-PSA stimulates potent anti-PSA T cell responses and causes the destruction of PSA-secreting tumor cells both in preclinical [112] and clinical trials [113, 114]. Furthermore, Ad5-PSA prolongs survival and was demonstrated safe in patients with recurrent and hormone refractory prostate cancer [113, 114].

### Ad-E6E7 in combination with and Ad-MAGEA3

Ad-E6E7 is another replication-deficient adenovirus-based anti-cancer vaccine, which expresses human papillomavirus (HPV) genes E6 and E7 [115, 116]. MG1-E6E7 is an oncolytic maraba virus strain which also expresses the HPV genes E6 and E7. The combination of Ad-E6E7 and MG1-E6E7 induced potent tumor-specific responses in various mouse cancer models [117]. In addition, the combination of Ad-E6E7 with MG1-E6E7 was proven to significantly prolong survival of mice with HPV-associated cancer [117].

### ChAdOx1-MVA

Cappuccini *et al* investigated the immunogenicity and efficacy of ChAdOx1-MVA against prostate cancer in mouse model [118]. They reported that the ChAdOx1-MVA induced tumor specific cell mediated immune response. Furthermore, the ChAdOx1-MVA was proven to prolong the survival of the

mice when used in combination with anti-PD-1 antibody [118].

### Adenovirus-transduced Dendritic cells

The other approach to use adenoviral vectors for anti-cancer vaccine is the use Dendritic cells (DC) -based adenoviral vaccines. In this approach, DCs are transduced *ex vivo* with Ads encoding cancer specific antigens. The advantageous of transducing DC *ex vivo* instead of injecting Ads *in vivo* include: 1. *Ex vivo* transduction of DC overcomes pre-existing anti-viral immunity and induce effective anti-tumor responses [119, 120] and 2. *Ex vivo* transduced DC induces lower anti-viral antibody responses than that of Ads injected *in vivo* [119].

Ad-transduced DCs have shown positive outcomes against both solid tumor and hematologic cancers [121, 122]. Butterfield *et al.* (2008) tested an autologous DCs transduced *ex vivo* with Ads encoding the full-length melanoma antigen MART-1/Melan-A. They reported that the autologous DC-based adenoviral vaccine significantly induced cell mediated immune response (CD+8-T cells response) in metastatic melanoma patients [121]. Similarly, in patients with advanced NSCLC, injections of autologous DCs resulted in induction of systemic tumor antigen-specific immune responses with enhanced CD8+T-cell infiltration [4].

### Challenges Of Using Adenoviral Vectors in Gene Therapy

The application of Adenoviral vectors in gene therapy and vaccine development is severely hampered by the widely prevalent pre-existing immunity to the most common Ad vectors infecting the human population [135, 136]. As Adenoviral vectors have high immunogenicity, their first use for vaccine can also leads to development of potent humoral and cellular immunity [137], which prevents the subsequent administration of adenoviral vector-based vaccines that requires repeated administration. Preexisting HAd immunity causes considerable reduction in immunogenicity of HAd5 vector vaccines in both humans and animals [137, 138].

In addition to preexisting immunity, there are also other obstacles in the use of adenoviral vectors in gene therapy and vaccine development, which include the possibility of the adenoviral vectors to reestablish replication competence, non-specificity, immunodominance of adenoviral antigens over the vaccine transgene antigen(s), and heterologous immunity with other pathogens such as hepatitis C virus [139, 140].

Table1: Clinical trials using replicating and non-replicating adenoviral vectors for cancer therapy

No	Ad vector	Transgene	Cancer	Phase	Reference
1	Ad5-SGE REIC/Dkk3	REIC/Dkks	Localized Prostate cancer	I/II	123
2	Ad5-PSA	PSA	Prostate cancer; recurrent/hormone refractory prostate cancer	I/II	113, 114
3.	Advexin (rAd-p53)	P53	squamous cell carcinoma of the oral cavity, oropharynx, hypopharynx, and larynx; colorectal cancer, HCC, NSCLC, prostate cancer, breast cancer, ovarian cancer, bladder cancer, glioma, and squamous cell carcinoma of the head and neck	I/II	39, 108
4.	Adv/tk (GMCI)	HSV-tk	Advanced non-metastatic pancreatic adenocarcinoma II	II	NCT02446093
5.	Ad5-yCD/ mut-TKSR39rep-hIL-12	Cytosine deaminase, HSV-tK, hIL-12	metastatic Prostate cancer	I	NCT03281382
6.	Ad5-SGE REIC/Dk3 (MTG201)	REIC/Dkk3	Relapsed malignant pleural mesothelioma	II	124
7	AdHSV-tk /GCV	HSV-tk Ad- hCMV- Flt3L	High-grade malignant gliomas	I/II	125
8	ETBX-011	CEA	Metastatic colorectal cancer	I/II	109, 110, 126
9	Adv/tk	HSV-tk	Advanced hepatocellular carcinoma	III	127
10	SCH-58500	P53	Primary ovarian cancer, fallopian cancer and peritoneal cancer	I	128
11	ONCOS-102	GM-CSF	Melanoma	I	64
12	Adv-tk (GMCI)	Adv-tk	Pediatric brain tumors	I	129
13	Ad-MAGEA3	MAGE-A3	Advanced/Met., MAGE-A3+ Solid Tumors, NSCLC	I/II	NCT02285816
14	rAd-IFN/Syn-3 (instiladrin)	INFα-2b	High grade non-muscle invasive bladder cancer	III	130
15	Ad-RTS-hIL-12	IL-12	Glioblastoma or malignant glioma; Advanced or metastatic breast cancer; recurrent or progressive melanoma	I	131
16	Ad-E6E7 and MG1-E6E7	HPV E6/E7	HPV-associated cancer	I	117

17	TILT-123	hTNF- $\alpha$ , hIL-2	Advanced melanoma	I	66
18	ADV/RSV-TK	HSV-TK	prostate cancer; glioma, retinoblastoma, mesothelioma	I	86-88
19	BG00001	INF- $\beta$	Pleural melanoma	I	87
20	LOAd703	CD40L, 4-1BBL	Pancreatic cancer, bacillary cancer, collateral cancer	I/II	NCT03225989
21	Adv/HSV-tk	HSV-tk	Metastatic non-small cell lung carcinoma and uveal melanoma	II	NCT02831933
22	DNX-2440	OX40L	Glioblastoma	I	NCT03714334
23	Ad / PNP + fludarabine	PNP	Head and neck squamous cell carcinoma	I	132
24	DNX-2401		Recurrent glioma	II	NCT02798406
25	Oncorine or H101		head and neck cancer	III	39
26	Ad-MAGEA3	MAGE-A3	NSCLC	I/II	NCT02879760
27	Gendicine (rAd-p53)	P53	head and neck squamous cell carcinoma, malignant glioma, HCC, NSCLC and epithelial ovarian carcinoma	III	133; 134
28	ONYX-015		Advanced cancer	Preclinical trial	52
29	ETBX-011, ETBX-061, and ETBX-051 (Tri-Ad vaccine)	TAA	Advanced cancer	I	111
30	ChAdOx1-MVA	STEAP1	Prostate cancer	Preclinical	118
31	Ad5-yCD/mutTKSR-39rep-hIL-12	Cytosine deaminase, HSV-tK, IL-12	Prostate cancer	I	NCT02555397
32	Ad-IFN/Syn 3	INF- $\alpha$	Bladder cancer	Preclinical trial	61

Several strategies have been employed in order to avoid the challenges of using adenoviral vectors, which includes the use of non-human adenoviral vectors [141], mucosal delivery of HAd5 vectors [142], engineering fiber- or hexon-chimeric HAd5 vectors [143], priming with recombinant DNA prior to boosting with a HAd5 vector [144], microencapsulation of HAd5 vectors in inert polymers [145], and use of vectors derived from HAd serotypes that rarely infect humans [135] and the use of gutless (helper-dependent Ad (HD-Ad) vectors [146].

## Conclusions

Adenoviruses viruses have become the promise of new therapeutic strategy for cancer treatment. Adenoviral vectors can be engineered in different ways so as to change the tumor microenvironment from cold tumor to hot tumor, including; 1. By modifying adenoviruses (Ads) to deliver transgenes that codes for tumor suppressor gene (p53) and other proteins whose expression result in cell cycle arrest 2. Ads can also be modified to express tumor specific antigens, cytokines, and other immune-modulatory molecules. The other strategy to use Ads in cancer therapy is to use oncolytic adenoviruses, which directly kills tumor cells after replication. Gendicine is a replication-defective recombinant human p53 adenoviral vector that was approved for treatment of squamous cell carcinoma of the head and neck by the Chinese FDA. The Chinese FDA agency has also approved Oncorin (oncolytic adenoviral vectors) for the treatment of head and neck cancer. Ads that were engineered to express immune-stimulatory cytokines and other immune-modulatory molecules have shown promising outcome in treatment of cancer. Adenoviruses can also improve therapeutic efficacy of adoptive cell therapy when used in combination with CAR T Cells. Adenoviruses can also improve therapeutic efficacy of immune checkpoint inhibitors when used in combination with anti-PD-L1, anti-PD-1 and anti-CTLA-4 antibodies. Furthermore, different replication-deficient adenoviral vectors that were used as anticancer vaccines have been shown to stimulate potent antitumor immune response. However, the use of adenoviral vectors in gene therapy is limited by several factors such as pre-existing immunity to adenoviral vectors; high immunogenicity of the viruses; the possibility of the adenoviral vectors to reestablish replication competence; non-specificity; immunodominance of adenoviral antigens over the vaccine transgene antigen(s); and heterologous immunity with other pathogens. Thus, innovative strategies must be continually developed so as to overcome the obstacles of using adenoviral vectors in gene therapy.

## List of Abbreviations

Ads: Adenoviruses; BiTE: Bispecific T cell engager; CAR: Chimeric Antigen Receptor; ChAd: chimpanzee adenoviral vectors; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; DC: Dendritic cells; DNA: deoxy ribonucleic acid; GM-CSF: Granulocyte macrophage colony-stimulating factor; HAd5 : Human adenoviruses; HCAd5: high capacity adenoviral vectors; HPV: human papillomavirus; HSV: herpes simplex virus; IFN: Interferon; IL-2: interleukin-2; MHC: Major Histocompatibility Complex; NSCLC: non-small cell lung cancer; PD1: Programmed Death 1 Receptor; PD-L1: Programmed death ligand-1; PSA: prostate specific antigen; TK: thymidine kinase gene; TME: tumor microenvironment; TNF- $\alpha$ : Tumor necrosis factor alpha; U.S. FDA: United States food and drug administrations

## Declarations

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## Not applicable

Consent for publication

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There is no conflict of interest in this work

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