LITERATURE REVIEW

Adenovirus Manipulation for Use as an Effective Delivery Vector

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Abstract

Adenoviruses are used as delivery vectors in many different biotherapeutic systems to provide treatment options in several clinical settings. Their relative safety, potent induction of an immune response, and ease of production have allowed these vectors to appear at the forefront of clinical medicine in recent times, with applications in gene therapies, cancer treatments, and vaccines (including those for SARS-CoV-2). Their ease of genome manipulation and large gene transduction abilities make them particularly attractive for use as delivery vectors.

This paper aims to show that, despite significant challenges, adenoviruses have generally been effective as delivery vectors for gene therapies and vaccination strategies. Taking advantage of their diversity and delineated viral tropism is critical to implementing effective clinical strategies, moderating the negative effects of pre-existing immunity, combatting transient action, and optimising target cell specificity. Overall, this paper argues that adenoviral vectors are a promising tool for use in a wide range of clinical applications.

Keywords: Adenovirus, Vector, Vaccine, Biotherapeutics

Introduction

Adenoviruses (Ads) are common viruses that are non-enveloped, icosahedral, and 90–100 nm in size. Adenoviruses also contain a double-stranded DNA genome. In recent years, Ads have been developed for use as vectors to transduce genes into host cells or to induce a robust host cell immune response. Clinical applications of Ad vectors include gene therapy, cancer gene therapy, and vaccination.

Ads are suitable viral vectors due to their size and they can be easily manipulated. Using recombinant DNA techniques, manipulation is efficient as the virus can produce progeny in permissive cells, elicit high levels of protein expression, and can hold up to 38kB of foreign DNA[1]. It is possible to remove regions of the Ad genome, particularly the E1 and E3 regions, in order to make space for exogenous DNA insertion (**Figure 1**).

These recombinant viruses, carrying foreign genes, can infect a greater percentage of cells than naked DNA insertion, generating the desired population of virus-infected cells more efficiently[1].

This paper discusses the applications of Ad vectors and their limitations. Ad transgenes are delivered efficiently[2], infecting both dividing and non-dividing cells[3]. However, they only have transient gene expression[4]. This is because Ad DNA cannot be integrated into host DNA and they are immunogenic, meaning they stimulate the immune clearance of the vector[5]. Transient gene expression in Ad vectors has been observed to be maximal during the first week of expression. Despite that, no transgene expression was found at all during the twenty-one days post-administration of Ad vectors in rat cardiomyocytes[6].

Another potential disadvantage of using Ad vectors is the high levels of pre-existing immunity in humans[7,8] This pre-existing immunity is due to the seroprevalence of Ads in the population, with up to 73.1% of 1,154 subjects in a trial in China showing the presence of human Ad5-neutralising antibodies[9]. Many of us may have already been exposed and are immune to infiltration by certain Ad serotypes. In addition, despite Ads generally being regarded as safe when used as vectors, complications have arisen in past clinical trials, which will be discussed later in this paper[10,11].

Adenovirus Vectors

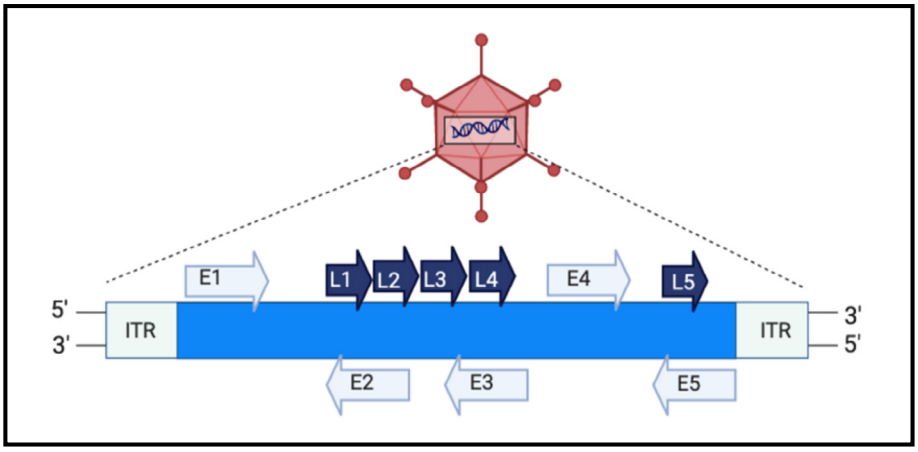
Vector Production

The production of Ad vectors is relatively easy, and high stocks of purified virus can be produced, making them attractive for clinical use[12,13]. Furthermore, they are compatible with industry-standard clinical manufacturing and thermostabilisation processes[13]. This thermostabilisation of the vector is also important for avoiding the use of cold chain technologies for storage, resulting in easier storage and improved shelf-life.

Several factors allow high titres of Ad vector production. These include their ability to be manufactured in mammalian cell cultures such as HEK293 cells, which provide trans-acting E1 proteins to allow viral replication[12]. Another factor is their stable genome, contributing to their ability to be amplified successfully. E1 proteins are essential for viral replication and early gene expression (**Figure 1**).

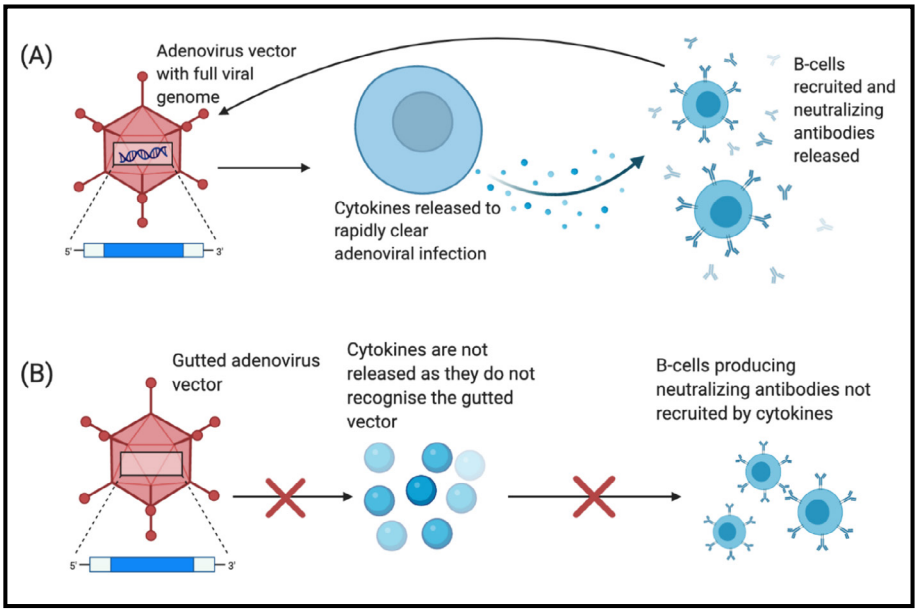
In addition to the ease of production of gene therapies and cancer therapies, studies aiming to develop vaccines against the recently emerged SARS-CoV-2 virus demonstrates that the development of Ad vectors is efficient and rapid. In terms of production, an easily reproducible murine model was developed within 2–3 weeks, that can be used to explore SARS-

Figure 1. **The Adenovirus Genome**



Adenoviruses contain a double stranded DNA genome. Early genes such as E1 are required for replication. As a result, they can be removed to render the virus replication-defective, a feature of many adenovirus vectors that contributes to the safety of their use, while expanding their inserted DNA capacity. E3 genes are also often removed to allow greater packaging space.

Figure 2. **Different Adenovirus Vector Constructions Lead to Differing Immune Cell Interactions**



A) Adenovirus vectors with full viral genomes stimulate immune cells and cytokines are released in response to the virus, leading to antibody release. B) Gutted adenovirus vectors containing only ITR regions at N and C-terminus domains can evade host immune response as their viral genome is not expressed and therefore cannot be recognized by the innate immune system to clear the virus. Thus, gutted vectors are a possible solution to the transient action of adenoviral vectors, along with pre-existing immunity to some adenovirus serotypes such as Ad5.

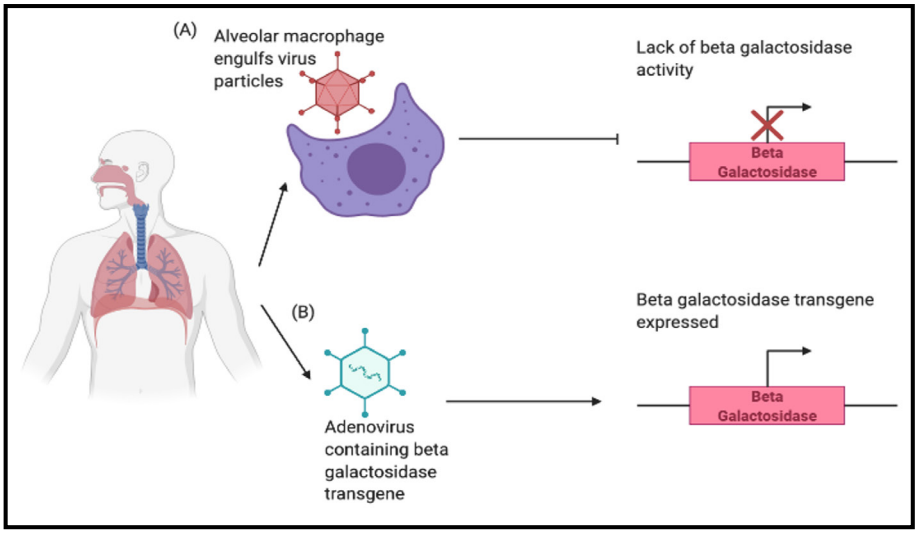
CoV-2 pathogenesis and potential vaccine strategies[14]. This method has progressed production a considerable amount in comparison to developing and breeding human ACE2-transgenic or human ACE2-knockin mice for experiments.

In some studies, using an Ad as the vector instead of an adeno-associated virus or lentivirus has been advantageous because Ad vector production does not require plasmid transfection on a grand scale. In a particular study, a single HDAd5/35++ vector stock could be used for numerous production cycles[15]. Viruses with small deletions can be propagated in cell cultures that have genetic defects to allow viral reproduction[16]. Gutless Ad vectors are defined as vectors that are manipulated to the point where it is essentially stripped of its genome, only retaining the inverted terminal repeat (ITR) regions (**Figure 2**). These gutless vectors can render the virus unrecognisable to hosts with pre-existing immunity, avoiding an anti-Ad response (**Figure 2**). However, their synthesis requires special producer cell lines[17]. In addition, gutless vectors exhibit reduced toxicity, immunogenicity, and a longer duration of transgene expression than vectors with a full or slightly removed genome (**Figure 2**)[18].

Ad vector production can be up-scaled successfully. Vectors have been scaled up to 3L bioreactors from shake-flasks[19]. Ad rabies vaccine AdRG1.3 has successfully been scaled up from 1 liter to 500 liters, while maintaining cost-effectiveness and efficacy[20]. The ability to up-scale vaccine vectors is important to maximise production and allow large doses of vaccine to be made available across the world.

Ads are easily manipulated for different treatment areas including vaccine strategies and cancer

Figure 3. **The Effect of Alveolar Macrophages on Virus Particles in the Lung**



A) Alveolar macrophages can phagocytose adenovirus particles, resulting in 70% virus particle degradation within 24 hours. B) Viral vectors containing beta galactosidase as a marker for transgene expression show that removal of these macrophages allows adenoviral gene expression.

treatments. Ad vectors were, however, originally developed as a gene therapy treatment strategy to combat cystic fibrosis (CF), with many subgroups of Ads showing an affinity for respiratory epithelia[21,22]. In fact, Ads carrying the cystic fibrosis transmembrane conductance regulator (CFTR) gene have been used in trials[23]. However, Ad vectors struggled to treat CF patients, showing only transient expression[24,25].

Vector Administration

In terms of administration, a lot is known about intravenous (i.v.) administration. However, it is not feasible for large scale vaccination, as it has been observed to stimulate a low immune response compared to intramuscular (i.m.) injections[26]. In addition to this, i.v. administration has been observed to cause anaphylaxis. Much less is known about i.m. or intranasal (i.n.) administration, so work should continue in these areas. Following i.n. administration, humoral and cellular immune responses have been observed[27]. However, alveolar macrophages have degraded the vector in some cases. Worgall et al.[28] showed that 70% of the Ad genome in the lung was degraded after 24 hours in the presence of alveolar macrophages. When these macrophages were removed, administration of Ad vectors encoding beta galactosidase showed a substantial increase in this transgene expression, showing vector degradation was macrophage-dependent in this case (**Figure 3**).

Vector Safety

One feature of Ads that make them so suitable as delivery vectors is their ability to transport their own DNA into the nucleus[29]. Along with this, replication-incompetent Ads have been shown to act with high accuracy[30]. Since these viral DNAs that enter the host nucleus cannot integrate into the host genome, they are remarkably safe. Ad vectors are easily rendered replication-defective, as the early genes rely on E1 gene expression, so replication can be inhibited by deletion of this single E1 gene. Such vectors have been the subject of a number of safe studies in both young children and older individuals who may be deemed "at risk"[31,32].

Non-replicating vectors are particularly safe, as observed in a phase I study of an Ad4 vector vaccine for H5N1 influenza[33], and the VXA-A1.1 vaccine phase II clinical trial for H1N1 influenza[34]. Oncolytic vectors on the other hand, are vectors that are used to treat cancer by using replication-competent virus inside of cancer cells to kill them. It is important for these replicating vectors to be target cell specific, focusing solely on cancerous cells. A replicating vector has the potential to be dangerous and cause harm if not produced to target very specific cancerous signals, including initial tumour enlargement[35].

Gene Therapy

Applications

Ad vectors have been proven to have a high rate of gene transfer in vivo. The fact that they can be gutted and store a lot of foreign DNA is of huge benefit for therapeutics and is one of the main contributors to success in gene therapies. The first gene therapy vector of any type to be approved for public use was Gendicine[36]. Gendicine is a recombinant Ad used to express wildtype p53 tumour suppressor genes. It has been used to treat patients with p53 gene mutations in cancer treatment[37].

An example of gene therapy using viral vectors is the delivery of the Cas9 gene and guide RNA (gRNA) to host cells to treat Duchenne muscular dystrophy (DMD)[38]. This study by Boucher et al. showed that multiple routes of gene insertion could be achieved using the flexibility of Ads[39]. The Cas9 and gRNA alone could induce gene editing via non-homologous end joining (NHEJ), while the use of a second vector containing homology directed repair (HDR) template DNA could allow the HDR pathway to be used[39]. NHEJ is prone to unwanted nucleotide deletion errors but is very efficient[39]. Alternatively, the HDR pathway was less efficient but was more precise in its insertion[39]. This Cas9 gene remedied DMD by causing

exon skipping in the host cell nucleus of the dystrophin gene[40].

Issues

Clinical risks. Gene therapies can be very effective but have some potential underlying issues. Ad vectors have been under public scrutiny after the death of Jesse Gelsinger in 1999. This death occurred after clinical trials aiming to treat ornithine transcarbamylase deficiency resulted in a cytokine storm, leading to multiple organ failure and, in turn, the death of Gelsinger[11]. This hampered viral vector development and threatened public faith in gene therapy, drawing specific criticisms of the 38 trillion particle dose of Ad the patient had received[10].

Tropism. At a molecular level, issues can also arise. Vector tropism—especially to the coxsackievirus and Ad receptor (CAR)—can be affected by low expression of CAR on the surface of target cancer cells and haematopoietic stem cells[41]. To solve this problem, studies are looking at the modification of Ad fibre domains to alter its tropism (**Figure 4**). This can be done through pseudotyping (replacing the entire fibre or knob domain with that of a different Ad serotype) or xenotyping (adding fibre elements of Ad serotypes that are nonhuman) the vector fibres[38].

The fibre proteins of Ads are the main determinant for their tropism. Two amino acid mutations in the AB loop of the Ad serotype 5 fibre knob showed reduced liver tropism, accompanied by increased gene transfer in low-CAR or CAR deficient cells[42]. Altered tropism was initially shown when Ad5 fibres were replaced with Ad7 fibres. The altered chimeric fibre knobs resulted in a difference in tropism in host cell binding (**Figure 4**)[43].

Liver tropism that is often seen in Ad vectors can damage their efficiency and ability to reach target cells elsewhere in the body. Recent studies have been focused on retargeting vectors through modifications of this discussed fibre domain. The fibre shaft contains a KKTK amino acid region that binds to coagulation factors and heparin sulfate molecules on the cell, found in abundance on liver cells. To combat this, studies have mutated this KKTK region to reduce liver tropism and increase gene transfer to target cells[44].

Despite Ad vector tropism modifications being limited today, some breakthroughs have been made. Even with transient expression, Ad5 vectors with recombinant Ad37 fibre knobs transduced NK-92 natural killer-derived cells to cancerous cells more efficiently than native Ad5 vectors[45]. This demonstrates the power of chimeric fibre knobs as Ad5 vectors often show not only transient expression, but much of the population has pre-existing immunity to this serotype.

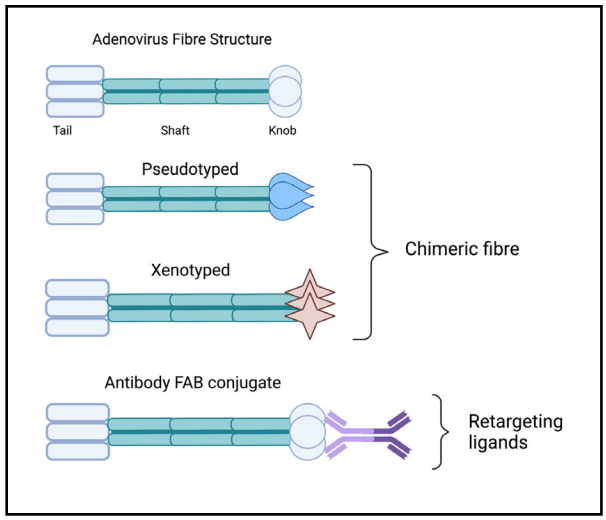
Cancer Gene Therapy

Applications

Ad vectors have been a focus of directed cancer treatments for specific human cancers. Gendicine was one such drug designed to treat cancer. In contrast to the replication-defective Ads being used in gene therapies and vaccines, cancer therapy uses replication-competent viruses. This strategy is used as it allows the viruses to lyse cancer cells through the lytic life cycle of the virus.

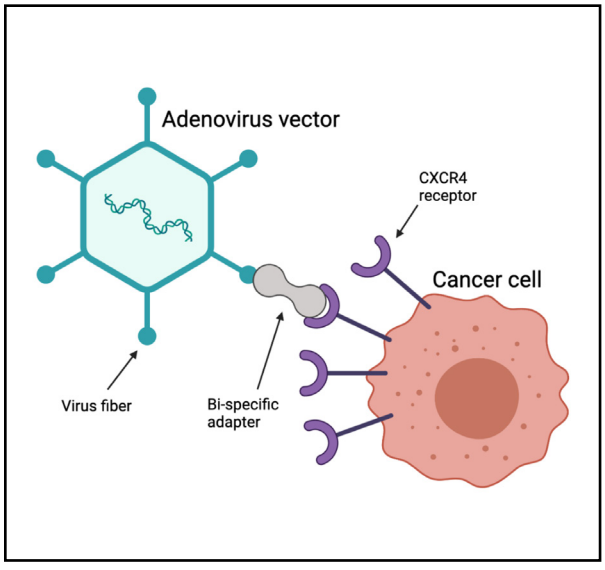
These vectors can have cancer-specific promoters

Figure 4. **Adenovirus Fibre Domain Structures**



Adenovirus fibre domains can be manipulated and modified in a number of ways to alter their receptor specificity to focus on target cells more efficiently. Most modifications take place in the fibre knob domain. Chimeric fibres alter virus tropism while retargeting ligands (adapters) physically link virus particles to host receptors such as CD40L. Peptide insertions (not shown) can alter fibre properties too.

Figure 5. **The Cellular Action of Bispecific Adapters in Retargeting Viral Vectors**



Adenovirus fibres can be retargeted with a tropism for cancer cells using the bispecific adapter sCAR-CXCL12. This retargeting allows the vector to bind the overexpressed CXCR4 chemokine receptors found on cancer cells.

that replace the E1A enhancer/promoter and/or the E4 promoter[36]. ONYX-015 was the first oncolytic Ad vector to be examined in clinical trials. It lacked the E1B-55K protein[46]. This E1 gene deletion in Ad vectors can be replaced with other genes that stimulate an immune response in only p53-deficient target cells. ONYX-015 targets cancer cells with different late RNA export mechanisms, rather than p53 inactivation[47]. ONYX-015 treats cancer cells using different mechanisms to chemotherapy, so it shows good potential in patients that have not responded to chemotherapeutic treatment[35]. There have been similar studies on E1-replaced vectors. For example, when the E1A promoter in the oncolytic CV706 Ad vector was replaced by the prostate-specific antigen (PSA) promoter-enhancer[48], CV706 demonstrated cancer-cell–specific tropism, selectively targeting and killing prostate cancer cells.

In general, cancer gene therapies use replication-competent Ads to lyse cells. Techniques used by Boucher et al.[38] have shown high-level selectivity in the delivery of CRISPR-Cas9 technology to mutated oncogenes by Ads, causing knockout mutants or inhibition. CRISPR-Cas9 deletions have reduced tumour growth in mouse lung cancer xenograft models using knockouts of L858R mutations in the EGFR-overexpressing lung cancers[30]. In some cancer treatments, the gene knockouts may be in the vector itself. The removal of E1B 19kDa is important to allow anti-tumour effects of p53-induced apoptosis in cancer cells[49].

Different approaches and techniques have been explored to combat cancer using viral vectors. The cancer gene therapy drug enadenotucirev was the first oncolytic Ad to be successfully designed using the directed evolution approach[50]. This approach aims to simulate natural selection using genetic diversification followed by phenotypic selection[50]. Enadenotucirev stimulates pro-inflammatory immune responses that stimulate an anticancer response. This is achieved through its transgenes expressing CD40 agonists, interferon-alpha, and chemokines CXCL9 and CXCL10, which promote a pro-inflammatory response. Enadenotucirev has a dual mechanism of action and including stimulating immune response, it binds CD46 or desmoglein 2,6, both of which are often found on carcinoma cells, causing ischemic cell death through ATP depletion[51]. Enadenotucirev has been trialed in concert with chemoradiotherapy to act in locally advanced rectal cancers, utilising its selective toxicity in carcinoma cells[52].

Issues

Tumour enlargement. These oncolytic Ad vectors have encountered issues in their progression to treat cancers. Reid et al.[35] found that transient enlargement of tumours was discovered in some patients, resulting in their removal from the clinical trial. It has been suggested that the inflammatory response as a result of viral sensing in the host may have led to tumour enlargement.

Tropism. Accompanying such limitations, the low expression of CAR receptor on cancer cells has been a treatment barrier. As discussed above, pseudotyping the Ad fibre is being attempted **(Figure 4)**[38]. An example of this occurring is expanding tropism to hematopoietic stem cells using Ad35 fibre targeting CD34+ cells, which binds to these cells independently of integrins[53]. Another technique that can be used is chimeric adapter proteins **(Figure 5)**. These proteins contain binding domains for the Ad fibre knob proteins and a binding domain for the cells of interest. CXCR4 chemokine receptors are upregulated in many cancer cells. As such, a bispecific adapter chemokine, sCAR-CXCL12 was developed to retarget Ad vectors to these receptors on specific cancer cells—particularly in breast cancer and melanoma—through direct interactions **(Figure 5)**[54].

Overcoming tropism specificity issues has become an increasing problem in Ad vector cancer treatment. Hexon interactions with coagulation factors (FX) have been hypothesised to be a major player in hepatocyte transduction by systemic delivery of Ads[2]. Different Ad serotypes have shown a range of binding affinities for coagulation factors. Some bind with high affinity, while other serotypes such as subgroup D do not bind to FX at all[55].

Vaccines

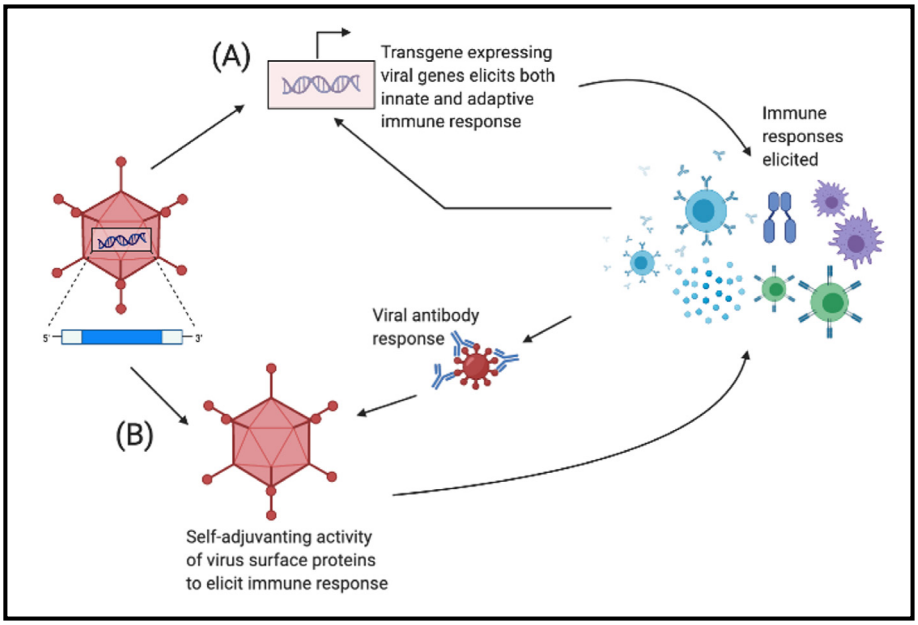
Applications

Ad vectors have increasingly been researched for use as vaccines, most recently in the fight against SARS-CoV-2. The Oxford/AstraZeneca ChAdOx1 vaccine, using a chimpanzee Ad, has a reported 62.1% efficacy[56]. This vector encodes the full spike protein of SARS-CoV-2[57], and the use of non-human Ads avoids pre-existing immunity. Similarly, the single dose Ad26 SARS-CoV-2 vector vaccine developed by Johnson & Johnson has completed phase 3 trials and has been licensed, distributed and administered worldwide[58]. Ad26 is a human Ad with strikingly low seroprevalence, yet another strategy to combat the threat of pre-existing immunity.

As of the writing of this paper, 9 of 27 vaccine candidates are currently in clinical trials to treat SARS-CoV-2 use viral vectors[59]. In many of these candidates, including the ChAdOx1 nCoV-19 vaccine, immunoglobulin G (IgG) response was higher in patients that received prime-boosted vaccine than in those who did not[59]. The immune response acted against the spike proteins within 28 days and showed both cellular and humoral immunity. Previous attempts to increase the immunogenicity of ChAdOx1 nCoV-19 saw rAd5 fibre and penton RGD motifs added. However, this did not increase the vaccine's immunogenicity[60]. Interestingly, a weaker first dose was given to some trial participants, conferring a higher efficacy (90.0%) than two full dose measures[56]. This shows that a high number of factors must trigger immune response when using vectors. This vaccine is very promising as it elicits a notable immune response in an older age group. Other viral vector vaccines in clinical trials have either shown reduced immunogenicity in older groups or have not yet been tested in these groups[61].

Other trials are underway regarding the use of a non-replicating type 5 Ad vectored SARS-CoV-2 vaccine[62]. The aim here was to express the entire spike

Figure 6. **Adenovirus Vector Vaccination**



Vaccination using adenovirus vectors can elicit immune response in two complimentary ways. A) Using transgene expression to allow access to host cells and elicit both innate and adaptive immune responses. B) The adenovirus itself, when at low seroprevalence, can have self-adjuvating properties through antiviral host antibody response to clear the adenoviral infection.

gene of SARS-CoV-2 to induce an immune response. Significant humoral and cellular immune responses were observed within 28 days, particularly in the younger populations[62].

In the case of SARS-CoV-2 vaccines, it is undeniable that Ad vectors have some advantages over other strategies. The main advantages are, while maintaining their efficacy, they have a low cost and an ability to be stored at regular refrigerator temperature. In contrast, the mRNA vaccine candidates must be stored at -20°C (Moderna) and -80°C (Pfizer-BioNTech)[63]. In addition, using human Ad vector technology is extremely cost efficient. For example, the European Union (EU) has paid 2.15 USD per dose of AstraZeneca ChAdOx1 vaccine while the Pfizer-BioNTech mRNA vaccine costs the EU 14.70 USD/dose[64]. These properties may prove to be of huge importance in facilitating mass vaccination by simplifying the logistics behind distribution and storage.

Along with delivering transgenes effectively to stimulate a strong humoral and cellular immune response, Ad vectors can behave in an adjuvant-like fashion, stimulating the immune system through toll-like receptor (TLR)-dependent and independent pathways[65]. TLRs are proteins that recognised conserved molecules in microbes and stimulate the innate immune response as a result. These pathogen recognition receptors (PRRs) recognise the virus and stimulate the host immune response to clear the virus. The vectors are attractive candidates for vaccines as they induce potent inflammatory responses after vaccination, both innate and adaptive, as shown in **Figure 6**. They can be enhanced using specific targeting such as having the Ad vector vaccine itself target dendritic cells[66]. Similar to the fibre changes in many gene therapies, Ad5 fibre is genetically modified to express hCD40L using FAB antibody conjugates (**Figure 4**), which in turn is what targets dendritic cells in this study by Sharma et al[66].

Vector vaccines have also come to the forefront of public health in recent years in the form of Zika virus vaccine candidates. The Ad vector vaccine ZIKV, uses Ad4-prM-E. One of the main advantages with this virus is its low seroprevalence, leading to anti-ZIKV T-cell response without eliciting many anti-ZIKV antibodies (**Figure 6**)[67]. The interesting aspect to this response was that it was a result of the serotype of Ad, not the transgene (**Figure 6B**). The same result was found using this vector to vaccinate against influenza hemagglutinin (HA) in this study. The study conducted by Bullard et al.[67] showed how Ads self-adjuvant properties can be utilised to create vaccines, almost irrespective of transgene effects (**Figure 6B**). Ad4 has been shown—due to its low seroprevalence—to be useful as a vaccine. It has been shown to induce anti-H1N1 immunity against influenza and was observed that Ad4 provided far superior protection in mice compared to Ad7 vectored vaccine strategies[68]. However, a major issue is the study lacks information on HA inhibition or virus neutralisation.

Issues

Clinical risks. The SARS-CoV-2 vaccine development path has not all been clear. The Sputnik V vaccine that uses an initial dose of Ad5 vector and a second dose of Ad26 vector has reported around a 91.6% efficacy[69], despite claims that their results are more compatible with an efficacy of around 60%[70]. The differences in reported efficacy appear to be a result of political controversy and the way in which the trials were conducted and published. Additionally, this vaccine is under scrutiny for its approval for use, as phase 3 of the clinical trial was still ongoing at the time of administration in Russia. Questions have been raised by immunologists about the efficacy of the two full-dose ChAdOx1 vaccines. Their

concerns are that a 62.1% efficacy is not high enough to confer herd immunity, along with adverse effects such as blood clotting being noted to be caused by this ChAdOx1 vaccine[71].

Ad vector vaccines against other viral infections have encountered development issues. Merck completed a test-of-concept study (STEP study) on the development of a MRKAd5 HIV-1 gag/pol/nef vaccine. Surprisingly, this replication-deficient Ad vector vaccine showed higher HIV-1 incidence than those who were treated with placebo[72]. What was completely unexpected was that those who had high titres of antibodies against Ads showed an increased incidence of HIV infection. The authors suggest a possible reason for this is that antibody and virus presence may lead to T cell activation, providing an environment that facilitates HIV infection[73].

Pre-existing immunity. A challenge to vaccination using Ad vectors is that the human population may have pre-existing immunity to specific Ad serotypes, preventing widespread protection. However, it is also worth noting that Ad vector vaccines have been shown to be able to overcome possible pre-existing immunity by increasing the dosage of the vaccine or using a different route of vaccination. Sayedahmed et al.[74] showed that along with the ability to overcome immunity, annual vaccines would be feasible. The challenges of pre-existing immunity to certain Ad serotypes in the human population have previously been outlined and can have hampering effects on their clinical use. Components of the immune system such as neutralizing antibodies and Ad-specific T cells can dampen the effects of Ad vectors in individuals with pre-existing immunity[75].

Overcoming pre-existing immunity. Sayedahmed et al.[73] showed that in mice, immunity levels decreased over time, with similar protection levels to mice with no immunity 10 months post-vaccination. What appears to be key is the time between vaccinations, allowing degradation of immunity. So, this pre-existing immunity may be seen to wane over time, allowing successful vaccination if apt time is allowed between vaccinations. However, this is yet to be studied in humans. The Ebola virus (EBOV) has caused many problems in Africa in recent years, similar to the Zika virus previously discussed. EBOV vaccine studies have used similar approaches to SARS-CoV-2 strategies, with both using viral surface proteins to elicit an immune response. In this case, EBOV glycoproteins are expressed as a transgene within an Ad26 viral vector[76]. Pre-existing immunity was very low in human trial participants (3.4%). In non-human primates, follow-up boost vaccines of Ad35 were given in response to EBOV glycoproteins[77]. This Ad35 boost provided a reasoning behind how immunity was bypassed, proving to be superior to Ad5 in binding dendritic cells. Additionally, Ad35 has a natural tropism for diverse primary human cell types[78]. Geisbert et al.[77] showed another interesting feature of Ad vectors in that they can be manipulated depending on administration techniques to confer either immediate immunity or longer lasting immunity. This may also diversify the possibilities of Ad vectors as vaccines, depending on whether emergency immediate immunity is required or a longer lasting protective cover is needed.

Conclusion

Based on the literature, Ads as delivery vectors have generally been effective for gene therapies and vaccination strategies. Despite setbacks in vector development and gaining public trust post-Jesse Gelsinger's death and the Merck STEP study, there is most certainly a future for Ad vector therapeutics. For example, the ChAdOx1 SARS-CoV-2 vaccine is immensely important (apart from their obvious immunogenicity) in providing a cost-efficient and accessible vaccine.

Ad diversity and delineated viral tropism is key to their success as vectors. Future studies of Ad vectors should look to develop these differing tropisms through complexes of different subtypes to adapt to their host, providing efficient transgene delivery and/or eliciting an immune response. These mosaic vectors help to overcome the issue of pre-existing immunity, while gutless vectors can also be used to avoid this anti-Ad immunity. Heterologous vectors using prime–boost strategies have also yielded successful results but require further development.

It is clear that studies should not become focused on specific Ad serotypes. If a single serotype was solely used in gene therapies and vaccination, immunity would be quickly developed by the global population. As a result of host adaptation, the library of Ads needs to include many chimeric surface proteins and mutated genotypes to allow for a broad range of possible treatment options. Pre-existing immunity is unavoidable but requires attention as Ads are encountered often in everyday settings, especially being implicated to sometimes cause the common cold[79,80].

In vaccination and cancer gene therapy, immunity must be managed. Vector transduction and its immunogenicity may be designed to elicit an immune response and act as a self-adjuvant. However, striking a balance of immune response—weak enough so the vector is not cleared too rapidly, while also strong enough to sufficiently stimulate adaptive immune memory cells—remains difficult. Evidence of this correct balance of induced immune response has been observed in the use of non-human and low seroprevalent Ad vectors.

Ad vectors that deliver CRISPR/Cas9 systems to target cells have shown promising results and could work to improve the efficiency and effectiveness of gene therapy using viral vector delivery. In gene therapy, it is important to be wary of possible vector contamination when using helper Ads, and to note that transient vector activity may arise.

Ad vectors demonstrate an encouraging source of gene delivery and bring about a new era of therapeutics. They show huge benefits when applied successfully, despite their many pitfalls. If these various pitfalls discussed (such as pre-existing immunity, transient action, exceedingly robust immune response, and target cell specificity) can be overcome, the potential of these delivery platforms is immense. Ad vector delivery could be transformative for therapeutic molecular biology and, indeed, for global human health. ◄

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Declarations

The author declares no conflicts of interest.

References

1. Flint S, Racaniello, V., Rall, G., Hatziioannou, T. and Skalka, A. Principles of Virology: American Society for Microbiology; 2015.
2. Beatty M, Curiel D. Adenovirus Strategies for Tissue-Specific Targeting. *Advances in Cancer Research*. 2012;115:39-67.
3. Kajon AE, Weinberg JB, Spindler KR. Adenoviruses. Reference Module in Biomedical Sciences: Elsevier; 2019.
4. Talmadge JE, Cowan KH. 31 - Gene Therapy in Oncology. In: Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE, editors. Abeloff's Clinical Oncology (Fifth Edition). Philadelphia: Churchill Livingstone; 2014. p. 493-507.e4.
5. Bollard CM, Russell C, Cruz Y, Brenner MK. CHAPTER 19 - Gene Therapy and Allergy. In: Leung DYM, Sampson HA, Geha R, Szefler SJ, editors. Pediatric Allergy: Principles and Practice (Second Edition). Edinburgh: W.B. Saunders; 2010. p. 211-22.
6. Gilgenkrantz H, Duboc D, Juillard V, Couton D, Pavirani A, Guillet JG, et al. Transient Expression of Genes Transferred In Vivo into Heart Using First-Generation Adenoviral Vectors: Role of the Immune Response. *Human Gene Therapy*. 1995;6(10):1265-74.
7. Mast TC, Kierstead L, Gupta SB, Nikas AA, Kallas EG, Novitsky V, et al. International epidemiology of human pre-existing adenovirus (Ad) type-5, type-6, type-26 and type-36 neutralizing antibodies: correlates of high Ad5 titers and implications for potential HIV vaccine trials. *Vaccine*. 2010;28(4):950-7.
8. Zhao H, Xu C, Luo X, Wei F, Wang N, Shi H, et al. Seroprevalence of Neutralizing Antibodies against Human Adenovirus Type-5 and Chimpanzee Adenovirus Type-68 in Cancer Patients. *Front Immunol*. 2018;9:335-.
9. 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. *J Med Virol*. 2013;85(6):1077-84.
10. Marshall E. Gene Therapy Death Prompts Review of Adenovirus Vector. *Science*. 1999;286(5448):2244-5.
11. Stolberg SG. The biotech death of Jesse Gelsinger. *NY Times Mag*. 1999;28:136-40.
12. Farnós O, Gelaye E, Trabelsi K, Bernier A, Subramani K, Kallel H, et al. Establishing a Robust Manufacturing Platform for Recombinant Veterinary Vaccines: An Adenovirus-Vector Vaccine to Control Newcastle Disease Virus Infections of Poultry in Sub-Saharan Africa. *Vaccines* (Basel). 2020;8(2).
13. Coughlan L. Factors Which Contribute to the Immunogenicity of Non-replicating Adenoviral Vectored Vaccines. *Front Immunol*. 2020;11:909.
14. Sun J, Zhuang Z, Zheng J, Li K, Wong RL, Liu D, et al. Generation of a Broadly Useful Model for COVID-19 Pathogenesis, Vaccination, and Treatment. *Cell*. 2020;182(3):734-43.e5.
15. Li C, Psatha N, Sova P, Gil S, Wang H, Kim J, et al. Reactivation of γ-globin in adult β-YAC mice after ex vivo and in vivo hematopoietic stem cell genome editing. *Blood*. 2018;131(26):2915-28.
16. Lukashev AN, Zamyatnin AA, Jr. Viral Vectors for Gene Therapy: Current State and Clinical Perspectives. *Biochemistry* (Mosc). 2016;81(7):700-8.
17. Lee CS, Bishop ES, Zhang R, Yu X, Farina EM, Yan S, et al. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. *Genes & Diseases*. 2017;4(2):43-63.
18. Sakhuja K, Reddy PS, Ganesh S, Cantaniag F, Pattison S, Limbach P, et al. Optimization of the generation and propagation of gutless adenoviral vectors. *Hum Gene Ther*. 2003;14(3):243-54.
19. Farnós O, Gelaye E, Trabelsi K, Bernier A, Subramani K, Kallel H, et al. Establishing a Robust Manufacturing Platform for Recombinant Veterinary Vaccines: An Adenovirus-Vector Vaccine to Control Newcastle Disease Virus Infections of Poultry in Sub-Saharan Africa. *Vaccines* (Basel). 2020;8(2).
20. Shen CF, Lanthier S, Jacob D, Montes J, Beath A, Beresford A, et al. Process optimization and scale-up for production of rabies vaccine live adenovirus vector (AdRG1.3). *Vaccine*. 2012;30(2):300-6.
21. Scaria A, St George JA, Jiang C, Kaplan JM, Wadsworth SC, Gregory RJ. Adenovirus-mediated persistent cystic fibrosis transmembrane conductance regulator expression in mouse airway epithelium. *Journal of Virology*. 1998;72(9):7302-9.
22. Zhang Y, Bergelson JM. Adenovirus receptors. *Journal of Virology*. 2005;79(19):12125-31.
23. Zabner J, Couture LA, Gregory RJ, Graham SM, Smith AE, Welsh MJ. Adenovirus-mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis. *Cell*. 1993;75(2):207-16.
24. Harvey BG, Leopold PL, Hackett NR, Grasso TM, Williams PM, Tucker AL, et al. Airway epithelial CFTR mRNA expression in cystic fibrosis patients after repetitive administration of a recombinant adenovirus. *J Clin Invest*. 1999;104(9):1245-55.
25. Knowles MR, Hohneker KW, Zhou Z, Olsen JC, Noah TL, Hu P-C, et al. A Controlled Study of Adenoviral-Vector–Mediated Gene Transfer in the Nasal Epithelium of Patients with Cystic Fibrosis. *New England Journal of Medicine*. 1995;333(13):823-31.
26. Zhang L, Wang W, Wang S. Effect of vaccine administration modality on immunogenicity and efficacy. *Expert Rev Vaccines*. 2015;14(11):1509-23.
27. Kim MH, Kim HJ, Chang J. Superior immune responses induced by intranasal immunization with recombinant adenovirus-based vaccine expressing full-length Spike protein of Middle East respiratory syndrome coronavirus. *PLoS One*. 2019;14(7):e0220196.
28. Worgall S, Leopold PL, Wolff G, Ferris B, Van Roijen N, Crystal RG. Role of alveolar macrophages in rapid elimination of adenovirus vectors administered to the epithelial surface of the respiratory tract. *Hum Gene Ther*. 1997;8(14):1675-84.
29. Greber UF, Flatt JW. Adenovirus Entry: From Infection to Immunity. *Annual Review of Virology*. 2019;6(1):177-97.
30. Koo T, Yoon A-R, Cho H-Y, Bae S, Yun C-O, Kim J-S. Selective disruption of an oncogenic mutant allele by CRISPR/Cas9 induces efficient tumor regression. *Nucleic Acids Research*. 2017;45(13):7897-908.
31. Green CA, Sande CJ, Scarselli E, Capone S, Vitelli A, Nicosia A, et al. Novel genetically-modified chimpanzee adenovirus and MVA-vectored respiratory syncytial virus vaccine safely boosts humoral and cellular immunity in healthy older adults. *J Infect*. 2019;78(5):382-92.
32. Mensah VA, Roetynck S, Kanteh EK, Bowyer G, Ndaw A, Oko F, et al. Safety and Immunogenicity of Malaria Vectored Vaccines Given with Routine Expanded Program on Immunization Vaccines in Gambian Infants and Neonates: A Randomized Controlled Trial. *Front Immunol*. 2017;8:1551.
33. Gurwith M, Lock M, Taylor EM, Ishioka G, Alexander J, Mayall T, et al. Safety and immunogenicity of an oral, replicating adenovirus serotype 4 vector vaccine for H5N1 influenza: a randomised, double-blind, placebo-controlled, phase 1 study. *The Lancet Infectious Diseases*. 2013;13(3):238-50.
34. Liebowitz D, Gottlieb K, Kolhatkar NS, Garg SJ, Asher JM, Nazareno J, et al. Efficacy, immunogenicity, and safety of an oral influenza vaccine: a placebo-controlled and active-controlled phase 2 human challenge study. *The Lancet Infectious Diseases*. 2020;20(4):435-44.
35. Reid TR, Freeman S, Post L, McCormick F, Sze DY. Effects of Onyx-015 among metastatic colorectal cancer patients that have failed prior treatment with 5-FU/leucovorin. *Cancer Gene Therapy*. 2005;12(8):673-81.
36. Wold WS, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Curr Gene Ther*. 2013;13(6):421-33.
37. Peng Z. Current Status of Gendicine in China: Recombinant Human Ad-p53 Agent for Treatment of Cancers. *Human Gene Therapy*. 2005;16(9):1016-27.
38. Boucher P, Cui X, Curiel DT. Adenoviral vectors for in vivo delivery of CRISPR-Cas gene editors. *Journal of Controlled Release*. 2020;327:788-800.
39. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature Biotechnology*. 2014;32(4):347-55.
40. Amoasii L, Hildyard JCW, Li H, Sanchez-Ortiz E, Mireault A, Caballero D, et al. Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. *Science*. 2018;362(6410):86-91.
41. Reynolds PN, Dmitriev I, Curiel DT. Insertion of an RGD motif into the HI loop of adenovirus fiber protein alters the distribution of transgene expression of the systemically administered vector. *Gene Ther*. 1999;6(7):1336-9.
42. Wang Z, Wang B, Lou J, Yan J, Gao L, Geng R, et al. Mutation in fiber of adenovirus serotype 5 gene therapy vector decreases liver tropism. *Int J Clin Exp Med*. 2014;7(12):4942-50.
43. Gall J, Kass-Eisler A, Leinwand L, Falck-Pedersen E. Adenovirus type 5 and 7 capsid chimera: fiber replacement alters receptor tropism without affecting primary immune neutralization epitopes. *J Virol*.

1996;70(4):2116-23.

44. Koski A, Karli E, Kipar A, Escutenaire S, Kanerva A, Hemminki A. Mutation of the fiber shaft heparan sulphate binding site of a 5/3 chimeric adenovirus reduces liver tropism. *PLoS One*. 2013;8(4):e60032.
45. Gao J, Zhang W, Mese K, Bunz O, Lu F, Ehrhardt A. Transient Chimeric Ad5/37 Fiber Enhances NK-92 Carrier Cell-Mediated Delivery of Oncolytic Adenovirus Type 5 to Tumor Cells. *Mol Ther Methods Clin Dev*. 2020;18:376-89.
46. Heise C, Sampson-Johannes A, Williams A, McCormick F, Von Hoff DD, Kirn DH. ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nature Medicine*. 1997;3(6):639-45.
47. O'Shea CC, Johnson L, Bagus B, Choi S, Nicholas C, Shen A, et al. Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity. *Cancer Cell*. 2004;6(6):611-23.
48. DeWeese TL, van der Poel H, Li S, Mikhak B, Drew R, Goemann M, et al. A phase I trial of CV706, a replication-competent, PSA selective oncolytic adenovirus, for the treatment of locally recurrent prostate cancer following radiation therapy. *Cancer Res*. 2001;61(20):7464-72.
49. Kim J, Cho JY, Kim JH, Jung KC, Yun CO. Evaluation of E1B gene-attenuated replicating adenoviruses for cancer gene therapy. *Cancer Gene Ther*. 2002;9(9):725-36.
50. Garcia-Carbonero R, Salazar R, Duran I, Osman-Garcia I, Paz-Ares L, Bozada JM, et al. Phase 1 study of intravenous administration of the chimeric adenovirus enadenotucirev in patients undergoing primary tumor resection. *J Immunother Cancer*. 2017;5(1):71.
51. Dyer A, Di Y, Calderon H, Illingworth S, Kueberuwa G, Tedcastle A, et al. Oncolytic Group B Adenovirus Enadenotucirev Mediates Non-apoptotic Cell Death with Membrane Disruption and Release of Inflammatory Mediators. *Mol Ther Oncolytics*. 2016;4:18-30.
52. O'Cathail SM, Davis S, Holmes J, Brown R, Fisher K, Seymour L, et al. A phase 1 trial of the safety, tolerability and biological effects of intravenous Enadenotucirev, a novel oncolytic virus, in combination with chemoradiotherapy in locally advanced rectal cancer (CEDAR). *Radiat Oncol*. 2020;15(1):151-.
53. Shayakhmetov DM, Papayannopoulou T, Stamatoyannopoulos G, Lieber A. Efficient gene transfer into human CD34(+) cells by a retargeted adenovirus vector. *Journal of Virology*. 2000;74(6):2567-83.
54. Bhatia S, O'Bryan SM, Rivera AA, Curiel DT, Mathis JM. CXCL12 retargeting of an adenovirus vector to cancer cells using a bispecific adapter. *Oncolytic Virother*. 2016;5:99-113.
55. Waddington SN, McVey JH, Bhella D, Parker AL, Barker K, Atoda H, et al. Adenovirus Serotype 5 Hexon Mediates Liver Gene Transfer. *Cell*. 2008;132(3):397-409.
56. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. *Lancet*. 2021;397(10269):99-111.
57. Fernandez-Garcia L, Pacios O, González-Bardanca M, Blasco L, Bleriot I, Ambroa A, et al. Viral Related Tools against SARS-CoV-2. *Viruses*. 2020;12(10).
58. Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19. *New England Journal of Medicine*. 2021;384(23):2187-201.
59. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *The Lancet*. 2020;396(10249):467-78.
60. Dicks MD, Spencer AJ, Coughlan L, Bauza K, Gilbert SC, Hill AV, et al. Differential immunogenicity between HAdV-5 and chimpanzee adenovirus vector ChAdOx1 is independent of fiber and penton RGD loop sequences in mice. *Sci Rep*. 2015;5:16756.
61. Ramasamy MN, Minassian AM, Ewer KJ, Flaxman AL, Folegatti PM, Owens DR, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *The Lancet*. 2020.
62. Zhu F-C, Guan X-H, Li Y-H, Huang J-Y, Jiang T, Hou L-H, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *The Lancet*. 2020;396(10249):479-88.
63. Mishra SK, Tripathi T. One year update on the COVID-19 pandemic: Where are we now? *Acta Trop*. 2020;214:105778-.
64. Dyer O. Covid-19: Countries are learning what others paid for vaccines. *BMJ*. 2021;372:n281.
65. 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):3170-80.
66. Sharma PK, Dmitriev IP, Kashentseva EA, Raes G, Li L, Kim SW, et al. Development of an adenovirus vector vaccine platform for targeting dendritic cells. *Cancer Gene Ther*. 2018;25(1-2):27-38.
67. Bullard BL, Corder BN, Gordon DN, Pierson TC, Weaver EA. Characterization of a Species E Adenovirus Vector as a Zika virus vaccine. *Scientific Reports*. 2020;10(1):3613-.
68. Weaver EA. Vaccines within vaccines: the use of adenovirus types 4 and 7 as influenza vaccine vectors. *Hum Vaccin Immunother*. 2014;10(3):544-56.
69. Logunov DY, Dolzhikova IV, Shcheblyakov DV, Tukhvatulin AI, Zubkova OV, Dzharullaeva AS, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *The Lancet*. 2021;397(10275):671-81.
70. Callaway E. Russia announces positive COVID-vaccine results from controversial trial. *Nature*. 2020.
71. Cari L, Fiore P, Naghavi Alhosseini M, Sava G, Nocentini G. Blood clots and bleeding events following BNT162b2 and ChAdOx1 nCoV-19 vaccine: An analysis of European data. *Journal of Autoimmunity*. 2021;122:102685.
72. McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *The Lancet*. 2008;372(9653):1894-905.
73. Sekaly R-P. The failed HIV Merck vaccine study: a step back or a launching point for future vaccine development? *J Exp Med*. 2008;205(1):7-12.
74. Sayedahmed EE, Kumari R, Shukla S, Hassan AO, Mohammed SI, York IA, et al. Longevity of adenovirus vector immunity in mice and its implications for vaccine efficacy. *Vaccine*. 2018;36(45):6744-51.
75. Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important? *Human Vaccines & Immunotherapeutics*. 2014;10(10):2875-84.
76. 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-Vectored Ebola Vaccines: A Randomized Clinical Trial. *JAMA*. 2016;315(15):1610-23.
77. 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 ebolavirus challenge. *J Virol*. 2011;85(9):4222-33.
78. Vogels R, Zuijdgeest D, van Rijnsoever R, Hartkoorn E, Damen I, de Béthune M-P, et al. Replication-deficient human adenovirus type 35 vectors for gene transfer and vaccination: efficient human cell infection and bypass of preexisting adenovirus immunity. *Journal of virology*. 2003;77(15):8263-71.
79. Ruohola A, Waris M, Allander T, Ziegler T, Heikkinen T, Ruuskanen O. Viral etiology of common cold in children, Finland. *Emerg Infect Dis*. 2009;15(2):344-6.
80. Heikkinen T, Järvinen A. The common cold. *The Lancet*. 2003;361(9351):51-9.