

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Adenoviral Vector-Based Vaccines and Gene Therapies: Current Status and Future Prospects

---

Shakti Singh, Rakesh Kumar and Babita Agrawal

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/intechopen.79697>

---

## Abstract

Adenoviruses are one of the most genetically diverse DNA viruses and cause non-life-threatening infections in the ocular, respiratory, or gastrointestinal epithelium of a diverse range of hosts. Adenoviruses are excellent vectors for delivering genes or vaccine antigens to the target host tissues and are being tested in several vaccine and gene therapy studies. Adenovirus-based vectors offer several advantages over other viral vectors such as broad range of tissue tropism, well-characterized genome, ease of genetic manipulation including acceptance of large transgene DNA insertions, inherent adjuvant properties, ability to induce robust transgene-specific T cell and antibody responses, non-replicative nature in host, and ease of production at large scale. However, several studies have highlighted major drawbacks to using adenovirus as vaccine and gene therapy vectors. These include pre-existing immunity in humans, inflammatory responses, sequestering of the vector to liver and spleen, and immunodominance of the vector genes over transgenes. In the same vein, recently discovered protein sequence homology and heterologous immunity between adenoviruses and hepatitis C virus have significant implications in the use of adenoviral vectors for vaccine development, especially for hepatitis C virus. This chapter focuses on the current scope and challenges in using adenoviral vector-based vaccines and gene therapies.

**Keywords:** adenoviruses, DNA viruses, viral vector, vaccine, gene therapy, immunity

---

## 1. Introduction

Adenoviruses (Ads) are non-enveloped, icosahedral DNA viruses with virion size ranges between 70 and 90 nm [1]. They belong to a diverse family (>50 serotypes) of DNA viruses called adenoviridae. Adenovirus was first isolated from human adenoid tissues in 1953 by

Rowe and his colleagues [2]. Adenoviruses usually cause non-symptomatic respiratory tract infections in both human and animals but can be life-threatening to immunocompromised individuals. Certain human adenovirus serotypes are ubiquitous in children, and most adults carry neutralizing antibodies to adenoviruses [3]. Nonetheless, since their initial use in gene therapy, they have gained wide recognition as a vaccine antigen delivery vehicle and have proven to be safe and efficient vaccine vectors for eliciting protective immune responses against transgene antigens in many animal and human studies. Recently, adenovirus vectors have been employed to attack cancer cells in cancer therapy [4]. In this chapter, we introduce different adenoviruses and their biology and potential for use in gene delivery, vaccine, and therapeutics in several human diseases. In addition, we will discuss their limitations and future prospects.

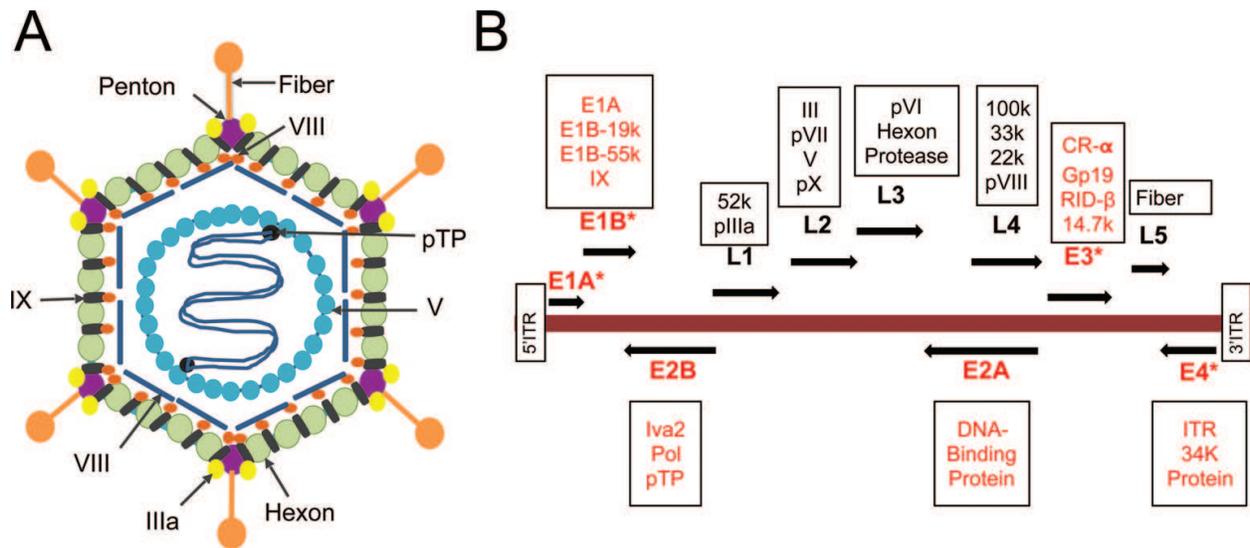
## 2. Adenoviruses

### 2.1. Genome and proteins

Adenoviruses contain a 26–45 kb size double-stranded DNA genome, inside their icosahedral virion [1]. The DNA genome of adenoviruses contains two inverted terminal repeats with 100–140 bp flanks on both the ends. Due to its small genome size, adenoviruses employ several strategies to maximally utilize its genome. For example, they encode proteins from both DNA strands, employ alternate-splicing, and use different poly A modifications of its mRNA. Adenoviral genes can be divided into five early and five late genes. Once internalized into target cells, the adenoviruses express the early genes E1A, E1B, E2, E3, and E4, which modulate host gene expression required for adenovirus protein synthesis and replication. The late transcriptional units include L1–L5 and are required in the assembly, release, and lysis of host cells [1, 5, 6] (**Figure 1**).

Structurally, adenovirus consists of a core of capsid and genome. The viral capsid consists of structural proteins hexon, penton, fiber, IIIa, VIII, and IX. Hexons are major surface structural proteins consisting of 270 trimers, which are arranged as 12 pentamers of pentons at the top of 12 icosahedral vertices. Hexons also contain several hypervariable regions and are the main targets of neutralizing antibodies. In adenoviral vectors, these sites can be engineered to carry vaccine antigen. Each icosahedral vertex gives rise to protruding fibers consisting of 12 trimers. Both penton and fiber proteins serve as ligands for host cell receptors and help in viral entry. The IIIa proteins are located in the inner surface of the capsid and help in the assembly and stabilization of vertex regions and also in the assembly of packaged viral genome. The VI proteins link the outer capsid shell to the inner icosahedral shell. The VIII proteins help in bonding hexons together and are critical for the stability of the viral capsid. The proteins V, VII, and X are associated with the DNA genome and make up the virion core. Terminal protein binds to each end of the DNA genome [6–8] (**Figure 1**).

The early gene first transcribes E1A protein, an essential protein for viral replication. The E1A protein activates the transcription of other viral genes responsible for viral DNA synthesis. In



**Figure 1.** Adenovirus structure and genome organization. (A) Graphical representation of adenovirus structure and various proteins. (B) Adenovirus genome organization showing various early (E) and late (L) transcripts and proteins encoded by each transcript. Regions indicated by red with (\*) are deleted in various adenoviral vectors. E1 and E3 regions were deleted in first generation and E1, E2, E3, and/or E4 were deleted in second-generation adenoviral vectors. Most recent adenoviral vectors called helper-dependent adenoviral vectors only contain ITRs and packaging signals. Figure is adapted from Ref. [209].

host cells, E1A stimulates apoptosis by both p53-dependent and -independent pathways [9]. In contrast, the E1B protein inhibits apoptosis by binding to several host cell proteins such as p53, Bak, and BAX proteins [8]. In non-replicating adenoviral vectors, the E1 gene is deleted to render them replication-defective so that it can infect the host cells but cannot multiply. However, for production of non-replicating adenoviral vectors, E1 transfected cells such as HEK293 and PER.C6 are used to allow production of replication-defective adenoviral vector [9].

## 2.2. Types of adenoviruses

Adenoviruses are grouped under the family Adenoviridae, which is divided into five genera: Mastadenovirus, Aviadenovirus, Siadenovirus, Atadenovirus, and Ichtadenovirus. Human adenoviruses, along with many animal adenoviruses (monkeys, cattle, sheep, swine, dogs), belong to the genus Mastadenovirus. Human adenoviruses (HAd) are classified into seven subgroups: A–G and further into 67 serotypes based on serological properties. The classification of serotypes into subgroups is based on their similarities in genome organization and DNA sequences, host tropism, carcinogenic potential in rodents, and growth properties in cell cultures. Adenoviral serotyping is based on viral surface antigen neutralizing antibodies and by phylogenetic distance (>10%) in the viral genes that encode viral protease, the protein pVIII, the hexon protein, and the DNA polymerase [10–12].

The genus Aviadenovirus contains bird adenoviruses, while other genera Siadenovirus, Atadenovirus, and Ichtadenovirus contain other adenoviruses of mammals, birds, reptiles, and fishes [13–15]. The adenoviruses isolated from sheep, cattle, deer, possum, and some birds differ from the adenoviruses of the genus Mastadenovirus and are classified under

the genus *Atadenovirus* [6, 16, 17]. The adenoviruses of the genus *Mastadenovirus* have high A + T (adenine and thymidine)-rich genomes and lack the early region 1 (E1) transcriptional unit. Adenoviruses isolated from many invertebrates are classified under the new genus *Siadenovirus*. Human and animal adenovirus infections are very common, and the majority of the population of host species contain neutralizing antibodies against the most prevalent serotypes of adenoviruses. Both human and non-human adenoviruses have been studied extensively and are the basis of adenoviral vector-based vaccine and gene therapies [18, 19]. In humans, infection by non-human adenovirus serotypes is not common. However, due to broad tissue tropism and structural and genomic similarity with human adenoviruses, non-human adenoviruses can infect various human tissue types. These properties of adenoviruses encouraged researchers to use non-human adenoviruses as gene or vaccine antigen delivery vectors to mitigate the pre-existing neutralizing immunity that commonly exists against human adenoviral vectors. Several non-human adenoviruses such as bovine Ad serotype 3 (BAd3); canine Ad serotype 2 (CAd2); chimpanzee Ad serotypes 1, 2, 3, 5, 6, 7 and 68 (ChAd1, ChAd2, ChAd3, ChAd5, ChAd6, ChAd7, ChAd68); ovine Ad serotype 7 (OAd7); porcine Ad serotype 3 and 5 (PAd3, PAd5); and fowl Ad serotypes 1, 8, 9, and 10 (FAd1, FAd8, FAd9, FAd10) are currently being tested as vaccine or gene delivery vectors [18, 20–22]. Extensive research in the molecular biology of both human and non-human Ads has helped in better understanding of the adenoviruses and designing of adenoviral vectors.

### 2.3. Immunity to adenoviruses

Initially, a host detects the invading virus by sensing unique pathogen-associated molecular patterns (PAMPs) present on the pathogen through pattern recognition receptors (PRRs). Once activated, these PRRs transmit signal to express type I interferons (IFNs) and proinflammatory cytokines which inhibit viral replication and recruit various innate immune cells to the site of infection [23–27]. These initial events ensure the efficient activation and presentation of viral antigens by the antigen-presenting cells to T cells and result in the induction of adaptive immune responses. In the following sections, we will discuss innate and adaptive immune responses to Adenoviruses in detail.

#### 2.3.1. Innate immunity

Adenoviruses are known to induce robust innate immune responses in their hosts. The adenovirus binds to its receptor(s) (such as Coxsackie adenovirus receptor or CAR, integrin  $\alpha\text{v}\beta\text{5}$  heparin sulfate proteoglycans, CD46, sialic acid, etc.) on host cells and gains entry into the cytoplasm [28–32]. However, phagocytic antigen-presenting cells such as macrophages and dendritic cells can also take up virus particles through scavenger receptors [33]. Inside a host cell, the virus can be recognized by various intracellular molecular sensors such as Toll-like receptors (TLRs), RIG-I like receptors (RLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), cytosolic DNA sensors, and effector molecules [34–36].

Cytokines such as IL-8 and TNF- $\alpha$  enhance the entry of human adenovirus type C by increasing the availability of CAR and integrin receptors, which facilitate the adenovirus to enter through clathrin-mediated dynamin-dependent endocytosis [27, 33, 37, 38]. The type B human adenoviruses use CD46 or desmoglein-2 and enter host cells through macropinocytosis [39, 40]; this also results in the suppression of IFN- $\gamma$ -induced production of proinflammatory cytokine IL-12 [41].

One of the major drawbacks of the use of adenovirus in gene therapy is the induction of undesired innate immune responses. In liver and spleen, the resident macrophages can sense and trap blood-borne adenovirus and induce inflammatory response mediators [42, 43]. Adenovirus also activates TLR2-dependent expression of chemokines such as MCP-1 and RANTES. In mice, TLR2 deficiency resulted in reduced NF- $\kappa$ B activation and humoral responses to HAd vector antigens and transgene-encoded antigens [42]. However, TLR2 deficiency did not result in complete inhibition of acute and adaptive responses to HAd, suggesting the involvement of an additional pathway [44]. The cellular  $\beta$ 3 integrins were recently reported to interact with arginine-glycine-aspartic acid (RGD) motifs of viral homo-pentameric penton base protein during viral entry, which results in the processing of inactive IL1 $\alpha$  into active cytokine in a MyD88-, TRIF-, and TRAF6-independent signaling pathway [43]. The IL1 $\alpha$  plays a major role in adenovirus-induced inflammatory responses. The IL1R-deficient mice or wild-type mice treated with anti-IL-1 antibodies demonstrated reduced inflammatory responses as well as hepatotoxicity in adenovirus infection [45]. Further, the interaction between the adenoviral RGD motif and host  $\beta$ 3 integrin mediates chemokine secretion, leukocyte infiltration, as well as corneal inflammation in human adenovirus serotype 37 infections [46].

TLR9 also plays a significant role in innate immunity against adenoviruses. Macrophages have been reported to sense adenovirus, helper-dependent adenoviral vector and recombinant E1- and E3-deleted adenovirus through TLR9 [47, 48]. The TLR9-deficient mice show reduced proinflammatory responses and IFN- $\alpha$  production upon adenoviral vector delivery. In a mouse model of keratitis, adenovirus induced TLR9-dependent IL6 production and monocyte infiltration of the cornea; however, chemokine secretion and keratitis development were TLR9-independent [49, 50]. Another study showed that recombinant adenovirus-induced type I IFN production in plasmacytoid dendritic cells (pDCs) is TLR9-MyD88-dependent but in myeloid DCs (mDCs) and macrophages, it is TLR9-independent [48].

The viral DNA also plays a critical role in the induction of innate immune responses as empty adenoviral particles are found to be poor inducers of innate responses [51]. The presence of double-stranded RNA with 5'-triphosphate groups in the cytoplasm of target cells is sensed by cytosolic PRR such as RIG-I, and viral DNA and RNA are recognized by intracellular PRRs such as TLR3, 7, and 8 present on the endosomal membrane [48, 52–55]. The double-stranded DNA is sensed by TLR9 in the intracellular environment and also by DNA-dependent activator of IRFs (DAI), DNA-dependent protein kinase (DNA-PK), IFN- $\gamma$ -inducible protein 16 (IFI16), DEAD (Asp-Glu-Ala-Asp) box polypeptide 41 (DDX41), and by cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) [34, 35, 56, 57]. Other cytosolic viral DNA sensors are NOD-like receptors (NLRs), which consist of a central nucleotide-binding

domain responsible for ATP-dependent self-oligomerization, a C-terminal leucine-rich repeat (LRR) domain that senses the presence of a ligand, and a variable N-terminal interaction domain that mediates protein-protein interactions. The NLR activation leads to the formation of inflammasomes with the help of microtubules [58]. The human adenovirus activates the formation of two types of inflammasomes in myeloid cells: absent in melanoma-2 (AIM2) and NLR-pyrin domain (PYD)-containing protein (NALP3). The activation of inflammasomes induces inflammatory responses via NF- $\kappa$ B signaling, converts pro-IL1 $\beta$  and pro-IL18 into IL1 $\beta$  and IL18, respectively, and can lead to DNA fragmentation, membrane pore formation, and eventually cell death by pyroptosis [59].

The early adenoviral proteins E1, E3, and E4 interfere with the innate immune signaling and help in evading host immune responses. The E1A protein allows hijacking of cell cycle, inducing apoptosis, evading immunizing, inducing tumorigenesis, and expressing viral genes [60, 61]. The E1A has been reported to block type 1 IFN-inducible gene expression [62–64], type 2 IFN- $\gamma$ -dependent HLA class II expression, and IFN- $\beta$  expression in response to double-stranded RNA due to inhibition of transcription complex formation [62]. Furthermore, E1A inhibits IFN-alpha-stimulated transcription factor 3 (ISGF3), IFN-stimulated genes (ISGs) [65, 66], and immunoproteasomes, resulting in reduced antigen presentation to T cells [67]. In addition, the early adenoviral proteins E1B-19K and E1B-55K antagonize p53-mediated apoptosis [68, 69], and E1B-55K interferes with the induction of IFN-inducible genes [70, 71]. The E1B-55K and E4 proteins induce proteasome-mediated degradation of defense factor death domain-associated protein (Daxx) resulting in the removal of viral transcription blocking allowing viral gene expression [72, 73]. E1B-55K and E4 protein complex also result in inhibition of antiviral innate immune responses [74–77]. The E3 protein has several immune modulatory functions. It blocks the surface transport of MHC-class I molecule and also reduces NK cell receptors on host cells, masking infected cells from detection by immune cells [78]. Furthermore, the E3 protein also inhibits apoptosis of adenovirus-infected cells by downregulation of death receptors [79].

The induction of innate immune responses is critical in adenoviral vector-based strategies. On the one hand, the gene transfer vector should have minimal activation of innate immune signaling to allow efficient gene delivery without immune activation. On the other hand, adenoviral vector-based vaccine antigen delivery could benefit from adenovirus's intrinsic property of innate immune activation that results in efficient activation of transgene-specific adaptive immune responses. Therefore, careful engineering of adenoviral vectors can serve the purpose of both gene and vaccine antigen delivery.

### 2.3.2. Adaptive immunity

The adaptive immune responses to adenoviruses are directed against both early and late viral proteins. They include both neutralizing antibodies and T cells against viral surface antigens such as hexon, penton, and fiber proteins. However, these adaptive immune responses against adenoviral antigens also present major obstacles in adenoviral vector development as gene delivery, and vaccine antigen carriers limit the number of

administrations that can be done and reduce the efficiency of transgene expression. Both humoral and cellular immune responses are discussed in detail in the following sections.

### 2.3.2.1. Humoral immunity

The surface antigens of adenovirus, penton, hexon, and fiber proteins are involved in host cell receptor interaction and can be neutralized by antibodies. The impact of neutralizing antibodies (nAbs) on adenoviral gene and antigen delivery has been studied extensively [80–82]. The passive transfer of serum from Ad immune mice or purified nAbs against adenoviruses decreases the vector transgene expression and induction of transgene-specific cellular and humoral responses. Depletion of antibodies against fiber, penton, and hexon by affinity chromatography has been shown to significantly enhance the transgene expression and induced immune responses. Furthermore, hexon-specific antibodies seem to play a relatively dominant role *in vivo* in comparison to other antigens. The hexon-specific nAbs are directed against exposed hypervariable loop-containing regions (HVR) on the surface of the virus particle. The pre-existing immunity to a prevalent human adenovirus 5 (HAd5) can be overcome by replacing the entire HAd5 hexon sequence of exposed epitopes with the HVR from a different serotype [6, 82–84]. Consequently, three amino acid substitutions in one of the HVRs significantly reduced neutralization by polyclonal serum raised against a chimpanzee Ad serotype 68 (ChAd68)-derived vector [83, 85]. Conversely, replacing SAd24 hexon with SAd23 into an SAd24/Pan7 vector resulted in reduced transgene expression in mice with pre-existing SAd23/Pan6 immunity [86]. Additionally, nAb against Ad fiber has a minimal effect *in vivo* as shown in a mouse study where nAbs induced by HAd7 administration weakly neutralized HAd7 fiber-expressing chimeric HAd5 vector, indicating that other non-fiber capsid protein-specific nAbs also have a major role in neutralization. Although these *in vivo* animal studies shed light on the relative importance of pre-existing fiber-specific nAbs, they do not accurately reflect the impact of preexisting immunity on adenovector efficiency in humans. In most of these animal studies, the pre-existing fiber-specific nAbs were induced by a single administration of Ad, which induced nAbs with poor breadth and at far lower levels than in humans exposed to repeated natural Ad infections. The nAbs against other surface antigens such as penton may also work against Ad in a synergistic fashion along with fiber-specific nAbs. Together, these factors contribute to more effective neutralization of Ad-based vectors and result in poor transgene expression and induced transgene antigen-specific immunity in humans. Importantly, Ad-specific nAbs are mostly serotype-specific and have very limited to no neutralization capability of other serotypes. This serotype-specificity is due to high sequence heterogeneity of epitopes in the hexon HVR and fiber knob among different Ad serotypes. Moreover, the nAbs are not the only factor in pre-existing Ad immunity; non-neutralizing Abs can also hamper Ad vector efficacy via Fc receptor-dependent cytotoxicity, complement-mediated lysis, and opsonization. In humans, Ad infections are very common and nearly everyone contains some levels of Ad-specific antibodies. The high seroprevalence of Ad-specific antibodies is a major roadblock in adenoviral vector development, and strategies to circumvent these must be examined.

### 2.3.2.2. Cellular immunity

In humans, Ad vector-specific CD4<sup>+</sup> Th1 cells have been detected, but the frequencies of these cells decrease with age [87]. The CD8<sup>+</sup> T cell responses to different structural proteins have also been detected in animals in response to adenovirus infection or adenovector administration [19, 80, 88]. Due to extensive homology between different adenoviral structural antigens, both human and mouse-derived CD4<sup>+</sup> and CD8<sup>+</sup> T cells cross react with human and simian Ad serotypes [20, 89, 90]. Similar to nAb, pre-existing Ad-specific T cells can also reduce Ad vector transgene expression and immunity. Furthermore, Ad-specific T cells have been detected in 80–100% of human subjects in various studies, which make them even more important in Ad vector development [89]. The human studies examining both nAbs and T cells demonstrated a higher proportion of individuals possessing T cell responses compared to nAbs against Ad. The pre-existing Ad-specific T cells have greater consequences for Ad vaccine vector development due to their cross-reactive nature, higher distribution in the human population, and their multifunctional nature [22]. Finally, human Ad vector has also recently been reported to induce cross-reactive hepatitis C virus-specific humoral and cellular immune responses [91]. Widespread use of adenoviral vectors in humans will induce such cross-reactive immune responses at high levels, which might be beneficial or detrimental in the development of natural immunity against HCV and affect the immunopathology and disease progression of HCV infection.

## 3. Adenoviral vectors

### 3.1. Construction of adenoviral vectors

Adenoviruses are engineered to make them safe and efficient for human use as vaccine and gene and cancer therapy vectors by deleting certain genome sequences. Initially, human adenoviruses, especially adenovirus 5 (HAd5), were developed as gene delivery vectors. Since the first generation of adenoviral vectors, based on E1 deletion, a number of different strategies have been employed to further improve the gene-carrying capacity and safety by deleting more genes. These strategies can be summarized under following three categories.

#### 3.1.1. First generation

First-generation adenoviral vectors were prepared by deleting early gene E1 to render them replication-defective and create space for a transgene sequence of up to 4.5 kb [92]. Since these vectors lack the E1 region essential for their replication, cell lines such as human embryonic kidney cells (HEK293) were engineered to incorporate viral E1 region [93]. The E1 region in the HEK293 cell line provides trans-complementation and allows the replication of the adenoviral vector [94–97]. These adenoviral vectors carry native tissue transduction capability and efficiently express the transgene in target host cells. However, there are possibilities of spontaneous homologous recombination between vector and E1

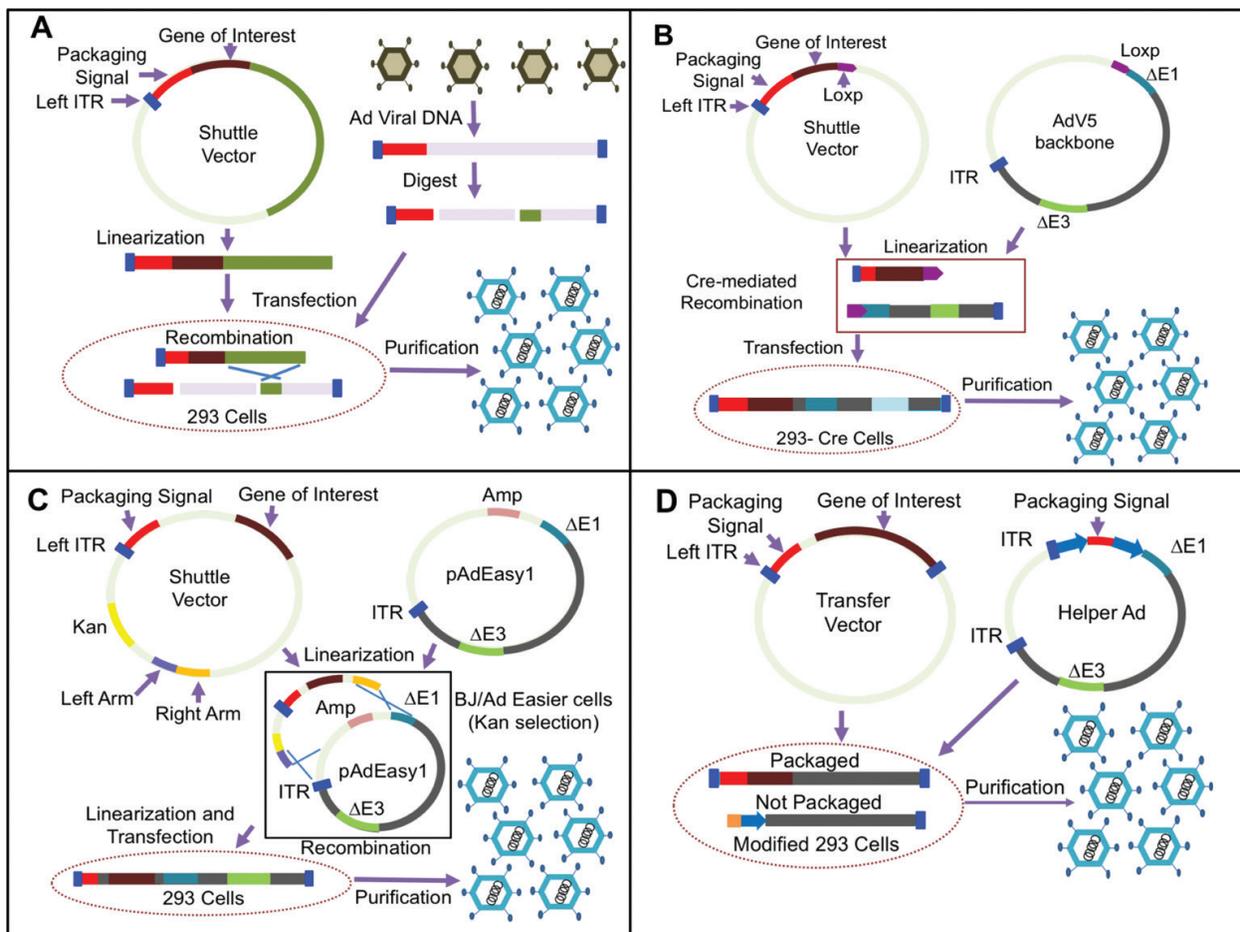
regions during amplification inside HEK293 cells, which might enable replication competent adenoviral (RCA) vectors to emerge [98]. To mitigate this problem, another cell line, human embryonic retinoblasts (PERC.6), was made by inserting an expression cassette for the adenoviral E1 region with its own promoter (ubiquitous phosphoglycokinase, PGK) [99]. This eliminates the adenoviral vector homologous regions from the E1 promoter and therefore the chances of recombination [100]. Adenoviral E3 region proteins are known to inhibit immunological pathways [101]. Therefore, the adenoviral E3 region was removed either partially or completely without affecting *in vivo* viral amplification [102]. These deletions in E1 and E3 regions allowed insertion of even larger cargo sequences (up to 8 kb) of two independent genes [103]. Due to the absence of the E1 region, adenoviral vectors are not able to transcribe other early and late viral proteins, although host cellular factors enable these proteins to be expressed at very low levels. This low-level expression of viral protein and subsequent presentation on the cell surface by MHC class I molecules induce robust cytotoxic T cell immune responses. Deletion of E1 is additionally beneficial since adenoviral proteins have toxic effects and induce cell death in a dose-dependent manner [104, 105] (**Figure 2A–C**).

### 3.1.2. Second generation

Second-generation adenoviral vectors possess deletions in E2 or E4 regions that encode for proteins required for replication in target cells [106–108]. These deleted proteins were complemented in trans by cell lines (such as HEK293) to allow for vector propagation. These second-generation vectors provided additional space for larger cargo sequences (10.5 kb) with up to four independent expression cassettes and eliminated the possibility of generating replication-competent adenoviruses during amplification. This deletion of early viral genes impacts the amplification of viral vector in cell culture and results in lower yields due to inefficient complementation by the producer cell lines [107, 109]. These vectors also have been reported to have lower transgene expression. Immunogenicity and cellular toxicity are still a major concern in the second-generation adenoviral vectors [110] (**Figure 2A–C**).

### 3.1.3. Third generation

Third-generation adenoviral vectors are also called “high capacity adenoviral vectors” (HCAds) because they can accept cargo sequences up to 36 Kb [111–113]. The HCAds were generated by deleting all viral sequences except the ITRs and the packaging signal [114]. For replication of third-generation adenovirus vectors in cell culture, instead of the complementation by the viral genes encoded by host cells, an additional adenoviral helper virus is provided. Therefore, the third-generation adenoviral vectors are also called helper-dependent or “gutless” adenoviral vectors [115–117]. The helper adenovirus is generated like a first-generation adenoviral vector and includes packaging signal flanking loxP sites. The vector is produced in HEK293 cells that constitutively express Cre recombinase by simultaneously transducing helper virus and the HCAd genome. This allows the synthesis of adenoviral proteins by the helper virus and enables assembly of viral capsids,



**Figure 2.** Methods of preparation of different types of adenoviral vectors. (A) First generation. The target gene is cloned into a shuttle vector containing 5'-ITR, a packaging signal, and the sequence for homologous recombination. This shuttle vector and an adenoviral backbone vector are transfected into HEK-293 cells, and adenoviral vector is created through homologous recombination between the two vectors. (B) First or second generation. The target gene is cloned into a shuttle vector that contains 5'-ITR, a packaging signal, and an LoxP site(s). This shuttle vector and a LoxP-containing adenoviral backbone vector are joined together through Cre recombinase-mediated recombination either *in vitro* or in HEK-293 cells. (C) First or second generation. The target gene is cloned into a shuttle vector containing 5'-ITR, a packaging signal, and a kanamycin-containing bacterial replication sequence flanked with two homologous arms. The homologous recombination between the linearized shuttle vector and ampicillin-resistant adenoviral backbone vector takes place in bacterial cells (BJ5183), and adenoviral plasmids are selected on kanamycin. This plasmid is linearized and transfected in HEK-293 cells for adenoviral vector production. (D) Third generation. The target gene is cloned into a transfer vector that only contains ITRs and a packaging signal. A helper adenovirus is used to generate the adenoviral vector. Modified HEK-293 cells are used for adenoviral production, which prevent packaging of helper adenovirus due to deletion of packaging signal. Figure is adapted from Ref. [209].

resulting in the packaging of HCAAd genome only. The helper virus genome-packaging signal is excised by Cre-mediated recombination of the loxP sites, thus preventing helper virus genomes from assembling into viral particles. In some production systems, other recombinases like *Saccharomyces cerevisiae*-derived FLP recombinase [118] or bacteriophage-derived phiC31 integrase [119] have also been used. Third-generation vectors have several benefits over first- and second-generation adenoviral vectors. These include less cellular toxicity and reduced immunogenicity [120, 121], thereby providing a flexible vector system that

efficiently transduces host cells due to reduced induction of anti-adenoviral neutralizing antibodies [118, 122]. The HCAdS can simultaneously encode multiple transgene cassettes. Although the HCAdS provide a much superior vector system, they are more complicated to generate compared to previous generations of adenoviral vectors and also have possibility of helper virus contamination due to inefficient Cre-mediated excision of the helper virus packaging signal [123] (**Figure 2D**).

### 3.2. Current applications of adenoviral vectors

Since their first use in gene therapy, adenoviral vectors have progressed significantly and are currently being tested clinically in several gene therapy, vaccine vector, and anticancer studies.

#### 3.2.1. Gene therapy

Adenoviruses have a unique ability to infect a broad range of cell types. Therefore, adenovirus-based vectors can be used to transduce and deliver transgenes to different cell types including both replicating and quiescent cell populations. This property of adenoviral vectors is extremely important in gene therapy and puts adenoviral vectors on top of viral vectors for gene delivery. Furthermore, adenovirus vectors do not integrate into host genomes but stay as episomal DNA in the nucleus of host cells. Modern adenoviral vectors can take multiple gene cassettes, up to 36 kb of foreign DNA, which make them suitable for delivering virtually any size of gene. In 1992, for the first time, a first-generation adenoviral vector was used to deliver and express alpha-1 antitrypsin (A1AT) in hepatocytes of a patient who had alpha-1 antitrypsin deficiency [124]. In another study, an E1–E3 deleted HAd5 adenoviral vector was used to deliver an A1AT gene to lung tissues [125]. Later, using adenoviral vectors, a number of attempts were made to deliver dysfunctional or deficient genes, which were responsible for several human genetic diseases and conditions. Cystic fibrosis is one such human genetic disease, in which the gene CFTR (cystic fibrosis transmembrane conductance regulator) becomes dysfunctional due to mutation. Adenoviral vector was used to deliver CFTR genes to lung tissues [126]. In another study, the adenoviral vector was used to deliver the gene for ornithine transcarbamylase, which is required in the urea cycle and is responsible for ornithine-transcarbamylase deficiency [127, 128]. These studies faced several challenges including humoral and cellular immunity to adenoviral vectors upon repeated administration of vector, cellular cytotoxicity, and oncogenesis [129]. These trials raised serious safety concerns for using adenoviral vectors in gene therapy and resulted in a sharp decline in their use. The reasons for these problems were studied extensively and addressed by constructing new adenoviral vectors. The adenoviral immunogenicity and cytotoxicity were suspected to be due to low-level expression of several viral proteins. The newer generations of adenoviral vectors had these adenoviral genes removed, and hence the vector immunogenicity and toxicity were significantly reduced. The new generations of adenoviral vectors have raised new hope in adenoviral vector-based gene delivery. Currently, a number of gene therapy clinical trials

are ongoing with adenoviral vectors (**Table 1**). The previous generation of adenoviral vectors is still in use for vaccine antigen delivery due to their inherent capability of inducing robust humoral and cellular immune responses.

### 3.2.2. Vaccine vector

As described earlier, adenoviruses activate several innate immune signaling pathways that result in the secretion of a number of proinflammatory cytokines. These proinflammatory cytokines pave the way for effective immune cell stimulation and result in the induction of robust adaptive humoral and cellular immune responses. To resolve infections with intracellular pathogens such as viruses, CD8<sup>+</sup> cytotoxic T lymphocyte (CTL) responses are critical. Transgene antigens carried by adenoviral vectors are presented to T cells via MHC class I molecules, and therefore, they induce efficient and robust CTL responses. The CTLs efficiently recognize and kill virus-infected cells, intracellular pathogens, and cancerous cells. These properties make adenoviral vectors promising as vaccine vectors. A number of human clinical trials have been conducted for adenoviral vector-based vaccines against different infectious diseases including Ebola virus, Zika virus, influenza viruses, HIV, *Mycobacterium tuberculosis*, and malaria [21, 130].

An HAd5 vector-based HIV vaccine containing clade B sequences of *gag/pol* and *nef* genes was tested in several clinical trials by Merck during 2003–2006 [131]. These studies demonstrated that a majority (80%) of vaccine recipients induced T cells with a magnitude of 275–300 IFN- $\gamma$  producing cells per million peripheral blood mononuclear cells (PBMCs), where about 50% vaccinees had detectable and durable HIV-specific CD8<sup>+</sup> T and CD4<sup>+</sup> T cells. This is far greater than any other T cell vaccine at that time [132–135]. These results were very encouraging and led to a multinational STEP trial involving about 3000 subjects in 2005 [136, 137]. The early results of this vaccine indicated that vaccine was well

S. no.	Adenoviral vector (biologic)	Modification	Transgene	Target/condition	Phase	ClinicalTrials identifier
1	HAd5-CB-CFTR	E1 deleted	Cystic fibrosis transmembrane conductance regulator (CFTR) gene	Cystic fibrosis	I	NCT00004779
2	HAd5-hAQP1	E1 deleted	Human aquaporin-1 (hAQP1)	Parotid salivary dysfunction	I	NCT00372320
3	HAd5-PDGF-B	E1 deleted	Platelet-derived growth factor B (PDGF-B)	Varicose ulcer	I	NCT00000431
4	HAd5-PEDF (AdGVPEDF.11D)	E1, E3 and E4 deleted	Pigment epithelium-derived factor (PEDF) protein	Macular degeneration	I	NCT00109499
5	HAd5-VEGF	E1–E3-deleted	Vascular endothelial growth factor D (VEGF-D) gene	Angina pectoris/ myocardial infarction	I	NCT01002430

**Table 1.** Gene therapy: adenoviral vectors in clinical trial.

tolerated upon repeated vaccination and induced robust T-cell responses to HIV antigens. Despite these early findings, the STEP trial had to be terminated prematurely in 2007 due to enhanced acquisition of HIV infection in the vaccine group compared to placebo [137, 138]. A total of 82 cases of HIV infection were recorded in the trial participants, 49 cases were in vaccine recipients and 33 were in placebo group. Another interesting observation was that the HIV infection rate was twofold higher in men with prior adenovirus type 5 infection (Ad5 titers >18) versus placebo recipients [135, 139]. The same HAd5 clade B *gag/pol* and *nef* genes-based vaccine was tested in another companion Phambili clinical trial in a South African population. The goal was to investigate whether this vaccine would be efficacious against clade C HIV infections. The participants had different prevalent modes of sexual transmission, different subtypes of HIV-1, and varying Ad5 seroprevalence. Unfortunately, this trial also had to be stopped due to acquisition of HIV infection in 9 females seropositive for HAd5 out of a total of 11 cases. Of these 9 cases, 6 were vaccinees [140, 141]. These results indicated that pre-existing immunity to the Ad5 vector is an important risk factor for HIV acquisition among vaccine recipients. Such profound effects of pre-existing Ad5 immunity on HIV acquisition were not observed in previous studies in non-human primates (NHP) using an adenovirus vector-based vaccine [142]. Several hypotheses were provided for the failure of the STEP trial but none of the hypotheses were proven after experimentation, and the mechanisms for higher HIV acquisition in vaccinees with pre-existing Ad immunity still remain unclear. These results shocked the vaccine community and raised serious questions regarding the fate of adenoviral vectors in vaccine approaches.

In 2009, another famous HIV vaccine clinical trial (HVTN phase II) was started [143]. It utilized a heterologous prime-boost strategy, in which vaccinees were first primed with a DNA-based vaccine expressing HIV proteins (*envA*, *envB*, *envC*, *gagB*, *polB*, *nefB*) followed by a booster Ad5 vector vaccine having matching HIV antigens as transgenes. This trial too met with the same fate as STEP, as the vaccine failed to reduce the HIV acquisition rate or attenuate the disease in infected subjects. It was terminated in 2013 prior to completion. However, this study and several other studies provided evidence of superior response rates, induction of broader T cell immune responses with well-accepted tolerance, by heterologous prime-boost vectors compared to homologous vaccination [144]. This started a new vaccination regimen involving priming with one type of adenoviral vector and boosting with another adenoviral vector derived from novel serotypes such as HAd26 and HAd35. This allowed for repeated vaccination and also vaccination in individuals with pre-existing vector immunity [145–149]. Currently, a number of non-human adenoviral vectors such as chimpanzee and bovine are also being utilized to avoid pre-existing vector-specific immunity [18, 21, 150–152].

Due to the emergence of life-threatening infectious diseases such as Ebola and Zika viruses, an immediate need for vaccines for these pathogens was recognized. These urgent needs attracted researchers toward viral vector platform-based vaccines, especially extensively studied and improved vector technology, and adenoviral vectors became the focus of several vaccines against these infectious diseases [21, 130]. Adenoviral vector-based vaccines are easy to design and to produce on a mass scale, which is of paramount significance for clinical

use. Therefore, three adenoviral vector-based vaccines encoding Ebola virus glycoprotein, ChAd3-ZEBOV1 from GlaxoSmithKline [153], Ad26-ZEBOV/MVA-BN-Filo2 from Johnson & Johnson [154], and HAd5 from the Chinese federal agency [155] were quickly generated and tested in macaques. Each of them proved to be well tolerated, immunogenic, and protective in macaques. All these vaccines were also well tolerated, safe, and immunogenic in phase I clinical trials, and the ChAd3- and ChAd26/MVA-based vaccines progressed further into phase II and phase III efficacy trials [156, 157]. The Chinese Ad5-based Ebola vaccine showed less efficacy in phase I clinical trial in individuals with pre-existing adenoviral immunity [158]. Beside these, a chimpanzee adenoviral vector ChAd63 prime/MVA boost-based malaria vaccine, which contains *Plasmodium falciparum*-derived ME-TRAP antigen, showed a significant enhancement in antigen-specific T cell responses and partial protection against malarial parasites in a phase I clinical trial [156, 159].

Despite initial setbacks, adenoviral vector-based vaccines are still very attractive and promising vaccine platforms. Currently, several adenoviral vector-based vaccines are in different stages of clinical development (**Table 2**).

### 3.2.3. Cancer immunotherapy

Several DNA viruses such as reo, measles, herpes simplex, Newcastle disease, and vaccinia have been tested in clinical trials for anticancer immunotherapy. The mechanism of anticancer activity of these viruses is multipronged. One mechanism involves selective infection and replication in tumor cells where expression of viral antigens or oncogenes inside the cancer cell changes the tumor microenvironment by inducing proinflammatory cytokines. These subsequently attract immune cells to the tumors eventually resulting in lysis of the tumor cells. Another mechanism uses vectors to deliver gene(s) whose expression results in the apoptosis of cancer cells or lysis of cancer cells due to replication by replication-competent viral vectors [160, 161]. Adenoviral vector technologies have progressed to the clinical stage for various cancers and also have been approved in some countries for use in human [162–164].

Various anticancer approaches have been tested using adenoviral vectors. One of these approaches depends on the induction of immune responses by delivering specific tumor-associated antigen as a vaccine, which activates immune cells against the tumor [165–168]. Due to immunogenic properties of adenoviral proteins such as capsid, adenoviral vectors induce robust CTL responses, which eventually kill the tumor cells expressing these tumor antigens. However, this vaccination strategy has shown limited success in cancer.

Another approach uses conditional replicative adenoviral vector (CRAd) to preferentially replicate inside a tumor cell and eventually lyse it through a lytic replication [162]. This strategy takes advantage of the conducive nature of cancer cells toward adenoviruses. Adenoviral vectors have been modified to efficiently carry out oncolytic replication in cancer cells while limiting their replication in healthy cells [9, 169]. An adenoviral vector with a partial E1B gene deficiency called ONYX-015 became the first ever adenoviral vector to enter clinical trial in 1996 [169]. The ONYX-015 is unable to replicate in healthy cells expressing p53 but replicates

S. no.	Adenoviral vector (biologic)	Modification	Transgene	Target/condition	Phase	ClinicalTrials identifier
1	HAd5-EBOV	E1-deleted	Glycoprotein	Ebola virus disease	II	NCT02575456
2	ChAd3-EBO-Z	E1-deleted	Glycoprotein	Ebola virus disease	I/II	NCT02289027
3	ChAd3-EBO-Z/ MVA-EBOV-Z-BN-Filo Prime/Boost	E1-deleted	Glycoprotein/envelope filovirus	Ebola virus disease	I	NCT02267109
4	Ad26-EBOV-Z/MVA- BN-Filo Prime/boost	E1 and E3 deleted	Glycoprotein	Ebola virus disease	I	NCT02891980
5	Ad26-EBOV-Z/MVA- BN-Filo Prime/boost	E1 and E3 deleted	Glycoprotein	Ebola virus disease	III	NCT02661464
6	HAd6-Nsmut/ VhAd3NSmut or CHAd3NSmut/ HAd6NSmut Prime/ boost	E1-deleted	Non-structural protein	Hepatitis C	I	NCT01070407
7	AdCh3NSmut/ Ad6NSmut	E1-deleted	Non-structural protein	Hepatitis C	I	NCT01094873
8	HAd5-HA (VXA-1.1)	E1-deleted	Hemagglutinin and double-stranded RNA as an adjuvant	Influenza H1N1	II	NCT02918006
9	HAd5-HA	E1/E3-deleted	Hemagglutinin	Influenza H5N1	I	NCT00755703
10	HAd4-HA5-Vtn HA	E3-partial deletion	Hemagglutinin	Influenza H5N1	I	NCT01006798
11	HAd35-CSP/HAd26- CSP Vectors Prime/ boost	E1 and E3 deleted	Circumsporozoite (CSP) antigen	Malaria	I/II	NCT01397227
12	HAd5 (NMRC-M3V-Ad-PfCA)	E1, E4 deleted, E3 partially deleted	Circumsporozoite (CSP) antigen, apical membrane antigen 1 (AMA1)	Malaria	I/II	NCT00392015
13	ChAd63-ME-TRAP/ MVA-ME-TRAP poxvirus Prime/boost	E1-deleted	ME-TRAP antigen (pre-erythrocytic thrombospondin- related adhesion protein)	Malaria	I	NCT01373879
14	HAd35-TB Antigens/ MVA85A Prime/boost (AERAS-402, BCG)	E1-deleted, HAd5 E4 orf6 replaced	TB antigens: Ag85A, Ag85B, and TB10.4	Tuberculosis	I	NCT01683773
15	HAd35-TB Antigens (AERAS-402)	E1-deleted, HAd5 E4 orf6 replaced	TB antigens: Ag85A, Ag85B, and TB10.4	Tuberculosis	II	NCT02414828

S. no.	Adenoviral vector (biologic)	Modification	Transgene	Target/condition	Phase	ClinicalTrials identifier
16	Four HAd5 vectors for four HIVAntigens (VRC-HIVADV014-00-VP/VRC-HIVADV014-00-VP)	E1, E4 and partial E3 deleted	HIV antigens gp140(A), gp140(B) dv12, gp140(C) and GagPol(B)	HIV infections	I	NCT01549509, NCT00119873, NCT00091416, NCT00709605, NCT00102089
17	Plasmid DNA expressing Gag, Plo and Nef +Four HAd5 vectors for four HIVAntigens (VRC-HIVDNA016-00-VP/VRC-HIVADV014-00-VP), DNA + HAd5/HAd5 Prime/boost	E1, E4 and partial E3 deleted	HIV antigens gp140(A), gp140(B) dv12, gp140(C) and GagPol(B)	HIV infections	I/II	NCT00123968, NCT00125970
18	Plasmid DNA vaccine/HAd5-HIV-1 (VRC-HIVDNA016-00-VP/VRC-HIVADV014-00-VP) prime/boost	E1, E4 and partial E3 deleted	HIV antigens gp140(A), gp140(B) dv12, gp140(C) and GagPol(B)	HIV infections	II	NCT00865566
19	DNA Vaccine/HAd35/HAd5 (VRC-HIVDNA044-00-VP/VRC-HIVADV027-00-VP/VRC-HIVADV038-00-VP) HAd35/HAd5 prime/boost or DNA/HAd5 prime/boost or DNA/HAd35 prime/boost	E1-deleted	Gag, pol and Nef antigens	HIV infections	I	NCT00801697
20	HAd26 (HAd26. ENVA.01)	E1 and E3 deleted	Env gp140	HIV infections	I	NCT00618605, NCT01103687
21	HAd4-mgag, HAd4-EnvC150 alone or combination	Replication competent	mosaic HIV Gag antigen, HIV clade C Env protein (gp150 1086.C)	HIV infections	I	NCT02771730, NCT01989533
22	rcAd26.MOS1.HIV-Env 1	E3 or E3/E4 deleted	HIV-1 Mos1Env	HIV infections	I	NCT02366013
23	Ad26.Mos.HIV (Ad26. Mos.1.Env + Ad26. Mos1.Gag-Pol + Ad26. Mos2.Gag-Pol)/MVA-Mosaic prime/boost	E3 or E3/E4 deleted	Hiv gag, pol and Env	HIV infections	I/II	NCT02919306

**Table 2.** Vaccine delivery: adenoviral vectors in clinical trials.

in p53-deficient tumor cells and results in the lysis of the cell, taking advantage of the cancer cell environment that supports vector replication [9]. The ONYX-015 has been proven to be safe and well tolerated in patients with various advanced cancers and is reported to be even

more effective when administered in combination with standard chemotherapy [169, 170]. A similar adenoviral vector named Oncorine or H101, developed by Shanghai Sunway Biotech, was approved by the Chinese Food and Drug Administration agency for the treatment of head and neck cancer [171, 172]. To further enhance the efficacy, potency, and specificity of the oncolytic adenoviral vectors, a new generation of adenoviral vectors is being tested. These new adenoviral vector systems carry a suicide gene like HSV thymidine kinase or a cytotoxic prodrug under the control of tumor gene/antigen promoter like prostate antigen promoter [173–175].

In some studies, replication-deficient or replication-competent adenoviral vectors were used to deliver transgenes, which express a tumor suppressor protein or cytotoxic/suicide protein that induces cell cycle arrest or a death cascade [176, 177]. More than 50% of cancers have a mutation in tumor suppressor gene p53. Advexin is a replication-deficient adenoviral vector that expresses p53 through a CMV promoter. It was tested in both pre-clinical and more than a dozen phase I/II clinical trials and proved to be well tolerated and efficacious against colorectal cancer, hepatocellular carcinoma (HCC), non-small cell lung cancer (NSCLC), prostate cancer, breast cancer, ovarian cancer, bladder cancer, glioma, and squamous cell carcinoma of the head and neck [171, 178, 179]. Gendicine, a similar adenovirus vector developed by a Chinese Biotech Company, Shenzhen SiBiono GeneTech, differs only in that its transgene promoter is from Rous Sarcoma Virus. In 2003, Gendicine was approved by the Chinese Food and Drug Administration agency as a first-ever gene therapy product to be used in combination with chemotherapy to treat head and neck squamous cell carcinoma [4, 178, 180]. Since then, Gendicine has been tested in a number of clinical trials against different types of cancers such as HCC, NSCLC, malignant glioma, and epithelial ovarian carcinoma. It is reported to be well tolerated and provide progression-free long-term survival benefits in combination regimens when compared to standard therapies alone [4, 178, 180, 181]. Therefore, adenoviral vectors have been clinically successful in anticancer therapy and have shown tremendous potential in the treatment of several cancer types [4, 171, 180, 181]. However, there is still scope for further improvements in clinical efficacy and safety (**Table 3**).

### 3.3. Challenges and solutions to adenoviral vectors use

Development of adenoviral vectors has come a long way since their first use. Currently, different types of adenoviral vectors are available for different applications. In the beginning, a high prevalence of pre-existing immunity to adenoviral vectors was considered as a serious concern for their use in mass vaccination and gene therapeutic applications. Further, immunogenicity, cellular toxicity, and oncogenesis were also major obstacles in gene therapy applications. Many other concerns, such as the possibility of vectors regaining replication competence, non-specificity, immunodominance of adenoviral antigens over the vaccine transgene antigen(s), immune modulation by viral antigens, heterologous immunity with other pathogens, are still evident in many adenoviral vector-based vaccine and gene therapy approaches [182]. These are discussed in the following sections.

S. no.	Adenoviral vector (biologic)	Modification	Transgene	Target/condition	Phase	ClinicalTrials identifier
1	ICOVIR-5	E2F-E1A Δ24 RGD	—	Solid tumors	I	NCT01864759
2	LOAd703	5/3 Δ24	CD40L & 4-1BBL	Pancreatic cancer	I/IIa	NCT02705196
3	HAd5-yCD/ mutTKSR39rep-hIL12	E1B-55K	Cytosine deaminase (CD)/ tyrosine kinase (TK) hIL12	Prostate cancer	I	NCT02555397
4	ONCOS-102 with cyclophosphamide	5/3 Δ24	GM-CSF	Advanced neoplasms	I	NCT01598129
5	VCN-01 with or without abraxane and gemcitabine	DM-1-E2F- E1A Δ24 RGD	Hyaluronidase	Advanced solid tumors	I	NCT02045602
6	VCN-01 with abraxane and gemcitabine	DM-1-E2F- E1A Δ24 RGD	Hyaluronidase	Advanced pancreatic cancer	I	NCT02045589
7	CG0070	E2F-E1A	Granulocyte macrophage colony- stimulating factor (GM-CSF)	Bladder cancer	III	NCT02365818
8	CG0070	E2F-E1A	GM-CSF	Bladder cancer	II/III	NCT01438112
9	Colo-Ad1	Ad11p/Ad3	—	Colon, non-small cell lung cancer, bladder, renal cancer	I	NCT02053220
10	DNX-2401 with Temozolomide	E1A Δ24 RGD	—	Glioblastoma multiforme	I	NCT01956734
11	DNX-2401 with IFN $\gamma$	E1A Δ24 RGD	—	Brain tumors	I	NCT02197169
12	Ad5-yCD/mutTKSR39rep- ADP with intensity- modulated radiation therapy (IMRT)	E1B-55K	CD/TK	Prostate carcinoma	II/III	NCT00583492
13	OBP-301	hTERT	—	Hepatocellular carcinoma	I/II	NCT02293850

**Table 3.** Oncolytic therapy: adenoviruses in clinical trial.

### 3.3.1. Tissue tropism and transgene expression

Adenoviruses can infect a diverse range of mammalian cell types. Infection to host cells is mediated by binding of adenoviral fiber protein to host cell surface receptors followed by recruitment of RGD motifs on penton bases to bind the host cell alpha-integrins. Most

common human adenoviruses (HAd5 and HAd2) bind to coxsackie adenovirus receptor (CAR) present on many different cell types including epithelial, endothelial, hepatocytes, myoblasts, and heart muscle cells. Some cells such as lymphocytes do not express CAR themselves but harbor CAR-recognizing adenoviruses. Adenovirus from subgroup B such as HAd35 do not bind to CAR but recognize another complement regulatory receptor CD46 present on most nucleated human cells, hematopoietic stem cells, and dendritic cells. Another subgroup B adenovirus HAd3 binds to CD80 and CD86 costimulatory molecules on antigen-presenting cells [8, 166, 183, 184]. Other cellular receptors such as integrin  $\alpha\beta_5$ , heparin sulfate proteoglycans, and sialic acid have also been reported to aid adenoviral entry to the cells [30–32, 185].

### 3.3.2. *Pre-existing adenoviral immunity*

The impact of pre-existing immunity against adenoviral vectors has been discussed in the previous section under adaptive immunity. To avoid the pre-existing immunity against adenoviral vectors, several strategies are being employed as discussed below.

#### 3.3.2.1. *Use of alternative less frequent adenoviruses for vector*

Several human adenoviruses with low seroprevalence such as HAd2, HAd26, and HAd35 were identified and developed into vectors [186]. The seroprevalence of these rare human adenovirus serotypes is very low, and hence the effect of pre-existing immunity is minimal [81, 187]. HAd26 and HAd35 vectors have been tested in phase I clinical trials and proven to be safe. However, the immunogenicity and efficacy of these low seroprevalence vectors are reported to be lower in comparison to more prevalent HAd5. These results are very concerning and warrant further investigation to find the reasons for the poor performance of these vectors. The nAb and T cells against HAd5 do not cross react with HAd35 but nAbs and T cells against another common serotype, HAd2, cross react with HAd35 and reduce the immunogenicity and efficacy of the HAd35 vectors [188, 189].

To avoid the cross-reactive immunity due to closely related serotypes of Ad, more genetically distant Ad serotypes including animal and bird Ad were developed as viral vectors. Among non-human adenovirus vectors, chimpanzee-derived adenovirus vector (ChAd) is the most widely used. In comparison to HAd, the nAbs against ChAd have been found to be less prevalent. For example, nAbs against ChAd7 were detected in only 15% of American, European, Chinese, and African population [186]. Similarly, nAbs against ChAd6 are also low in these populations except Africans, which have about 40% ChAd6-specific nAbs [81, 186]. Several chimpanzee adenoviral vector-based vaccines, such as ChAd7 for Ebola virus, ChAd6 for rabies, and ChAd6, ChAd7, and ChAd9 for malaria, have shown high efficacy in animal models [190]. Furthermore, ChAd63-based malaria and ChAd3-based hepatitis C virus vaccines have shown to be safe and highly immunogenic in phase I clinical trial [191, 192]. Despite low seroprevalence of ChAd vectors in humans, pre-existing cross-reactive T cells against many conserved viral antigens are still a major concern. The HAd-induced ChAd cross-reactive T cells have been reported against ChAd6, ChAd7, ChAd24, ChAd32, and ChAd68 [22, 80, 89, 90]. The negative effects of these cross-reactive T cells on ChAd have been demonstrated in several animal models [22, 89]. The

impact of the pre-existing cross-reactive T cells could be far greater in the clinical setting where humans are repeatedly exposed to various serotypes of adenovirus and carry a far broader diversity and a higher frequency of cross-reactive T cells.

Apart from rare human and chimpanzee adenoviruses, several other adenoviruses derived from animals such as bovine, porcine, ovine, canine, and fowl are also being explored for vector development [151, 193–195]. The human population lack nAbs against these adenoviruses, and therefore, the vectors derived from these adenoviruses could be more efficacious in comparison to HAd and ChAd. The mouse models with experimentally induced pre-existing immunity by the administration of HAd vector demonstrated a lack of nAbs and CD4<sup>+</sup> T cells against PAd3 and BAd3 [18]. Furthermore, BAd3- or PAd3-based influenza virus vaccine demonstrated high efficacy even in the presence of pre-existing HAd5 immunity. There was also no effect of pre-existing HAd5 immunity on transgene expression, immunogenicity, and efficacy in animal models. However, their potential benefits still need to be proven in humans.

#### *3.3.2.2. Routes of immunization*

Several studies have reported that different routes of immunizations can negate the detrimental effect of pre-existing immunity. This outcome could be at least partly due to evasion of tissue-resident Ad-specific T cells when using different routes of immunization. Tissue-resident CD8 memory T cells remain confined to a specific tissue. As such, they are not systemic and do not prevent Ad vector infection in distant tissues. These tissue-resident T cells are induced by tissue-derived migratory dendritic cells during priming, which activate T cells with specific tissue-homing molecules [196–198]. In a non-human primate model, HAd5-induced protective immune responses by intranasal/intratracheal immunization were not affected by pre-existing HAd5 immunity that had been induced by intramuscular administration of an unrelated HAd5 vector [199]. Although this strategy has shown promising results in animal models, it has not been verified in humans. This strategy is however restricted by limited routes feasible for administration in humans. Moreover, the nAbs induced by adenoviral infection or adenoviral vector-based vaccination can be detected in any part of the body, which may affect the transgene expression and immune responses irrespective of the alternative route used for subsequent adenovector administration. However, nAbs are generally present in blood and are in most cases not cross-reactive to different serotypes or subtypes and Ads from different host species [200].

#### *3.3.2.3. Heterologous prime-boost strategy*

Another strategy to avoid pre-existing adenovector immunity involves the use of heterologous prime-boost regimens. In this strategy, the priming and boosting are done by using different antigen delivery vehicle and/or vectors derived from either different serotypes of the same species or vectors from completely different host species, for example, priming with DNA and boosting with HAd5 or priming with ChAd68 and boosting with ChAd1 [201]. Studies have shown that the heterologous prime-boost induces more robust immune

responses compared to single vaccination or homologous prime-boost immunizations. The cellular immune responses induced by DNA prime and HAd boost were not affected by pre-existing HAd5 immunity. These findings were further confirmed in a clinical trial. Another preclinical study involving *Plasmodium* or SARS antigens encoded by Modified Vaccinia Ankara (MVA)/adenoviral vector as prime/boost showed induction of robust T cell and Ab responses of higher magnitude compared to Ad/DNA regimens. Finally, a ChAd63/MVA prime-boost strategy is now being evaluated in clinical trials as a vaccine against malaria, HIV, and HCV [191, 202–204]. The heterologous prime/boost strategy seems very effective in circumventing the pre-existing immunity against adenoviral vectors in studies conducted so far; however, the vehicle for priming and/or boosting must be carefully designed and selected.

### 3.3.3. Immunodominance over transgene immunity

Adenoviral antigens induce robust antibody and T cell immune responses. Recent studies have shown that adenoviral-derived epitopes can dominate over the transgene-derived epitopes and hinder the induction of transgene-specific immunity. This impairment of transgene-specific immune responses in naive vaccinees is due to immune competition. Epitopes derived from an adenovirus vector were shown to inhibit the induction of HIV GagL85-93-specific CD8<sup>+</sup> T cells [205]. This study demonstrated that competition occurs at the level of responding CD8<sup>+</sup> T cells, and co-immunization with an interleukin 2-encoding plasmid restored GagL85-93-specific CD8<sup>+</sup> T cell responses in the presence of an adenoviral hexon486-494 epitope. The IL2, however, could not restore GagL85-93 responsiveness in Ad-based immunization, likely due to the presence of other epitopes in the Ad vector [206]. Another study demonstrated that plasmid DNA, but not adenovirus vector-encoding hepatitis B surface antigen (HBsAg), primed CD8 T cells against subdominant HBsAg epitopes [207]. These studies suggest that adenoviral antigen-specific T-cell immunity is primed efficiently during adenoviral vector-based immunization, which can limit the immunogenicity of adenoviral vector-encoded transgenic antigens. These studies highlight the need for modifications of the vector or the transgene used in immunization to circumvent or dominate over the adenoviral vector-specific epitopes and induce more effective transgene-specific immunity.

### 3.3.4. Heterologous immunity induced by adenoviral antigens

We discovered an unusual and interesting phenomenon that non-recombinant HAd5 vector induces robust cross-reactive immune responses toward hepatitis C virus (HCV) antigens [91]. Upon further investigation, we found that adenoviral proteins contain extensive homologies with various peptide epitopes derived from HCV antigens. These observations led us to investigate the adenoviral vector-induced HCV cross-reactive immune responses in detail in both mice and humans. In mice, we demonstrated that Ad vector alone can induce potent, broad anti-HCV cross-reactive immunity that can significantly reduce viral load upon challenge with infectious chimeric Vaccinia-HCV. Furthermore, we also detected

HCV cross-reactive antibodies and HCV antigen-dependent expression of IFN- $\gamma$  in T cells from a cohort of HCV-naïve but Ad-immune human individuals. Previous studies have also reported that one pathogen can induce cross-reactive immunity against an unrelated pathogen [91, 208]. This kind of immunity is known as heterologous immunity. Heterologous immunity is a double-edged sword, which can modulate the breadth of the T cell repertoire, influence the memory T cell pool and/or the immune dominance of a specific epitope, and lead to enhanced or diminished immune responses against a pathogen. These observations have significant clinical implications on natural history, immunopathogenesis, and disease outcome in HCV infection. The widespread use of adenoviral vectors in mass vaccination programs might change the immune hierarchy and natural T cell responses against HCV antigens and deviate and/or alter the incidence of HCV infection and immune pathogenesis in an at-risk population. However, a careful evaluation of adenoviral-induced cross-reactive immune responses and their impact on HCV immunity and immunopathology is needed to more accurately ascertain the impact of this phenomenon.

#### **4. Conclusions and future prospects**

Since their first use as gene delivery vehicles, adenoviral vectors have been extensively studied in a number of applications and have improved substantially over time. Issues such as toxicity, pre-existing immunity in humans, and challenges in construction are continually being addressed. More recently, outbreaks of newly emerging infectious diseases, such as SARS, Ebola, and Zika, and the continuing threat of bioterrorism have increased the requirement of novel vaccine platforms which can be designed and produced in large scale within a short period of time. Adenoviral vectors, due to their versatility, ease of construction, adeptness to rapid mass production, and induction of robust transgene-specific humoral and cellular immune responses, have proven to be valuable in the development of vaccines for emerging viral infectious diseases. Furthermore, extensive knowledge about the adenoviral vector biology and the induced immune responses in animals have tremendously helped in developing effective vaccine candidates against several viral pathogens, which have progressed to advanced clinical stages. The adenoviruses are now at the forefront of vaccinology and have shown huge potential in both pre-clinical and clinical studies for HIV, malaria, Ebola virus, and Zika virus vaccines. Apart from infectious disease, adenoviral vectors have been approved for human use as cancer and gene therapy. Therefore, adenoviral vectors have opened new avenues in gene delivery, vaccine antigen delivery, and cancer molecular therapy.

#### **Acknowledgements**

We acknowledge Bharti Singh for designing and creating figures for this chapter. This work was supported by the Canadian Institutes of Health Research (CIHR) operating grant to BA (MOP 79327).

## Conflict of interest

The authors SS, BA, and RK are co-inventors of a patent on adenoviral vector-based method of inducing immune responses against hepatitis C virus.

## Author details

Shakti Singh<sup>1,3</sup>, Rakesh Kumar<sup>2</sup> and Babita Agrawal<sup>3\*</sup>

\*Address all correspondence to: [bagrawal@ualberta.ca](mailto:bagrawal@ualberta.ca)

1 Division of Infectious Disease, Los Angeles Biomedical Research Institute, Harbor-University of California Medical Center, Torrance, California, USA

2 Department of Laboratory Medicine and Pathology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

3 Department of Surgery, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

## References

- [1] Wold W, Horwitz M. Adenoviruses. In: Fields BN, Knipe DM, Howley PM, editors. *Fields Virology*. 6th ed. 5th ed. Philadelphia: Lippincott-Raven; 2007. pp. 2395-2436
- [2] Rowe W, Hueer R, Gilmore L, Parrot R, Ward T. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proceedings of the Society for Experimental Biology and Medicine Society for Experimental Biology and Medicine* (New York, NY). 1953;**84**(3):570-573
- [3] Yu B, Wang Z, Dong J, Wang C, Gu L, Sun C, et al. A serological survey of human adenovirus serotype 2 and 5 circulating pediatric populations in Changchun, China, 2011. *Virology Journal*. 2012;**9**:287
- [4] Zhang W, Li L, Li D, Liu J, Li X, Li W, et al. The first approved gene therapy product for cancer Ad-p53 (gencicine): 12 years in the clinic. *Human Gene Therapy*. 2018;**29**(2):160-179
- [5] McConnell M, Imperiale MJ. Biology of adenovirus and its use as a vector for gene therapy. *Human Gene Therapy*. 2004;**15**(11):1022-1033
- [6] Crawford-Miksza L, Schnurr D. Analysis of 15 adenovirus hexon proteins reveals the location and structure of seven hypervariable regions containing serotype-specific residues. *Journal of Virology*. 1996;**70**(3):1836-1844

- [7] CMMF, Rosa-Calatrava M, Conway J, Zubieta C, Cusack S, Ruigrok R, et al. A quasi-atomic model of human adenovirus type 5 capsid. *The EMBO Journal*. 2005;**24**(9):1645-1654
- [8] Wickham T, Carrion M, Kovesdi I. Targeting of adenovirus penton base to new receptors through replacement of its RGD motif with other receptor-specific peptide motifs. *Gene Therapy*. 1995;**2**(10):750-756
- [9] Bischoff J, Kirn D, Williams A, Heise C, Horn S, Muna M, et al. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science (New York, NY)*. 1996;**274**(5286):373-376
- [10] Benkő M, Virus-Host H. Molecular evolution of adenoviruses. *Current Topics in Microbiology and Immunology*. 2003;**272**:3-35
- [11] Wadell G. Adenoviridae: The adenoviruses. In: *Laboratory Diagnosis of Infectious Diseases Principles and Practice*. New York, NY: Springer; 1988
- [12] Ginsberg HS. Identification and classification of adenoviruses. *Virology*. 1962;**18**:312-319
- [13] Mase M, Mitake H, Inoue T, Imada T. Identification of group I-III avian adenovirus by PCR coupled with direct sequencing of the hexon gene. *The Journal of Veterinary Medical Science*. 2009;**71**(9):1239-1242
- [14] Calnek B, Shek W, Menendez N, Stiube P. Serological cross-reactivity of avian adenovirus serotypes in an enzyme-linked immunosorbent assay. *Avian Diseases*. 1982;**26**(4):897-906
- [15] Kawamura H, Shimizu F, Tsubahara H. Avian adenovirus: Its properties and serological classification. *National Institute of Animal Health Quarterly*. 1964;**4**:183-193
- [16] Bürki F, Wege H, Reich-Rohrwig C, Hinaidy B. Bovine adenoviruses. I. Characterization and serological classification as types 4 of two isolates from latently infected calf testicles. *Zentralblatt für Veterinärmedizin. Reihe B*. 1978;**25**(7):555-565
- [17] Pereira HG, Huebner RJ, Ginsberg HS, Van Der Veen J. A short description of the adenovirus group. *Virology*. 1963;**20**:613-620
- [18] Guo J, Mondal M, Zhou D. Development of novel vaccine vectors: Chimpanzee adenoviral vectors. *Human Vaccines & Immunotherapeutics*. 2018;**14**(7):1679-1685
- [19] Cheng C, Wang L, Ko S, Kong W, Schmidt S, Gall J, et al. Combination recombinant simian or chimpanzee adenoviral vectors for vaccine development. *Vaccine*. 2015;**33**(51):7344-7351
- [20] Colloca S, Barnes E, Folgari A, Ammendola V, Capone S, Cirillo A, et al. Vaccine vectors derived from a large collection of simian adenoviruses induce potent cellular immunity across multiple species. *Science Translational Medicine*. 2012;**4**(115):115ra2-115ra2
- [21] Ewer K, Sebastian S, Spencer A, Gilbert S, Hill A, Lambe T. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. *Human Vaccines & Immunotherapeutics*. 2017;**13**(12):3020-3032

- [22] Iampietro M, Larocca R, Provine N, Abbink P, Kang Z, Bricault C, et al. Immunogenicity and cross-reactivity of rhesus adenoviral vectors. *Journal of Virology*. 2018;**92**(11): e00159-e00118
- [23] Suomalainen M, Nakano MY, Boucke K, Keller S, Greber UF. Adenovirus-activated PKA and p38/MAPK pathways boost microtubule-mediated nuclear targeting of virus. *The EMBO Journal*. 2001;**20**(6):1310-1319
- [24] Tibbles LA, Spurrell JC, Bowen GP, Liu Q, Lam M, Zaiss AK, Robbins SM, Hollenberg MD, Wickham TJ, Muruve DA. Activation of p38 and ERK signaling during adenovirus vector cell entry lead to expression of the CXC chemokine IP-10. *Journal of Virology*. 2002;**76**(4):1559-1568
- [25] Basner-Tschakarjan E, Gaffal E, O'Keeffe M, Tormo D, Limmer A, Wagner H, Hochrein H, Tüting T. Adenovirus efficiently transduces plasmacytoid dendritic cells resulting in TLR9-dependent maturation and IFN- $\alpha$  production. *The Journal of Gene Medicine*. 2006;**8**(11):1300-1306
- [26] Hartman Z, Appledorn D, Amalfitano A. Adenovirus vector induced innate immune responses: Impact upon efficacy and toxicity in gene therapy and vaccine applications. *Virus Research*. 2008;**132**(1-2):1-14
- [27] Lütschg V, Boucke K, Hemmi S, Greber UF. Chemotactic antiviral cytokines promote infectious apical entry of human adenovirus into polarized epithelial cells. *Nature Communications*. 2011;**2**:391
- [28] Amberg N. Adenovirus receptors: Implications for targeting of viral vectors. *Trends in Pharmacological Sciences*. 2012;**33**(8):442-448
- [29] Wolfrum N, Greber UF. Adenovirus signalling in entry. *Cellular Microbiology*. 2013;**15**(1):53-62
- [30] Lyle C, McCormick F. Integrin  $\alpha\beta 5$  is a primary receptor for adenovirus in CAR-negative cells. *Virology Journal*. 2010;**7**(1):1-13
- [31] Lenman A, Liaci MA, Liu Y, Årdahl C, Rajan A, Nilsson E, et al. Human adenovirus 52 uses sialic acid-containing glycoproteins and the coxsackie and adenovirus receptor for binding to target cells. *PLOS Pathogens*. 2015;**11**(2):e1004657
- [32] Amberg N, Pring-Åkerblom P, Wadell G. Adenovirus type 37 uses sialic acid as a cellular receptor on Chang C cells. *Journal of Virology*. 2002;**76**(17):8834-8841
- [33] Meier O, Gastaldelli M, Boucke K, Hemmi S, Greber UF. Early steps of clathrin-mediated endocytosis involved in phagosomal escape of Fc $\gamma$  receptor-targeted adenovirus. *Journal of Virology*. 2005;**79**(4):2604-2613
- [34] Rathinam VA, Fitzgerald KA. Cytosolic surveillance and antiviral immunity. *Current Opinion in Virology*. December 2011;**1**(6):455-462

- [35] Rathinam V, Fitzgerald KA. Innate immune sensing of DNA viruses. *Virology*. 2011; **411**(2):153-162
- [36] Takeuchi O, Akira S. Innate immunity to virus infection. *Immunological Reviews*. 2009; **227**(1):75-86
- [37] Wang X, Bergelson JM. Coxsackievirus and adenovirus receptor cytoplasmic and transmembrane domains are not essential for coxsackievirus and adenovirus infection. *Journal of Virology*. 1999; **73**(3):2559-2562
- [38] Wang K, Huang S, Kapoor-Munshi A, Nemerow G. Adenovirus internalization and infection require dynamin. 1998. *Journal of Virology*. 1998; **72**(4):3455-3458
- [39] Gaggar A, Shayakhmetov DM, Lieber A. CD46 is a cellular receptor for group B adenoviruses. 2003. *Nature Medicine*. 2003; **9**(11):1408-1412
- [40] Amstutz B, Gastaldelli M, Kälin S, Imelli N, Boucke K, Wandeler E, Mercer J, Hemmi S, Greber UF. Subversion of CtBP1-controlled macropinocytosis by human adenovirus serotype 3. *The EMBO Journal*. 2008; **27**(7):956-969
- [41] Iacobelli-Martinez M, Nepomuceno RR, Connolly J, Nemerow GR. CD46-utilizing adenoviruses inhibit C/EBP $\beta$ -dependent expression of proinflammatory cytokines. *Journal of Virology*. 2005; **79**(17):11259-11268
- [42] Appledorn DM, Patial S, McBride A, Godbehere S, Van Rooijen N, Parameswaran N, Amalfitano A. Adenovirus vector-induced innate inflammatory mediators, MAPK signaling, as well as adaptive immune responses are dependent upon both TLR2 and TLR9 in vivo. 2008. *Journal of Immunology*. 2008; **181**(3):2134-2144
- [43] Di Paolo NC, Miao EA, Iwakura Y, Murali-Krishna K, Aderem A, Flavell RA, Papanopoulou T, Shayakhmetov DM. Virus binding to a plasma membrane receptor triggers interleukin-1 $\alpha$ -mediated proinflammatory macrophage response in vivo. *Immunity*. 2009; **31**(1):110-121
- [44] Fejer G, Drechsel L, Liese J, Schleicher U, Ruzsics Z, Imelli N, et al. Key role of splenic myeloid DCs in the IFN- $\alpha\beta$  response to adenoviruses in vivo. *PLoS Pathogens*. 2008; **4**(11):e1000208
- [45] Di Paolo NC, van Rooijen N, Shayakhmetov DM. Redundant and synergistic mechanisms control the sequestration of blood-born adenovirus in the liver. *Molecular Therapy*. 2009; **17**(4):675-684
- [46] Chintakuntlawar A, Astley R, Chodosh J. Adenovirus type 37 keratitis in the C57BL/6J mouse. *Investigative Ophthalmology & Visual Science*. 2007; **48**(2):781-788
- [47] Cerullo V, Seiler M, Clarke C, Erez A, Barry M, Lee B. 150. Metabolically biotinylated helper dependent adenovirus: A new and rapid approach for targeting of high-capacity adenoviral vector. *Molecular Therapy*. 2006; **13**:S59
- [48] Zhu J, Huang X, Yang Y. Innate immune response to adenoviral vectors is mediated by both Toll-like receptor-dependent and -independent pathways. *Journal of Virology*. 2007; **81**(7):3170-3180

- [49] Chintakuntlawar A, Chodosh J. Chemokine CXCL1/KC and its receptor CXCR2 are responsible for neutrophil chemotaxis in adenoviral keratitis. *Journal of Interferon & Cytokine Research: The Official Journal of the International Society for Interferon and Cytokine Research*. 2009;**29**(10):657-666
- [50] Chintakuntlawar A, Zhou X, Rajaiya J, Chodosh J. Viral capsid is a pathogen-associated molecular pattern in adenovirus keratitis. *PLoS Pathogens*. 2010:e1000841
- [51] Iacobelli-Martinez M, Nemerow GR. Preferential activation of Toll-like receptor nine by CD46-utilizing adenoviruses. *Journal of Virology*. 2007;**81**(3):1305-1312
- [52] Weber C, Armbruster N, Scheller C, Kreppel F, Kochanek S, Rethwilm A, et al. Foamy virus-adenovirus hybrid vectors for gene therapy of the arthritides. *The Journal of Gene Medicine*. 2013;**15**(3-4):155-167
- [53] Johnsen IB, Nguyen TT, Ringdal M, Tryggestad AM, Bakke O, Lien E, Espevik T, Anthonsen MW. Toll-like receptor 3 associates with c-Src tyrosine kinase on endosomes to initiate antiviral signaling. 2006. *The EMBO Journal*. 2006;**25**(14):3335-3346
- [54] Shayakhmetov DM, Di Paolo NC, Mossman KL. Recognition of virus infection and innate host responses to viral gene therapy vectors. *Molecular Therapy*. 2010;**18**(8):1422-1429
- [55] Crozat K, Beutler B. TLR7: A new sensor of viral infection. *Proceedings of the National Academy of Sciences of the United States of America*. 2004;**101**(18):6835-6836
- [56] Lam E, Stein S, Falck-Pedersen E. Adenovirus detection by the cGAS/STING/TBK1 DNA sensing cascade. *Journal of Virology*. 2014;**88**(2):974-981
- [57] Orazio N, Naeger C, Karlseder J, Weitzman. The adenovirus E1b55K/E4orf6 complex induces degradation of the Bloom helicase during infection. *Journal of Virology*. 2010;**85**(4):1887-1892
- [58] Misawa T, Takahama M, Kozaki T, Lee H, Zou J, Saitoh T, et al. Microtubule-driven spatial arrangement of mitochondria promotes activation of the NLRP3 inflammasome. *Nature Immunology*. 2013;**14**:454-460
- [59] Fink S, Cookson B. Apoptosis, pyroptosis, and necrosis: Mechanistic description of dead and dying eukaryotic cells. *Infection and Immunity*. 2005;**73**(4):1907-1916
- [60] Berk A. Functions of adenovirus E1A. *Cancer Surveys*. 1986;**5**(2):367-387
- [61] Berk A. Adenovirus promoters and E1A transactivation. *Annual Review of Genetics*. 1986;**20**:45-79
- [62] Kalvakolanu D, Bandyopadhyay S, Harter M, Sen G. Inhibition of interferon-inducible gene expression by adenovirus E1A proteins: Block in transcriptional complex formation. *Proceedings of the National Academy of Sciences*. 1991;**88**(17):7459-7463
- [63] Ackrill A, Foster G, Laxton C, Flavell D, Stark G, Kerr I. Inhibition of the cellular response to interferons by products of the adenovirus type 5 E1A oncogene. *Nucleic Acids Research*. 1991;**19**(16):4387-4393

- [64] Gutch M, Reich N. Repression of the interferon signal transduction pathway by the adenovirus E1A oncogene. *Proceedings of the National Academy of Sciences of the United States of America*. 1991;**88**(18):7913-7917
- [65] Fonseca G, Cohen M, Nichols A, Barrett J, Mymryk J. Viral retasking of hBre1/RNF20 to recruit hPaf1 for transcriptional activation. *PLoS Pathogens*. 2013;**9**(6):e1003411
- [66] Fonseca G, Thillainadesan G, Yousef A, Ablack J, Mossman K, Torchia J, et al. Adenovirus evasion of interferon-mediated innate immunity by direct antagonism of a cellular histone posttranslational modification. *Cell Host & Microbe*. 2012;**11**(6):597-606
- [67] Ferreon AM, Ferreon JC, Wright PE, Deniz AA. Modulation of allostery by protein intrinsic disorder. *Nature*. 2013;**498**(7454):390
- [68] Teodoro J, Branton P. Regulation of p53-dependent apoptosis, transcriptional repression, and cell transformation by phosphorylation of the 55-kilodalton E1B protein of human adenovirus type 5. *Journal of Virology*. 1997;**71**(5):3620-3627
- [69] Sabbatini P, Chiou S, Rao L, White E. Modulation of p53-mediated transcriptional repression and apoptosis by the adenovirus E1B 19K protein. *Molecular and Cellular Biology*. 1995;**15**(2):1060-1070
- [70] Chahal J, Flint S. Timely synthesis of the adenovirus type 5 E1B 55-kilodalton protein is required for efficient genome replication in normal human cells. *Journal of Virology*. 2012;**86**(6):3064-3072
- [71] Chahal J, Qi J, Flint S. The human adenovirus type 5 E1B 55 kDa protein obstructs inhibition of viral replication by type I interferon in normal human cells. *PLoS Pathogens*. 2012;**8**(8):e1002853
- [72] Schreiner S, Bürck C, Glass M, Groitl P, Wimmer P, Kinkley S, et al. Control of human adenovirus type 5 gene expression by cellular Daxx/ATRAX chromatin-associated complexes. *Nucleic Acids Research*. 2013;**41**(6):3532-3550
- [73] Schreiner S, Wimmer P, Sirma H, Everett R, Blanchette P, Groitl P, et al. Proteasome-dependent degradation of Daxx by the viral E1B-55K protein in human adenovirus-infected cells. *Journal of Virology*. 2010;**84**(14):7029-7038
- [74] Stracker T, Lee D, Carson C, Araujo F, Ornelles D, Weitzman M. Serotype-specific reorganization of the Mre11 complex by adenoviral E4orf3 proteins. *Journal of Virology*. 2005;**79**(11):6664-6673
- [75] Stracker T, Carson C, Weitzman M. Adenovirus oncoproteins inactivate the Mre11–Rad50–NBS1 DNA repair complex. *Nature*. 2002;**418**:348-352
- [76] Evans J, Hearing P. Relocalization of the Mre11-Rad50-Nbs1 complex by the adenovirus E4 ORF3 protein is required for viral replication. *Journal of Virology*. 2005;**79**(10):6207-6215

- [77] Evans J, Hearing P. Distinct roles of the adenovirus E4 ORF3 protein in viral DNA replication and inhibition of genome concatenation. *Journal of Virology*. 2003;**77**(9):5295-5304
- [78] McSharry B, Burgert H, Owen D, Stanton R, Prod'homme V, Sester M, et al. Adenovirus E3/19K promotes evasion of NK cell recognition by intracellular sequestration of the NKG2D ligands major histocompatibility complex class I chain-related proteins A and B. *Journal of Virology*. 2008;**82**(9):4585-4594
- [79] Cianciola N, Carlin C. Adenovirus RID-alpha activates an autonomous cholesterol regulatory mechanism that rescues defects linked to Niemann-Pick disease type C. *The Journal of Cell Biology*. 2009;**187**(4):537-552
- [80] Fausther-Bovendo H, Kobinger G. Pre-existing immunity against Ad vectors: Humoral, cellular, and innate response, what's important? *Human Vaccines & Immunotherapeutics*. 2014;**10**(10):2875-2884
- [81] Zhang S, Huang W, Zhou X, Zhao Q, Wang Q, Jia B. Seroprevalence of neutralizing antibodies to human adenoviruses type-5 and type-26 and chimpanzee adenovirus type-68 in healthy Chinese adults. *Journal of Medical Virology*. 2013;**85**(6):1077-1084
- [82] Sumida, Truitt D, Lemckert A, Vogels R, JHH C, Addo M, et al. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *Journal of Immunology (Baltimore, Md: 1950)*. 2005;**174**(11):7179-7185.
- [83] Roberts D, Nanda A, Havenga M, Abbink P, Lynch D, Ewald BA, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature*. 2006;**441**(7090):239-243
- [84] Olive M, Eisenlohr L, Flomenberg N, Hsu S, Flomenberg P. The adenovirus capsid protein hexon contains a highly conserved human CD4+ T-cell epitope. *Human Gene Therapy*. 2002;**13**(10):1167-1178
- [85] Roy S, Shirley P, McClelland A, Kaleko M. Circumvention of immunity to the adenovirus major coat protein hexon. *Journal of Virology*. 1998;**72**(8):6875-6879
- [86] Roy S, Clawson D, Calcedo R, Lebherz C, Sanmiguel J, Wu D, et al. Use of chimeric adenoviral vectors to assess capsid neutralization determinants. *Virology*. 2005;**333**(2):207-214
- [87] Calcedo R, Vandenberghe L, Roy S, Somanathan S, Wang L, Wilson J. Host immune responses to chronic adenovirus infections in human and nonhuman primates. *Journal of Virology*. 2009;**83**(6):2623-2631
- [88] Sumida SM, Truitt D, Kishko M, Arthur J, Jackson S, Gorgone D, et al. Neutralizing antibodies and CD8+ T lymphocytes both contribute to immunity to adenovirus serotype 5 vaccine vectors. *Journal of Virology*. 2004;**78**(6):2666-2673
- [89] Veltrop-Duits L, Heemskerk B, Sombroek C, Vreeswijk T, Gubbels S, REE T, et al. Human CD4+ T cells stimulated by conserved adenovirus 5 hexon peptides recognize cells infected with different species of human adenovirus. *European Journal of Immunology*. 2006;**36**(9):2410-2423

- [90] Leen A, Sili U, Vanin E, Jewell A, Xie W, Vignali D, et al. Conserved CTL epitopes on the adenovirus hexon protein expand subgroup cross-reactive and subgroup-specific CD8<sup>+</sup> T cells. *Blood*. 2004;**104**(8):2432-2440
- [91] Singh S, Vedi S, Samrat S, Li W, Kumar R, Agrawal B. Heterologous immunity between adenoviruses and hepatitis C virus: A new paradigm in HCV immunity and vaccines. *PLoS One*. 2016;**11**(1):e0146404
- [92] McGrory W, Bautista D, Graham F. A simple technique for the rescue of early region I mutations into infectious human adenovirus type 5. *Virology*. 1988;**163**(2):614-617
- [93] Akusjärvi G. Proteins with transcription regulatory properties encoded by human adenoviruses. *Trends in Microbiology*. 1993;**1**(5):163-170
- [94] Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. *The Journal of General Virology*. 1977;**36**(1):59-74
- [95] Shaw G, Morse S, Ararat M, Graham FL. Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. *The FASEB Journal*. 2002;**16**(8):869-871
- [96] Benihoud K, Yeh P, Perricaudet M. Adenovirus vectors for gene delivery. *Current Opinion in Biotechnology*. 1999;**10**(5):440-447
- [97] Zhang P-X, Fuleihan R. Transfer of activation-dependent gene expression into T cell lines by recombinant adeno-associated virus. *Gene Therapy*. 1999;**6**(2):3300803
- [98] Hehir K, Armentano D, Cardoza L, Choquette T, Berthelette P, White G, et al. Molecular characterization of replication-competent variants of adenovirus vectors and genome modifications to prevent their occurrence. *Journal of Virology*. 1996;**70**(12):8459-8467
- [99] Kovesdi I, Hedley SJ. Adenoviral producer cells. *Viruses*. 2010;**2**(8):1681-1703
- [100] Fallaux FJ, Bout A, van der Velde I, van den Wollenberg DJ, Hehir KM, Keegan J, et al. New helper cells and matched early region 1-deleted adenovirus vectors prevent generation of replication-competent adenoviruses. *Human Gene Therapy*. 1998;**9**(13):1909-1917
- [101] Wold W, Tollefson A, Hermiston T. E3 transcription unit of adenovirus. *Current Topics in Microbiology and Immunology*. 1995;**199**(1):237-274
- [102] Wold W, Doronin K, Toth K, Kuppuswamy M, Lichtenstein DL, Tollefson AE. Immune responses to adenoviruses: Viral evasion mechanisms and their implications for the clinic. *Current Opinion in Immunology*. 1999;**11**(4):380-386
- [103] Bett A, Haddara W, Prevec L, Graham F. An efficient and flexible system for construction of adenovirus vectors with insertions or deletions in early regions 1 and 3. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;**91**(19):8802-8806
- [104] Yang Y, Nunes F, Berencsi K, Gönczöl E, Engelhardt J, Wilson J. Inactivation of E2a in recombinant adenoviruses improves the prospect for gene therapy in cystic fibrosis. *Nature Genetics*. 1994;**7**:362-369

- [105] Yang Y, Nunes F, Berencsi K, Furth E, Gönczöl E, Wilson J. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proceedings of the National Academy of Sciences of the United States of America*. 1994;**91**(10):4407-4411
- [106] Engelhardt JF, Litzky L, Wilson JM. Prolonged transgene expression in cotton rat lung with recombinant adenoviruses defective in E2a. *Human Gene Therapy*. 1994;**5**(10):1217-1229
- [107] Lusky M, Christ M, Rittner K, Dieterle A, Dreyer D, Mourot B, et al. In vitro and in vivo biology of recombinant adenovirus vectors with E1, E1/E2A, or E1/E4 deleted. *Journal of Virology*. 1998;**71**(3):2022-2032
- [108] Schaack J. Adenovirus vectors deleted for genes essential for viral DNA replication. *Frontiers in Bioscience: A Journal and Virtual Library*. 2005;**1**(10):1146-1155
- [109] Bett A, Krougliak V, Graham F. DNA sequence of the deletion/insertion in early region 3 of Ad5 dl309. *Virus Research*. 1995;**30**(1):75-82
- [110] Wang Q, Finer M. Second-generation adenovirus vectors. *Nature Medicine*. 1996;**2**:714-716
- [111] Kochanek S, Clemens P, Mitani K, Chen H, Chan S, Caskey C. A new adenoviral vector: Replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. *Proceedings of the National Academy of Sciences*. 1996;**93**(12):5731-5736
- [112] Ishizaki M, Kawano R, Uchida Y, Kimura E, Uchino M, Maeda Y. Gene therapy for duchenne muscular dystrophy by the helper-dependent adenovirus vector (HDA<sub>dv</sub>)—Mediated full-length dystrophin expression. *Molecular Therapy*. 2018;**15** (Supplement 1):S52
- [113] Puntel M, Muhammad A, Candolfi M, Salem A, Yagiz K, Farrokhi C, et al. A novel bicistronic high-capacity gutless adenovirus vector that drives constitutive expression of herpes simplex virus type 1 thymidine kinase and tet-inducible expression of Flt3L for glioma therapeutics. *Journal of Virology*. 2010;**84**(12):6007-6017
- [114] Parks R, Chen L, Anton M, Sankar U, Rudnicki M, Graham F. A helper-dependent adenovirus vector system: Removal of helper virus by Cre-mediated excision of the viral packaging signal. *Proceedings of the National Academy of Sciences of the United States of America*. 1996;**93**(24):13565-13570
- [115] Fisher KJ, Choi H, Burda J, Chen S-J, Wilson J. Recombinant adenovirus deleted of all viral genes for gene therapy of cystic fibrosis. *Virology*. 1996;**217**(1):11-22
- [116] Hardy S, Kitamura M, Harris-Stansil T, Dai Y, Phipps M. Construction of adenovirus vectors through Cre-lox recombination. *Journal of Virology*. 1997;**71**(3):1842-1849
- [117] Hartigan-O'Connor D, Amalfitano A, Chamberlain J. Improved production of gutted adenovirus in cells expressing adenovirus preterminal protein and DNA polymerase. *Journal of Virology*. 1999;**73**(9):7835-7841

- [118] Ng P, Beauchamp C, Eveleigh C, Parks R, Graham F. Development of a FLP/frt system for generating helper-dependent adenoviral vectors. *Molecular Therapy: The Journal of the American Society of Gene Therapy*. 2001;**3**(5):809-815
- [119] Alba R, Hearing P, Bosch A, Chillon M. Differential amplification of adenovirus vectors by flanking the packaging signal with attB/attP-PhiC31 sequences: Implications for helper-dependent adenovirus production. *Virology*. 2007;**367**(1):51-58
- [120] Schiedner G, Morral N, Parks RJ, Wu Y, Koopmans SC, Langston C, et al. Genomic DNA transfer with a high-capacity adenovirus vector results in improved in vivo gene expression and decreased toxicity. *Nature Genetics*. 1998;**18**(2):180-183
- [121] Morral N, Parks R, Zhou H, Langston C, Schiedner G, Quinones J, et al. High doses of a helper-dependent adenoviral vector yield supraphysiological levels of alpha1-antitrypsin with negligible toxicity. *Human Gene Therapy*. 1998;**9**(18):2709-2716
- [122] Chen H, Mack L, Kelly R, Ontell M, Kochanek S, Clemens P. Persistence in muscle of an adenoviral vector that lacks all viral genes. *Proceedings of the National Academy of Sciences of the United States of America*. 1997;**94**(5):1645-1650
- [123] Palmer D, Ng P. Improved system for helper-dependent adenoviral vector production. *Molecular Therapy: The Journal of the American Society of Gene Therapy*. 2003;**8**(5):846-852
- [124] Lemarchand P, Jaffe H, Danel C, Cid M, Kleinman H, Stratford-Perricaudet L, et al. Adenovirus-mediated transfer of a recombinant human alpha 1-antitrypsin cDNA to human endothelial cells. *Proceedings of the National Academy of Sciences*. 1992;**89**(14):6482-6486
- [125] Davies JC, Geddes DM, Alton E. Prospects for gene therapy in lung disease. *Current Opinion in Pharmacology*. 2001;**1**(3):272-278
- [126] Griesenbach U, Ferrari S, Geddes D, Alton E. Gene therapy progress and prospects: Cystic fibrosis. *Gene Therapy*. 2002;**9**(20):3301791
- [127] Raper S, Wilson J, Yudkoff M, Robinson M, Ye X, Batshaw M. Developing adenoviral-mediated in vivo gene therapy for ornithine transcarbamylase deficiency. *Journal of Inherited Metabolic Disease*. 1998;**21**(Suppl 1):119-137
- [128] Batshaw M, Wilson J, Raper S, Yudkoff M, Robinson M. Recombinant adenovirus gene transfer in adults with partial ornithine transcarbamylase deficiency (OTCD). *Human Gene Therapy*. 1999;**10**(14):2419-2437
- [129] Bramson J, Hitt M, Gauldie J, Graham F. Pre-existing immunity to adenovirus does not prevent tumor regression following intratumoral administration of a vector expressing IL-12 but inhibits virus dissemination. *Gene Therapy*. 1997;**4**(10):3300508
- [130] Sullivan NJ, Sanchez A, Rollin PE, Yang Z, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. *Nature*. 2000;**408**(6812):605-609

- [131] Corey L, McElrath JM, Kublin JG. Post-Step modifications for research on HIV vaccines. *AIDS*. 2009;**23**(1):3
- [132] Shiver JW, Fu T-M, Chen L, Casimiro DR, Davies M-E, Evans RK, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. *Nature*. 2002;**415**(6869):415331a
- [133] Catanzaro AT, Koup RA, Roederer M, Bailer RT, Enama ME, Moodie Z, et al. Phase 1 safety and immunogenicity evaluation of a multiclade HIV-1 candidate vaccine delivered by a replication-defective recombinant adenovirus vector. *The Journal of Infectious Diseases*. 2006;**194**(12):1638-1649
- [134] Gómez-Román RV, Florese RH, Peng B, Montefiori DC, Kalyanaraman VS, Venzon D, et al. An adenovirus-based HIV subtype B prime/boost vaccine regimen elicits antibodies mediating broad antibody-dependent cellular cytotoxicity against non-subtype B HIV strains. *JAIDS Journal of Acquired Immune Deficiency Syndromes*. 2006;**43**(3):270
- [135] Fitzgerald D, Janes H, Robertson M, Coombs R, Frank I, Gilbert P, et al. An Ad5-vectored HIV-1 vaccine elicits cell-mediated immunity but does not affect disease progression in HIV-1-infected male subjects: Results from a randomized placebo-controlled trial (The Step Study). *The Journal of Infectious Diseases*. 2011;**203**(6):765-772
- [136] O'Brien KL, Liu J, King SL, Sun Y-H, Schmitz JE, Lifton MA, et al. Adenovirus-specific immunity after immunization with an Ad5 HIV-1 vaccine candidate in humans. *Nature Medicine*. 2009;**15**(8):873-875
- [137] Harro C, Sun X, Stek JE, Leavitt RY, Mehrotra DV, Wang F, et al. Safety and immunogenicity of the merck adenovirus serotype 5 (MRKAd5) and MRKAd6 human immunodeficiency virus type 1 trigene vaccines alone and in combination in healthy adults. *Clinical and Vaccine Immunology*. 2009;**16**(9):1285-1292
- [138] Patterson L. The "STEP-wise" future of adenovirus-based HIV vaccines. *Current Medicinal Chemistry*. 2011;**18**(26):3981-3986
- [139] Richie TL, Villasante EF. Use of adenovirus serotype 5 vaccine vectors in seropositive, uncircumcised men: Safety lessons from the step trial. *The Journal of Infectious Diseases*. 2013;**207**(4):689-690
- [140] Gray G, Buchbinder S, Duerr A. Overview of STEP and Phambili trial results: Two phase IIb test-of-concept studies investigating the efficacy of MRK adenovirus type 5 gag/pol/nef subtype B HIV vaccine. *Current Opinion in HIV and AIDS*. 2010;**5**(5):357-361
- [141] Michael NL, Robb ML. Phambili: Moving forward without the blindfold. *The Lancet Infectious Diseases*. 2014;**14**(5):361-362
- [142] Pine SO, Kublin JG, Hammer SM, Borgerding J, Huang Y, Casimiro DR, et al. Pre-existing adenovirus immunity modifies a complex mixed Th1 and Th2 cytokine response to an Ad5/HIV-1 vaccine candidate in humans. *PLoS One*. 2011;**6**(4):e18526

- [143] Churchyard GJ, Morgan C, Adams E, Hural J, Graham BS, Moodie Z, et al. A phase IIA randomized clinical trial of a multiclade HIV-1 DNA prime followed by a multiclade rAd5 HIV-1 vaccine boost in healthy adults (HVTN204). *PLoS One*. 2011;**6**(8):e21225
- [144] Janes HE, Cohen KW, Frahm N, Rosa SC, Sanchez B, Hural J, et al. Higher T-cell responses induced by DNA/rAd5 HIV-1 preventive vaccine are associated with lower HIV-1 infection risk in an efficacy trial. *The Journal of Infectious Diseases*. 2017; **215**(9):1376-1385
- [145] Omosa-Manyonyi G, Mpendo J, Ruzagira E, Kilembe W, Chomba E, Roman F, et al. A phase I double blind, placebo-controlled, randomized study of the safety and immunogenicity of an adjuvanted HIV-1 Gag-Pol-Nef fusion protein and adenovirus 35 Gag-RT-Int-Nef vaccine in healthy HIV-uninfected African adults. *PLoS One*. 2015; **10**(5):e0125954
- [146] Keefer MC, Gilmour J, Hayes P, Gill D, Kopycinski J, Cheeseman H, et al. A phase I double blind, placebo-controlled, randomized study of a multigenic HIV-1 adenovirus subtype 35 vector vaccine in healthy uninfected adults. *PLoS One*. 2012;**7**(8):e41936
- [147] Baden LR, Karita E, Mutua G, Bekker L-G, Gray G, Page-Shipp L, et al. Assessment of the safety and immunogenicity of 2 novel vaccine platforms for HIV-1 prevention: A randomized trial. *Annals of Internal Medicine*. 2016;**164**(5):313-322
- [148] Baden LR, Walsh SR, Seaman MS, Tucker RP, Krause KH, Patel A, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD001). *The Journal of Infectious Diseases*. 2013;**207**(2):240-247
- [149] Baden LR, Walsh SR, Seaman MS, Cohen YZ, Johnson JA, Licona HJ, et al. First-in-human randomized controlled trial of mosaic HIV-1 immunogens delivered via a modified vaccinia Ankara vector. *The Journal of Infectious Diseases*. 2018;**218**(4):633-644
- [150] Paris R, Kuschner RA, Binn L, Thomas SJ, Colloca S, Nicosia A, et al. Adenovirus Type 4 and 7 vaccination or adenovirus Type 4 respiratory infection elicits minimal cross-reactive antibody responses to nonhuman adenovirus vaccine vectors. *Clinical and Vaccine Immunology*. 2014;**21**(5):783-786
- [151] Moffatt S, Hays J, HogenEsch H, Mittal S. Circumvention of vector-specific neutralizing antibody response by alternating use of human and non-human adenoviruses: Implications in gene therapy. *Virology*. 2000;**272**(1):159-167
- [152] Earl PL, Americo JL, Wyatt LS, Eller L, Montefiori DC, Byrum R, et al. Recombinant modified vaccinia virus Ankara provides durable protection against disease caused by an immunodeficiency virus as well as long-term immunity to an orthopoxvirus in a non-human primate. *Virology*. 2007;**366**(1):84-97
- [153] Tapia MD, Sow SO, Lyke KE, Haidara F, Diallo F, Doumbia M, et al. Use of ChAd3-EBO-Z Ebola virus vaccine in Malian and US adults, and boosting of Malian adults with MVA-BN-Filo: A phase 1, single-blind, randomised trial, a phase 1b, open-label and

- double-blind, dose-escalation trial, and a nested, randomised, double-blind, placebo-controlled trial. *The Lancet Infectious Diseases*. 2016;**16**(1):31-42
- [154] Milligan ID, Gibani MM, Sewell R, Clutterbuck EA, Campbell D, Plested E, et al. Safety and immunogenicity of novel adenovirus type 26- and modified vaccinia Ankara–vectorized Ebola vaccines: A randomized clinical trial. *Journal of the American Medical Association*. 2016;**315**(15):1610-1623
- [155] Wu S, Kroeker A, Wong G, He S, Hou L, Audet J, et al. An adenovirus vaccine expressing Ebola virus variant makona glycoprotein is efficacious in Guinea pigs and nonhuman primates. *The Journal of Infectious Diseases*. 2016;**214**(suppl\_3):S326-S332
- [156] Kennedy SB, Bolay F, Kieh M, Grandits G, Badio M, Ballou R, et al. Phase 2 placebo-controlled trial of two vaccines to prevent Ebola in Liberia. *The New England Journal of Medicine*. 2017;**377**(15):1438-1447
- [157] Santis O, Audran R, Pothin E, Warpelin-Decrausaz L, Vallotton L, Wuerzner G, et al. Safety and immunogenicity of a chimpanzee adenovirus-vectorized Ebola vaccine in healthy adults: A randomised, double-blind, placebo-controlled, dose-finding, phase 1/2a study. *The Lancet Infectious Diseases*. 2016;**16**(3):311-320
- [158] Wu L, Zhang Z, Gao H, Li Y, Hou L, Yao H, et al. Open-label phase I clinical trial of Ad5-EBOV in Africans in China. *Human Vaccines & Immunotherapeutics*. 2017;**13**(9):2078-2085
- [159] Ogwang C, Afolabi M, Kimani D, Jagne Y, Sheehy SH, Bliss CM, et al. Safety and immunogenicity of heterologous prime-boost immunisation with *Plasmodium falciparum* malaria candidate vaccines, ChAd63 ME-TRAP and MVA ME-TRAP, in healthy Gambian and Kenyan Adults. *PLoS One*. 2013;**8**(3):e57726
- [160] Jhawar SR, Thandoni A, Bommareddy PK, Hassan S, Kohlhapp FJ, Goyal S, et al. Oncolytic viruses—Natural and genetically engineered cancer immunotherapies. *Frontiers in Oncology*. 2017;**7**:202
- [161] Warner SG, O’Leary MP, Fong Y. Therapeutic oncolytic viruses: Clinical advances and future directions. *Current Opinion in Oncology*. 2017;**29**(5):359
- [162] Shaw A, Suzuki M. Recent advances in oncolytic adenovirus therapies for cancer. *Current Opinion in Virology*. 2016;**21**:9-15
- [163] Wold W, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Current Gene Therapy*. 2014;**13**(6):421-433
- [164] Yu W, Fang H. Clinical trials with oncolytic adenovirus in China. *Current Cancer Drug Targets*. 2007;**7**(2):141-148
- [165] Cerullo V, Capasso C, Vähä-Koskela M, Hemminki O, Hemminki A. Cancer-targeted oncolytic adenoviruses for modulation of the immune system. *Current Cancer Drug Targets*. 2018;**18**(2):124-138

- [166] Yamamoto Y, Nagasato M, Yoshida T, Aoki K. Recent advances in genetic modification of adenovirus vectors for cancer treatment. *Cancer Science*. 2017;**108**(5):831-837
- [167] Cerullo V, Vähä-Koskela M, Hemminki A. Oncolytic adenoviruses. *OncoImmunology*. 2012;**1**(6):979-981
- [168] Cerullo V, Diaconu I, Romano V, Hirvinen M, Ugolini M, Escutenaire S, et al. An oncolytic adenovirus enhanced for toll-like receptor 9 stimulation increases antitumor immune responses and tumor clearance. *Molecular Therapy*. 2012;**20**(11):2076-2086
- [169] Heise C, Williams A, Xue S, Propst M, Kirn D. Intravenous administration of ONYX-015, a selectively replicating adenovirus, induces antitumoral efficacy. *Cancer Research*. 1999;**59**(11):2623-2628
- [170] Khuri FR, Nemunaitis J, Ganly I, Arseneau J, Tannock IF, Romel L, et al. A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nature Medicine*. 2000;**6**(8):nm0800\_879
- [171] Rätty J, Pikkarainen J, Wirth T, Ylä-Herttuala S. Gene therapy: The first approved gene-based medicines, molecular mechanisms, and clinical indications. *Current Molecular Pharmacology*. 2008;**1**(1):13-23
- [172] Guo W, Song H. Development of gene therapeutics for head and neck cancer in China: From bench to bedside. *Human Gene Therapy*. 2018;**29**(2):180-187
- [173] Sangro B, Mazzolini G, Ruiz M, Ruiz J, Quiroga J, Herrero I, et al. A phase I clinical trial of thymidine kinase-based gene therapy in advanced hepatocellular carcinoma. *Cancer Gene Therapy*. 2010;**17**(12):837
- [174] Zhan Y, Yu B, Wang Z, Zhang Y, Zhang H-H, Wu H, et al. A fiber-modified adenovirus co-expressing HSV-TK and Coli.NTR enhances antitumor activities in breast cancer cells. *International Journal of Clinical and Experimental Pathology*. 2014;**7**(6):2850-2860
- [175] Barton KN, Paielli D, Zhang Y, Koul S, Brown SL, Lu M, et al. Second-generation replication-competent oncolytic adenovirus armed with improved suicide genes and ADP gene demonstrates greater efficacy without increased toxicity. *Molecular Therapy*. 2006;**13**(2):347-356
- [176] Saito H, Kitagawa K, Yoneda T, Fukui Y, Fujsawa M, Bautista D, et al. Combination of p53-DC vaccine and rAd-p53 gene therapy induced CTLs cytotoxic against p53-deleted human prostate cancer cells in vitro. *Cancer Gene Therapy*. 2017;**24**(7):289-296
- [177] Zhou J, Huang Z, Wang Z, Liu S, Grandien A, Ernberg I, et al. Tumor suppressor BLU promotes TRAIL-induced apoptosis by downregulating NF- $\kappa$ B signaling in nasopharyngeal carcinoma. *Oncotarget*. 2014;**8**(27):43853-43865
- [178] Gabilovich DI. INGN 201 (Advexin): Adenoviral p53 gene therapy for cancer. *Expert Opinion on Biological Therapy*. 2006;**6**(8):823-832

- [179] Senzer N, Nemunaitis J. A review of contusugene ladenovec (Advexin) p53 therapy. *Current Opinion in Molecular Therapeutics*. 2009;**11**(1):54-61
- [180] Chen G, Zhang S, He X, Liu S, Ma C, Zou X-P. Clinical utility of recombinant adenoviral human p53 gene therapy: Current perspectives. *OncoTargets and Therapy*. 2014;**7**:1901-1909
- [181] Xia Y, Du Z, Wang X, Li X. Treatment of uterine sarcoma with rAd-p53 (Gendicine®) followed by chemotherapy—Clinical study on TP53 gene therapy. *Human Gene Therapy*. 2018;**29**(2):242-250
- [182] Vitelli A, Folgori A, Scarselli E, Colloca S, Capone S, Nicosia A. Chimpanzee adenoviral vectors as vaccines—Challenges to move the technology into the fast lane. *Expert Review of Vaccines*. 2017;**16**(12):1241-1252
- [183] Short J, Pereboev A, Kawakami Y, Virology V. Adenovirus serotype 3 utilizes CD80 (B7. 1) and CD86 (B7. 2) as cellular attachment receptors. *Virology*. 2004;**322**(2):349-359
- [184] Zhang C, Yang Y, Chi Y, Yin J, Yan L, Ku Z, et al. Hexon-modified recombinant E1-deleted adenoviral vectors as bivalent vaccine carriers for Coxsackievirus A16 and Enterovirus 71. *Vaccine*. 2015;**33**(39):5087-5094
- [185] Tuve S, Wang H, Jacobs JD, Yumul RC, Smith DF, Lieber A. Role of cellular heparan sulfate proteoglycans in infection of human adenovirus serotype 3 and 35. *PLoS Pathogens*. 2008;**4**(10):e1000189
- [186] Chen H, Xiang Z, Li Y, Kurupati R, Jia B, Bian A, et al. Adenovirus-based vaccines: Comparison of vectors from three species of adenoviridae. *Journal of Virology*. 2010;**84**(20):10522-10532
- [187] Geisbert TW, Bailey M, Hensley L, Asiedu C, Geisbert J, Stanley D, et al. Recombinant adenovirus serotype 26 (Ad26) and Ad35 vaccine vectors bypass immunity to Ad5 and protect nonhuman primates against Ebola virus challenge. *Journal of Virology*. 2011;**85**(9):4222-4233
- [188] Barouch DH, Pau MG, Custers JH, Koudstaal W, Kostense S, Havenga MJ, et al. Immunogenicity of recombinant adenovirus serotype 35 vaccine in the presence of pre-existing anti-Ad5 immunity. *The Journal of Immunology*. 2004;**172**(10):6290-6297
- [189] Holterman L, Vogels R, van der Vlugt R, Sieuwerts M, Grimbergen J, Kaspers J, et al. Novel replication-incompetent vector derived from adenovirus type 11 (Ad11) for vaccination and gene therapy: Low seroprevalence and non-cross-reactivity with Ad5. *Journal of Virology*. 2004;**78**(23):13207-13215
- [190] Capone S, Meola A, Ercole B, Vitelli A, Pezzanera M, Ruggeri L, et al. A novel adenovirus type 6 (Ad6)-based hepatitis C virus vector that overcomes preexisting anti-Ad5 immunity and induces potent and broad cellular immune responses in rhesus macaques. *Journal of Virology*. 2006;**80**(4):1688-1699

- [191] Swadling L, Capone S, Antrobus RD, Brown A, Richardson R, Newell EW, et al. A human vaccine strategy based on chimpanzee adenoviral and MVA vectors that primes, boosts, and sustains functional HCV-specific T cell memory. *Science Translational Medicine*. 2014;**6**(261):261ra153
- [192] Biswas S, Choudhary P, Elias SC, Miura K, Milne KH, de Cassan SC, et al. Assessment of humoral immune responses to blood-stage malaria antigens following ChAd63-MVA immunization, controlled human malaria infection and natural exposure. *PLoS One*. 2014;**9**(9):e107903
- [193] Reddy PS, Idamakanti N, Zakhartchouk AN, Baxi MK, Lee JB, Pyne C, Babiuk LA, Tikoo SK. Nucleotide sequence, genome organization, and transcription map of bovine adenovirus type 3. *Journal of Virology*. 1998;**72**(2):1394-1402
- [194] Hammond JM<sup>1</sup>, McCoy RJ, Jansen ES, Morrissy CJ, Hodgson AL, Johnson MA. Vaccination with a single dose of a recombinant porcine adenovirus expressing the classical swine fever virus gp55 (E2) gene protects pigs against classical swine. *Vaccine*. 2000;**18**(11-12):1040-1050
- [195] Wüest T, Both GW, Prince AM, Hofmann C, Löser P. Recombinant ovine adenovirus induces a strong and sustained T cell response against the hepatitis C virus NS3 antigen in mice. *Vaccine*. 2004;**22**(21-22):2717-2721
- [196] Xiang Z, Gao G, Reyes-Sandoval A, Li Y, Wilson J, Ertl H. Oral vaccination of mice with adenoviral vectors is not impaired by preexisting immunity to the vaccine carrier. *Journal of Virology*. 2003;**77**(20):10780-10789
- [197] Lin SW, Cun AS, Harris-McCoy K, Ertl HC. Intramuscular rather than oral administration of replication-defective adenoviral vaccine vector induces specific CD8<sup>+</sup> T cell responses in the gut. *Vaccine*. 2007;**25**(12):2187-2193
- [198] Mackay LK, Stock AT, Ma JZ, Jones CM, Kent SJ, Mueller SN, et al. Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**(18):7037-7042
- [199] Richardson JS, Pillet S, Bello AJ, Kobinger GP. Airway delivery of an adenovirus-based Ebola virus vaccine bypasses existing immunity to homologous adenovirus in nonhuman primates. *Journal of virology*. 2013;**87**(7):3668-3677
- [200] Croyle MA<sup>1</sup>, Patel A, Tran KN, Gray M, Zhang Y, Strong JE, Feldmann H, Kobinger GP. Nasal delivery of an adenovirus-based vaccine bypasses pre-existing immunity to the vaccine carrier and improves the immune response in mice. *PLoS One*. 2008;**3**(10):e3548
- [201] McCoy K, Tatsis N, Koriath-Schmitz B, Lasaro MO, Hensley SE, Lin S-W, et al. Effect of preexisting immunity to adenovirus human serotype 5 antigens on the immune responses of nonhuman primates to vaccine regimens based on human- or chimpanzee-derived adenovirus vectors. *Journal of Virology*. 2007;**81**(12):6594-6604

- [202] Afolabi MO, Tiono AB, Adetifa UJ, Yaro J, Drammeh A, Nébié I, et al. Safety and immunogenicity of ChAd63 and MVA ME-TRAP in West African children and infants. *Molecular Therapy*. 2016;**24**(8):1470-1477
- [203] Mensah VA, Gueye A, Ndiaye M, Edwards NJ, Wright D, Anagnostou NA, et al. Safety, immunogenicity and efficacy of prime-boost vaccination with ChAd63 and MVA encoding ME-TRAP against *Plasmodium falciparum* infection in adults in Senegal. *PLoS One*. 2016;**11**(12):e0167951
- [204] Hayton E-J, Rose A, Ibrahimsa U, Sorbo M, Capone S, Crook A, et al. Safety and tolerability of conserved region vaccines vectored by plasmid DNA, simian adenovirus and modified vaccinia virus Ankara administered to human immunodeficiency virus type 1-uninfected adults in a randomized, single-blind phase I trial. *PLoS ONE*. 2014;**9**(7):e101591
- [205] Kaulfuß M, Wensing I, Windmann S, Hrycak C, Bayer W. Induction of complex immune responses and strong protection against retrovirus challenge by adenovirus-based immunization depends on the order of vaccine delivery. *Retrovirology*. 2017;**14**(1):8
- [206] Schöne D, Hrycak CP, Windmann S, Lapuente D, Dittmer U, Tenbusch M, et al. Immunodominance of adenovirus-derived CD8+ T cell epitopes interferes with the induction of transgene-specific immunity in adenovirus-based immunization. *Journal of Virology*. 2017;**91**(20):e01184-17
- [207] Schirmbeck R, Reimann J, Kochanek S, Kreppel F. The immunogenicity of adenovirus vectors limits the multispecificity of CD8 T-cell responses to vector-encoded transgenic antigens. *Molecular Therapy*. 2008;**16**(9):1609-1616
- [208] Agrawal B, Singh S, Gupta N, Li W, VEDI S, Kumar R. Unsolved puzzles surrounding HCV immunity: Heterologous immunity adds another dimension. *International Journal of Molecular Sciences*. 2017;**18**(8):1626-1639
- [209] Lee CS et al. Adenovirus mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. *Genes and Diseases*. 2017;**4**(2):43-63. DOI: 10.1016/j.gendis.2017.04.001

