REVIEW

Editorial Process: Submission:12/11/2024 Acceptance:11/17/2025 Published:11/20/2025

Virotherapy as Gene Deliver for Anti-Cancer Therapy: A Review Article

Fakhren Nukha Zalfa, Nurul Hikma Suciani, Putu Ananda Arviana Dewi, Anissafa Atul Khusna, Rima Nurlita, Dion Bishma Daniswara, Anisa Oktaviana Putri, Muh. Ade Artasasta*

Abstract

This review identifies Adenovirus (AdV) as a leading candidate for gene therapy due to its high transduction efficiency and large gene-carrying capacity, supported by evidence of its progression to phase III clinical trials. However, the interpretation of findings is limited by variations in study designs, potential publication bias, and possible exclusion of relevant studies. As the field continues to evolve with new vector technologies, future research should focus on improving vector safety, specificity, and long-term outcomes to maximize the therapeutic potential of virotherapy. **Objective:** Cancer is the second leading cause of death worldwide. Treatments like chemotherapy and radiotherapy are commonly used, but they have side effects and complications. Gene therapy, using viral vectors like retroviruses, Adenovirus (AdV), and Adeno-Associated Virus (AAV), offers a new approach to overcome these limitations by specifically targeting cancer cells' genetics. This article was developed as a review to compare the effectiveness of different viruses in virotherapy for cancer treatment. **Methods:** This article employed a narrative literature review and qualitative content analysis to synthesize current knowledge on the topic. More than 85 peer-reviewed articles published between 2016 and 2024 were collected from databases such as ScienceDirect, PubMed, Scopus, and Nature. The search used relevant keywords with Boolean operators (AND, OR) to refine results. Articles were selected based on language (English), publication type (peer-reviewed), and relevance to the topic. The selected literature was analyzed to identify recurring patterns, key themes, and significant insights. **Result:** The research results indicate that viruses demonstrated vary levels of efficacy. Retroviruses have a 40%-60% transduction efficiency, can integrate into the host genome, and infect only dividing cells. Adenovirus (AdV) has a 98% efficiency in hepatocellular carcinoma and 70%-80% in other cancers, delivers genetic material without integration, infects both dividing and non-dividing cells, and has a gene capacity of 37 kb. Adeno-Associated Virus (AAV) has a 30%-50% efficiency, a gene capacity of 4.8 kb, and also shows therapeutic potential. **Conclusion:** Each virotherapy agent used in gene therapy exhibits varying efficacy against cancer cells, indicating specific mechanisms unique to each virotherapy agent.

Keywords: Adenovirus- Adeno-Associated Virus- Cancer- Retrovirus- Viral Vector

Asian Pac J Cancer Prev, **26 (11)**, 3895-3907

Introduction

Cancer is one of the diseases with the second highest death rate in the world with 8.97 million deaths after ischemic heart disease and is expected to continue to increase. The number of cancer cases recorded is almost 20 million new cases in 2022 and 9.7 million deaths due to cancer worldwide, this figure has increased from the previous year which was 18 million cases in 2018 and continuing to increase every year.

In the last few decades, several combinations of therapies have been suggested and are currently used to treat various types of cancer [1]. Chemotherapy and conventional radiotherapy are now widely utilized, which were not common a few years ago [2]. Although chemotherapy and radiotherapy are the primary treatments for cancer, their effectiveness and application are often hindered by severe side effects [3].These side effects directly reduce patients' quality of life and may also lead to long-term complications in cancer survivor [4], [5].

Gene therapy is a cutting-edge treatment method that involves introducing new genes into cancerous cells or surrounding tissues to induce cell death or slow the progression of cancer [6, 7], This therapy works by focusing on genetic modifications that interfere with the cellular mechanisms responsible for the rapid growth and division of these cancer cells [8]. By disrupting these processes, gene therapy has the potential to directly

Department of Applied Science, Faculty of Mathematics and Natural Science, Universitas Negeri Malang, Malang City, Indonesia.
**For Correspondence: muh.ade.artasasta.fmipa@um.ac.id*

eliminate cancer cells while simultaneously suppressing their ability to survive and thrive within the body [9].

This therapy can be implemented using both viral and non-viral methods. Non-viral approaches offer significant advantages in terms of safety and avoiding immune reactions despite being less commonly used than viral vectors [7]. Viruses are most commonly used vector for gene therapy, utilizing native or modified viruses to trigger an anti-cancer response where these are known as virotherapy [10]. The main reason for using a virus as a vector for gene delivery is to take advantage of its natural ability to infect cells and effectively transport the desired genetic material into host cells [11, 12]. These viruses include retrovirus, adenovirus, and adeno-associated virus (AAV) [13]. The use of viral groups such as retroviruses, adenoviruses, and adeno-associated viruses (AAVs) as virotherapy agents is well-founded. These three types of viruses possess key characteristics that support their effectiveness in virotherapy, including high transduction efficiency, large gene packaging capacities, the ability to integrate into the host genome, and relatively low immunogenicity [14-16]. These features make them ideal candidates for the efficient and targeted delivery of genetic material to cancer cells. Moreover, the selection of these viral vectors is further supported by clinical trial data. According to the Wiley Gene Therapy Trials Database, adenoviruses, lentiviruses (a subclass of retroviruses), and AAVs are the most commonly used viral vectors in gene therapy clinical trials, accounting for approximately 50%, 28%, and 22% of all trials, respectively [17].

However, despite the numerous approaches that have been explored, there remains uncertainty regarding which method is the most effective Consequently, this article was developed as a review to compare the effectiveness of different viruses in virotherapy for cancer treatment. By evaluating their capacities, efficiency transductions, structures, mechanisms, cell lines, advantages and disadvantages, conclusions can be drawn about which viruses have the greatest potential for use as gene delivery agents in cancer therapy.

Materials and Methods

The methodology employed in this article consists of a comprehensive literature review and qualitative content analysis. The literature review focuses on gathering and synthesizing relevant information from more than 85 scientific articles published between 2016 and 2024, sourced from reputable databases, including ScienceDirect, PubMed, Scopus, and Nature. The qualitative content analysis was conducted to identify patterns, themes, and insights relevant to the research topic, ensuring a thorough and systematic evaluation of the available data.

Results

The review evaluates Retroviruses, Adenoviruses (AdVs), and Adeno-Associated Viruses (AAVs) as viral vectors in virotherapy, comparing their safety, transduction efficiency, and clinical trial outcomes. Retroviruses exhibit moderate transduction efficiency, ranging from 40% to 60%, and their ability to integrate into the host genome ensures stable, long-term gene expression. However, retroviruses can only infect dividing cells and carry risks of insertional mutagenesis, limiting their safety. In clinical trials, retroviral vectors demonstrated therapeutic potential, such as significant tumor size reduction in osteosarcoma, but their application is hindered by production challenges and receptor dependency.

Adenoviruses, in contrast, deliver genetic material without integrating into the host genome, enabling temporary but effective gene expression. With transduction efficiency reaching up to 98% in hepatocellular carcinoma and around 70–80% in other cancers, Adenoviruses outperform retroviruses in efficiency. They can infect both dividing and non-dividing cells and offer a large gene capacity (37 kb), making them suitable for delivering complex therapeutic genes. Clinical trials in pancreatic cancer reported an overall response rate (ORR) of 50%, with manageable side effects, supporting AdVs as effective

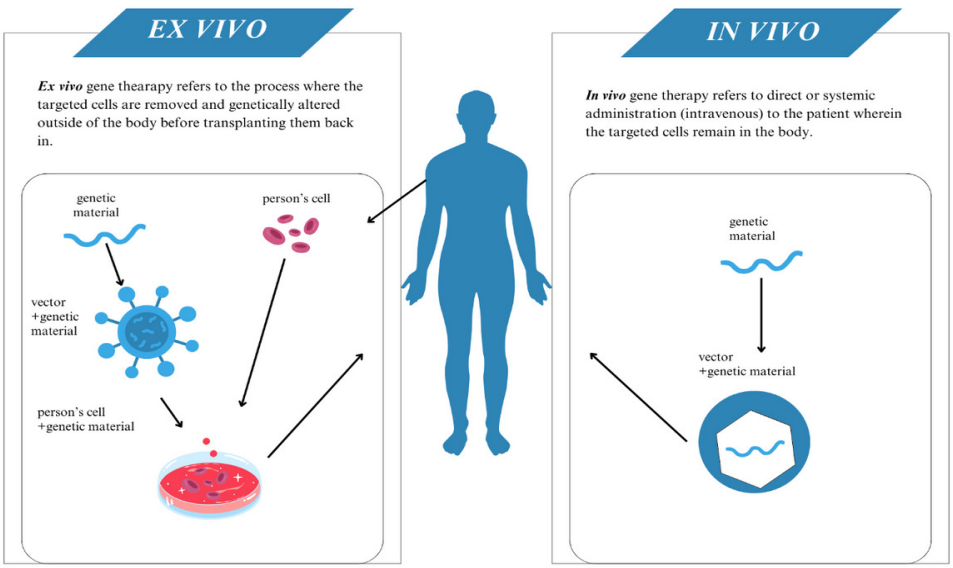

Figure 1. Types of Gene Therapy [99]

and relatively safe vectors. However, immune responses triggered by Adenoviruses capsids remain a notable drawback.

Adeno-Associated Viruses provide the advantage of long-term gene expression and minimal immune responses, making them highly safe for therapeutic use. However, their transduction efficiency is lower, at around 30–50% depending on the target cell, and their limited genetic capacity (4.8 kb) restricts the delivery of larger genes. Preclinical studies on AAVs have shown effective tumor inhibition with minimal side effects, though risks such as liver cell damage in some cases highlight areas for improvement.

Among these vectors, Adenoviruses stand out due to their superior transduction efficiency, versatility in infecting various cell types, and strong clinical trial outcomes. While each vector has unique strengths and weaknesses, Adenoviruses currently show the most promise for broader applications in cancer virotherapy (the results are presented in Table 1).

Discussion

Basics of Gene Therapy

Definition of Gene Therapy

Gene therapy is defined as the treatment of disease by inserting genetic material into cells which can correcting defective genes that cause diseases [18]. This medical approach focuses on modifying the genes in cells to achieve a therapeutic outcome or treat disease by repairing or reconstructing faulty genetic material (as shown in Figure 1) [19].

The Mechanism of Gene Therapy

Healthy genes are inserted into the genome to replace abnormal genes contributing to specific diseases in gene therapy [20]. However, a significant challenge in this process is the efficient insertion of these genes into stem cells. To address this challenge, molecular carriers known as "vectors" are utilized for gene transfer [21, 22].

Virus Vector-Based Delivery

Virus vector is a modification of a virus that is used to deliver genetic material or a target gene into a host cell [23]. Genetic modification for virotherapy aims to create oncolytic viruses that can specifically attack cancer cells without harming normal cells [24]. This process involves several important steps, such as deleting or modifying the virus genes that usually cause disease [25, 26]. In virotherapy, several viruses are commonly used and modified as therapeutic agents, primarily for gene delivery in cancer therapy. These vectors are integrated into the DNA of the host cell and express the genes they carry. Viruses can express their genes efficiently in host cells, making them very suitable as delivery vectors (as shown in Figure 2) [23]. One of the parameters to determine how effective the virus is in delivering target genes into host cells is based on the gene transduction efficiency value. Transduction efficiency is an indicator that measures the number of target cells that successfully receive new genetic material via the viral vector used in therapy [27, 28]. Transduction efficiency in virus vectors is usually calculated by calculating the percentage of cells that successfully receive and express the target gene after infection by the viral vector [29].

$$Transduction\ efficiency = \frac{Total\ Number\ of\ Infected\ Cells}{Number\ of Transduced\ Cells}(x100)$$

This efficiency values can increase the potential success of gene therapy from viral vectors. It can be

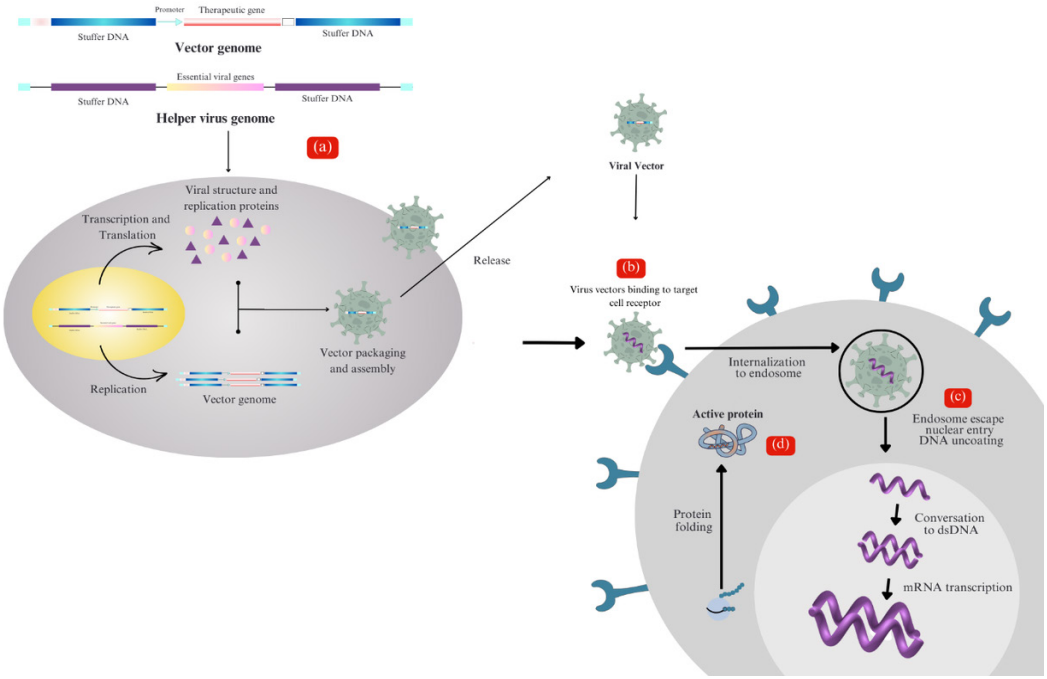

Figure 2. Viral Vector Expression Systems [23] A). Converting virus into a vector B). The process of binding the viral vector to the target cell receptor C). Endosome escape from viral vector and nuclear DNA entry D). Protein release from viral vector expression [94]

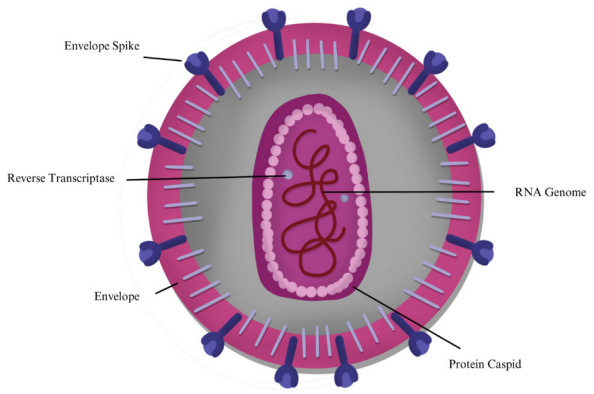

Figure 3. Structure of Retrovirus [39]

known if the higher the transduction efficiency value, the more cells will receive the therapeutic gene. The more therapeutic genes that are successfully integrated into target cells, the success of therapeutic treatment will also increase [30]. The choice of the vector to be used as based delivery depends upon the type of genetic material to be delivered, capacity, efficiency transduction, structure, mechanism of genetic transmission parameters, and also the advantages-disadvantages of each virus as a vector [31].

Retrovirus Mechanisms and Applications in Gene Therapy
Definition of Retrovirus

Retrovirus is a type of RNA virus that replicates itself through a process called reverse transcription. Unlike most viruses, which directly use their RNA or DNA to create proteins, retroviruses use an enzyme called reverse transcriptase to convert their RNA genome into DNA after infecting a host cell [32]. Retroviruses have a genetic capacity of approximately 8 kilobases (kb), allowing them to carry and integrate relatively large fragments of DNA into the host genome, thereby supporting applications in gene therapy and vaccine development [33].

Structure of Retrovirus

The structure of retroviruses allows them to attach to the host cell, inject their genome, and replicate through infection [34]. Surrounding the capsid is a viral envelope, derived from the host cell membrane during the budding process, which contains glycoproteins and lipid molecules. This envelope not only shields the nucleocapsid but also aids in the virus's entry into and exit from host cells [35]. Embedded in the envelope are glycoprotein spikes, which are essential for binding to specific receptors on the host cell membrane [34]. The structure of the retrovirus can be seen in Figure 3.

Mechanism of Retrovirus Infection

The retroviral infection process starts with the virus binding to specific receptors on the target cell, ensuring selective targeting and minimizing effects on healthy cells, crucial for gene therapy [36, 37]. After binding, the viral envelope fuses with the host cell membrane, allowing the viral core (RNA and enzymes) to enter the cytoplasm [38]. Reverse transcription then converts viral RNA into complementary DNA (cDNA) via reverse transcriptase, a key retrovirus feature [36-38]. The cDNA is transported to the nucleus, where integrase integrates it into the host genome, enabling long-term gene expression [39]. The host transcribes the integrated DNA into mRNA, which is translated into therapeutic proteins in the cytoplasm, [40, 41]. Subsequently, viral RNA and newly synthesized proteins in the cytoplasm begin assembling into new viral particles. This process involves RNA-protein binding, ensuring proper packaging of the viral genome [40, 41]. New viral particles assemble in the cytoplasm and are transported to the host membrane, where they bud off using the host's lipid bilayer [42]. To avoid uncontrolled replication, gene therapy vectors are engineered to be non-infectious while delivering therapeutic genes [42, 43]. Finally, viral proteases mature the virus, but most vectors are designed to limit replication to targeted delivery [44]. The mechanism of the retrovirus infection can be seen in Figure 4.

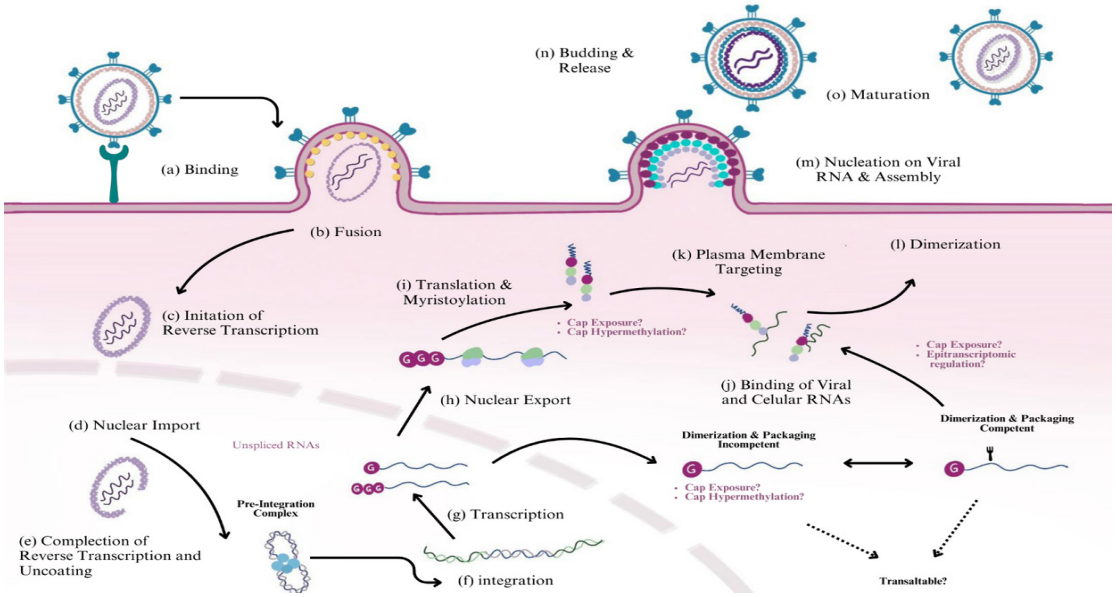

Figure 4. Mechanism of Retrovirus Infection [95]

Transduction Efficiency of Retrovirus

Transduction efficiency of retroviruses (RV) in in vitro-activated CD8+ T cells typically ranges from 40% to 80%, depending on factors such as RV titer, gene of interest (GOI) insert size, and the activation status of the target T cells. Enriching activated CD8+ T cells, which are more susceptible to RV transduction, increases the yield of transduced cells. After spin-transduction, approximately 50% of the initial cell population remains viable, with around 1 million RV-transduced T cells recovered from 10 million cells [45]. Lentiviruses, a subtype of retroviruses, also demonstrate high transduction efficiency in lung cancer cells, achieving over 50% transduction in most lung cancer cell lines with a low Multiplicity of Infection (MOI) of around 5 [44].

CAR-T therapy, which often uses retrovirus-based vectors for genetic modification, has shown strong efficacy in treating challenging cancers [46, 47]. In the ZUMA-1 trial for large B-cell lymphoma, CAR-T therapy with axi-cel achieved an Overall Response Rate (ORR) of 83%, with a Complete Remission (CR) rate of 53%, indicating significant tumor reduction or disappearance in most patients with remission duration exceeded two years [47]. The ZUMA-2 trial, which focused on mantle cell lymphoma, reported an ORR of 91% and a CR rate of 68%, further highlighting the therapy's effectiveness [48]. Adverse effects like Cytokine Release Syndrome (CRS) and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) occurred in 55.3% and 37.2% of cases, respectively, underscoring the need to manage these toxicities alongside treatment [49, 50]. The FDA has approved CAR-T products, including axi-cel, tisa-cel, and liso-cel, for relapsed or refractory high-grade B-cell lymphomas, with approvals spanning from 2017 to 2021 [46]. Additionally, CAR-T has been studied in phase I and II trials [46].

Types of Cancer Cell Lines for Retrovirus Applications

Cancer cell lines are populations of cells derived from tumours or cancerous tissues that have been isolated and cultured in the laboratory [51]. These cells possess the ability to divide and multiply continuously, making them suitable for research over extended periods [51]. Cancer cell lines can originate from various types of cancer, including breast cancer, lung cancer, colon cancer, melanoma, and others. They provide researchers with essential tools for understanding cancer and developing more effective treatment strategies. Several examples of cancer cell lines used for retrovirus applications include the melanoma cell line A375, the breast cancer cell line MCF-7, the breast cancer cell line T47-D, and the colon cancer cell line HT-29 [38, 52-55]

Advantages and Disadvantages of Retrovirus

Retroviruses offer advantages in gene therapy, such as the higher availability of transfer vector mRNA during packaging, which optimizes production and enhances gene delivery efficiency [53]. Their ability to integrate genetic material into the host cell's chromosomes ensures long-term therapeutic effects by replicating alongside the host DNA during cell division [11]. However, retroviruses have limitations, including infecting only dividing cells, which restricts their use in tissues with quiescent cells [38]. Additionally, their random integration into the host chromosome poses a risk of insertional mutagenesis, potentially leading to tumorigenesis [39, 42]. These drawbacks require careful consideration and further research for safe therapeutic use.

Clinical Trials and Success Rates of Retrovirus

The study conducted by Shoji Kubo et al. [56] examines an in vivo trial in mice aimed at evaluating the effectiveness of Retroviral Replicating Vectors (RRVs) in cancer therapy, specifically targeting human osteosarcoma. This research employs two types of RRVs-AMLV (amphotropic murine leukemia virus) and GALV (gibbon ape leukemia virus) that have been engineered to carry the prodrug-activating cytosine deaminase (CD) gene [56]. Mice with subcutaneous tumors were injected with RRVs, followed by administration of the prodrug 5-fluorocytosine (5FC), which is converted into an active chemotherapeutic agent within tumor cells infected by RRVs [56]. The findings indicate that AMLV-CD RRV achieved significant tumor growth inhibition compared to GALV-CD and control groups. The therapeutic efficacy was demonstrated by a significant reduction in tumor size in mice treated with AMLV-CD and 5FC [56]. Although no major toxic side effects were reported in the mice, the effectiveness of RRVs depends on the specific receptor expression within tumor cells, which represents a primary limitation, as low receptor expression reduces transduction efficiency [56]. Additionally, large-scale RRV production remains challenging, and further development is needed to enable effective application of this therapy for cancers with systemic metastasis.

Adenovirus (AdV) Virrus Mechanism and Applications in Gene Therapy

Definition of Adenovirus (AdV)

AdV are part of the notable adenoviridae family, non-enveloped viruses with double-stranded DNA that frequently cause respiratory infections in people of all ages [57]. The absence of viral coding sequences in the genomes of HC-AdVs increases the cloning capacity to 37 Kb [55]. Over the past 30 years, viral vectors like AdV have been widely researched for their potential in gene therapy. Their gene expression is temporary because the DNA remains separate from the host genome, and they can penetrate both dividing and non-dividing cells [12].

Structure of Adenovirus (AdV)

AdV is a non-enveloped virus with a size of approximately 70-90 nm. AdV have a structure that consists of a capsid shaped like an icosahedron or polyhedron [58]. AdV contains a double-stranded DNA (dsDNA) genome that is surrounded by a protein capsid. The main structure of the capsid consists of large proteins such as hexon and penton, which are responsible for the stability of the capsid [58-60]. Therefore, the capsid serves as a protective barrier for the viral genome while also facilitating fusion with the host cell membrane [58].

Between the hexons and pentons, there are other

proteins in the form of fibers [60]. Each vertex of the adenovirus has one fiber equipped with a knob [61]. Additionally, there is a core, which is the inner part of the capsid containing the viral genetic material and several proteins involved in replication and infection. The core plays a crucial role in ensuring that the genetic material can be safely delivered into the host cell and expressed to produce more viral particles [60]. The structure of the adenovirus can be seen in Figure 5.

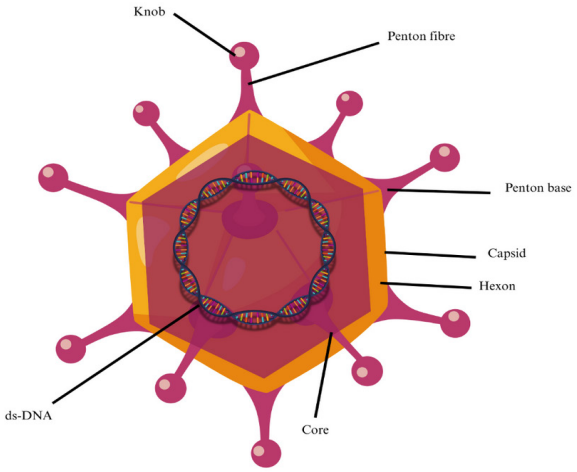

Figure 5. Structure of Adenovirus [96]

Mechanism of Adenovirus (AdV) Infection

AdV enters host cells in multiple stages, facilitated by its capsid. The capsid protects the viral genome and controls intracellular movement, releasing the genome at specific cell locations [55]. Infection begins with binding to receptors on the plasma membrane, where the AdV fiber's terminal knob interacts with receptors like CAR, desmoglein-2, CD46, and sialic acid-containing glycans [62]. This binding induces structural changes, making the fiber more flexible to interact with CAR and integrin αv, triggering dynamic uncoating within the virus [55]. Components like fiber and protein VI detach, signaling the virus to penetrate the cell via endocytosis [63]. Integrin αv on the cell surface interacts with the AdV penton base's RGD motif, triggering temporary endocytosis and forming a clathrin-coated vesicle [62]. Adaptor proteins like AP2 and EPS15 further recruit scaffolding proteins and clathrin, forming a clathrin-coated pit. GTPase dynamins constrict and cleave this pit, releasing the vesicle. The clathrin coat sheds quickly with help from Hsc70 and auxilin, allowing the virus to move within a membrane-enclosed compartment [62]. AdV can also enter through macropinocytosis, a non-specific endocytic process involving actin filament rearrangement and lamellipodia formation triggered by AdV binding to integrin αv [62].

Inside the endosome, AdV undergoes maturation but must escape before being degraded by lysosomes. Protein VI is activated, destabilizing the endosomal membrane and allowing the virus to escape into the cytoplasm [62]. To avoid degradation, AdV recruits the E3 ubiquitin ligase Nedd4.2 via the PPXY motif on Protein VI, inhibiting autophagy and preventing destruction [62]. Finally, AdV uses a microtubule network and motor proteins like dynein to move toward the nucleus, where it undergoes uncoating to deliver its genome, initiating replication [64]. The mechanism of the adenovirus infection can be seen in Figure 6.

Transduction Efficiency of Adenovirus (AdV) in Cancer

Adenoviral (AdV) vectors are used as a transduction vehicle because they can infect both dividing and non-dividing cells, and they have minimal restrictions on the packaging capacity of their genetic material [65]. The transduction efficiency of adenovirus can reach up to 98,37% in hepatocellular carcinoma [16, 65]. In pancreatic cancer, adenovirus vectors displaying PFW and SYE demonstrated high gene transduction efficiency when tested with clinical pancreatic cancer samples. Specifically, vectors with PFW and SYE enhanced transduction

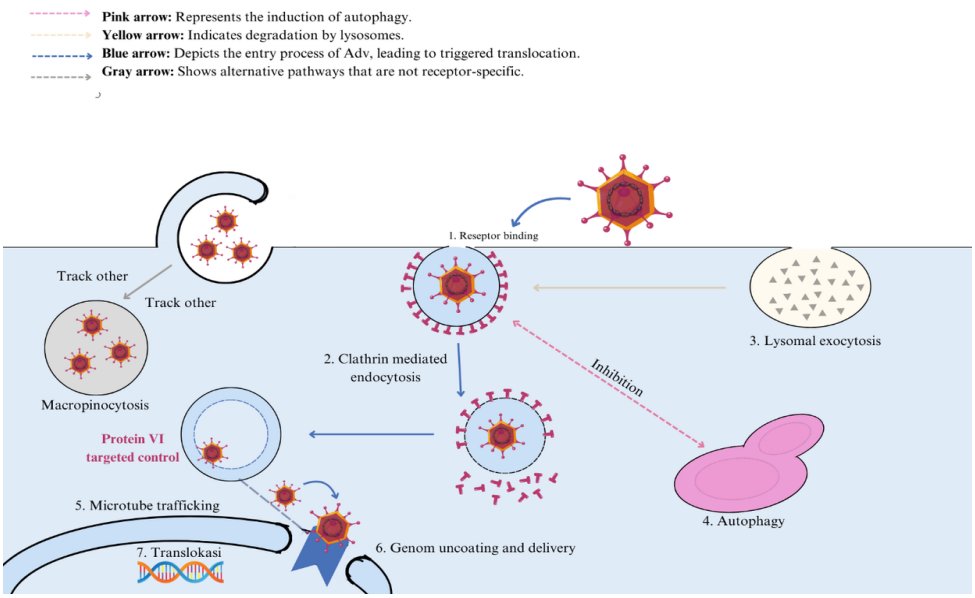

Figure 6. Mechanism of Adenovirus Infection [62]

Table 1. Comparision of Retrovirus, Adenovirus, and Adeno Associated Virus (AAV) for Anti-Cancer Therapy

Virus	Capacity	Structure	Mechanism	Cell Line and It's Efficiency Transduction (%)	Clinical Trial	Advantages and Disadvantages
Retrovirus	8 kb [99]	Figure 3 [100]	Converts RNA to DNA via reverse transcriptase, integrates into host genome [99]	Mouse CD8+ T cells 60% [45], Lung cancer NCI-H69 and NCI-H1155 cells 40% [44],	Shoji Kubo et al. [56]: AMLV & GALV vectors for osteosarcoma; reduced tumor size, low toxicity	Advantages High integration and gene expression 52], [11] Disadvantages Retrovirus can only infect dividing cells [101] & random integration into host chromosome [54]
Adeno Virus (AdV)	37 kb [55]	Figure 5 [58]	Receptor binding, endocytosis, trafficking, genome delivery, replication [63]	Hepatocellular Carcinoma HepG2 98,37% [70] Ovarian Cancer OVCAR-3 95% [108] Hamster CHO-CAR 70-80% [65] Esophageal carcinoma EC109 67% [70]	VCN-01 used for pancreatic cancer; 50% response rate in phase II–III trials, mild side effects [74].	Advantages High titers and broad transduction [70] Disadvantages A potent inflammatory response is mediated by the capsid [16], [68].
Adeno Associated Virus (AAV)	4,8 kb [102]	Figure 7 [97]	Enters via endocytosis, replicates with helper virus or stays as episome [103]	Lung Cancer A549 70% [82] Ovarian Cancer NIH:OVCAR-3 65 70% [83] Carniocoma Caki-2 70% [82] Prostate Cancer PC-3.35% [82]	2018–2023 studies: reduced tumor size and growth in mice, no side effects [91]	Advantages Stable long-term expression and infects various cells [82]. Disadvantages Small capacity limits large gene delivery [9].

efficiency by 4.4-fold and 4.3-fold, respectively [66]. The efficiency of adenovirus transduction also shows positive results in ovarian cancer. Ad5eYFP had a multiplicity of infection (MOI) of 1000, Ad5-TR3DAFeYFP had an MOI of 8750, and Ad5-TR3GPIeYFP had an MOI of 5000, all resulting in similar transduction rates of 70% to 80%, as measured by the proportion of YFP-positive cells [65]. Overall, these findings suggest that adenoviral vectors hold significant promise for effective gene therapy in various cancer types.

Types of Cancer Cell Lines for Adenovirus (AdV) Applications

In the past few decades, gene therapy for diseases such as cancer using adenoviral vectors has been significantly advanced [67]. In 2018, adenoviral (AdV) vectors were mainly used in clinical applications for cancer treatment, accounting for 80% of their total use [68], [69]. Adenoviral vectors have been designed to replicate oncolytically in cancer cells while avoiding replication in healthy cells [67]. Several studies have demonstrated that adenoviruses can treat ovarian cancer, esophageal carcinoma, hepatocellular carcinoma, pancreatic cancer, and Chinese Hamster Ovary (CHO) cell line modified to express the Coxsackievirus Adenovirus Receptor (CAR) [65]. The cell lines that have been successfully researched include the ovarian cancer cell line OVCAR-3 [66], the esophageal carcinoma cell line EC-109 [70], and hepatoblasma cell line HepG2 [70]. Oncolytic Adenoviral vector technologies have been approved in some countries for treatment of cancer in humans [71].

Advantages and Disadvantages of Adenovirus (AdV)

AdV offers several advantages for gene therapy, including high transduction ability, which allows efficient infection of various cell types, both dividing and non-dividing [68]. This ability will help adenoviruses to be flexible in targeting gene delivery to various tissues and increase the potential effectiveness of gene therapy or gene delivery [68, 72]. This flexibility enhances its potential for targeting diverse tissues. AdV also has a large genetic payload capacity, enabling it to carry complex or multiple genes, and is genetically stable, reducing the likelihood of mutation. Additionally, it is easy to produce in large quantities [13, 16]. However, AdV has notable drawbacks. A major disadvantage is the strong immune response triggered by its capsid, which can neutralize the virus before gene delivery, reducing therapy effectiveness [58]. Although AdV does not integrate its genetic material into the host DNA, there remains a risk of mutagenic effects from random integration into the host chromosome. These factors require further research to ensure the safety and efficacy of AdV in gene delivery and therapeutic applications.

Clinical Trials and Success Rates of Adenovirus (AdV)

The potential of Adv as a gene therapy for cancer can be reviewed based on an in vivo study that explored the therapeutic potential of Apolipoprotein A1-based oncolytic adenovirus to treat triple-negative breast cancer (TNBC). In a TNBC mouse model, ADV-ApoA1 was found to inhibit tumor growth, reduce lung metastasis, and prolong survival, while showing good tolerance in rhesus monkeys and Syrian hamsters at high doses [73].

As for clinical testing, it can be seen based on a study by Garcia-Carbonero et all. [74] using adenovirus type VCN-01 (AdV type 5) in 12-16 patients with pancreatic adenocarcinoma who had not undergone previous treatment. The results showed that in patients with pancreatic adenocarcinoma, the overall response rate reached 50% in phases II and III. Despite adverse events such as grade 4 aspartate aminotransferase elevation in one patient (Phase I), grade 4 febrile neutropenia in one patient and grade 5 thrombocytopenia plus enterocolitis in another patient (Phase II), treatment with VCN-O1 was feasible and safe [74].

From these examples, it can be concluded that the use of adenovirus as gene therapy has been well tested in vivo up to phase III with an acceptable level of safety, making it one of the potential approaches for cancer treatment in the future.

Adeno-Associated Virus Mechanism and Applications in Gene Therapy

Definition of Adeno-Associated Viruses (AAV)

Adeno-associated viruses (AAV) are small viruses with a single-stranded DNA (ssDNA) genome that is encapsulated in a random mixture of VP1, VP2, or VP3 proteins and belongs to the genus Dependoparvovirus and rely on co-infection with helper viruses, such as herpesviruses or adenoviruses, in order to replicate which is reliance on helper viruses, along with their low immunogenic profile, meaning they do not easily trigger an immune response, makes AAVs ideal candidates as gene therapy vectors [75]. The natural genome capacity of AAV is around 4.8 kilobases (kb) [76].

Structure of AAV

AAV consists of a single-stranded DNA (ssDNA) genome that is 4.8 kb in size, with two inverted terminal repeats (ITRs) on either end of the genome [76]. These ITRs form a T-shaped hairp in structure that initiates the replication process. The ITRs flank the rep and cap genes. Within the rep region, there are three promoters: p5, p19, and p40 [77]. These promoters drive the transcription of six different mRNA transcripts. The p5 promoter produces two large Rep proteins through alternative splicing, while p19 drives the transcription of two smaller Rep proteins. The AAV capsid is composed of 60 viral protein (VP) molecules [78]. The structure of the AAV can be seen in Figure 7.

Mechanism of AAV Infection

Adeno-Associated Virus (AAV) initiates infection by binding to specific receptors on the surface of the target cell, which vary depending on the AAV serotype [78]. The virus then enters the cell through endocytosis, where

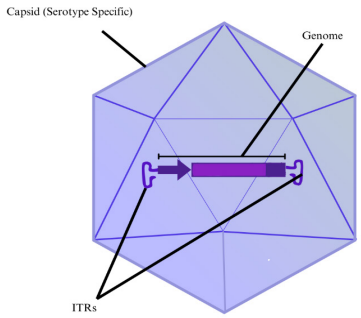

Figure 7. Structure of Adeno Assosiated-Virus (AAV) [97]

it is transported into the cytoplasm and subsequently to the cell nucleus. Once inside, AAV becomes trapped in an endosomal vesicle. The endosome carrying AAV then undergoes maturation, during which its internal pH decreases, creating an acidic environment that triggers structural changes in the AAV capsid, allowing the virus to escape from the endosome into the cytoplasm [79]. After successfully exiting the endosome, AAV moves toward the nucleus to deliver its genetic material.

The uncoating phase of Adeno-Associated Virus (AAV) infection involves the release of the viral genome from its protective capsid after the virus has entered the host cell [80]. After the uncoating process, the ssDNA can be converted into a double-stranded form for further integration into the host genome or remain in episomal form for replication [80]. Then, AAV can proceed to the replication cycle. In the absence of a helper virus, the AAV genome can persist in a latent state as an episome or may integrate into the host DNA chromosome. Then, dsDNA can integrate into the host genome, leading to stable expression of the encoded genes, or it may remain in episomal form, allowing for transcription and translation processes [80]. The mechanism of the AAV infection can be seen in Figure 8.

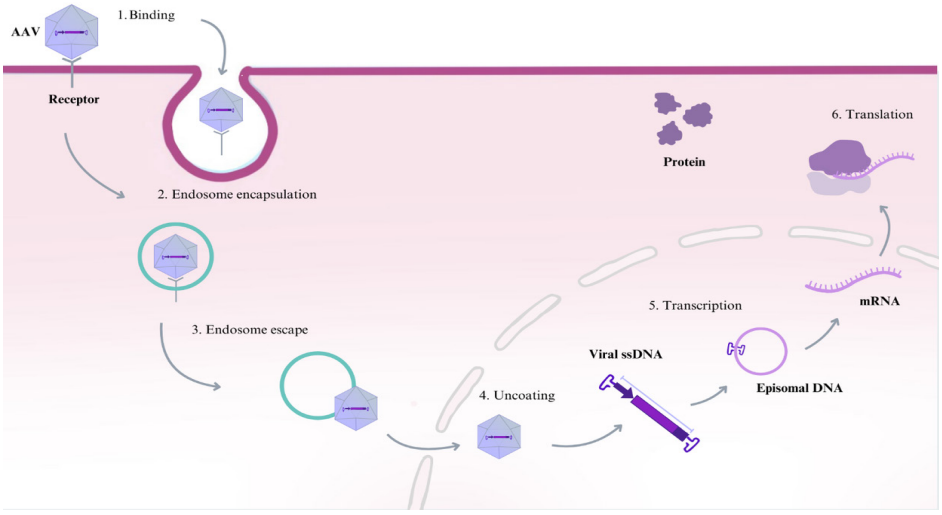

Figure 8. Mechanism of Adeno Assosiated-Virus (AAV) Infection [98]

Transduction Efficiency of Adeno-Associated Virus in Cancer

AAV (Adeno-Associated Virus) has proven to be an effective virus in inducing gene transduction in lung cancer cells, with transduction rates ranging from 30% to 50% [81]. Research shows that using the AAV2/1 virus type, the highest transduction rate achieved is between 30% and 50% when using a Multiplicity of Infection (MOI) of 100 [81]. This means that from 100 AAV2/1 virus particles added to each target cell, only 30% to 50% of those cells successfully get infected and receive genetic material from the virus. Although using a higher MOI can increase the likelihood of cell being infected, the efficiency rate achieved by AAV2/1 still indicates limitations in its ability to optimally induce gene transduction.

Additionally, another study reported that transduction mediated by recombinant AAV (rAAV) in A549 cells demonstrated that sTRAIL (soluble Tumor necrosis factor-Related Apoptosis-Inducing Ligand) was quite effective in inducing apoptosis [82]. The transduction rate of AAV in A549 lung cancer cells can reach approximately 70% when using a very high MOI of 5 × 10^4 [83]. The following research indicates that AAV transduction efficiency in NIH:OVCAR-3 ovarian cancer cells ranges from 65–70%, depending on cell temperature conditions [83]. AAV also shows varying transduction rates in other cancer cell lines such, achieving 70% in Caki-2 renal carcinoma cells and 75% in PC-3 prostate cancer cells [82].

Types of Cancer Cell Lines for Assosiated Adeno-Virus (AAV) Applications

Adeno-associated virus (AAV) gene therapy is emerging as a powerful approach for treating various types of cancer, leveraging the ability to deliver therapeutic genes directly to cancer cells . Known for its high efficiency as a vector and minimal pathogenicity, AAV is well-suited for targeted gene delivery, allowing researchers to design therapies that address specific genetic abnormalities or critical pathways within different types of cancer [84]. By tailoring the AAV approach to individual cancer cell lines, it's possible to target genetic mutations or cellular mechanisms unique to each cancer type, enhancing the precision and effectiveness of treatment [85]. For instance, the approach can be applied to the Lung Cancer cell line A549, the Chronic Ovarian Cancer cell line NIH:OVCAR-3 , Carcinoma cell line Caki-2, and Prostate cancer cell line PC-3 [82, 83] . In these models, AAV therapy shows potential in slowing cancer progression by targeting cancer cells directly, promoting cell death, and enhancing immune responses against the tumour. This ability to address both cell proliferation and immune activation provides a comprehensive strategy that underscores AAV's versatility and effectiveness as a gene therapy option for multiple cancer types, paving the way for more personalized and targeted cancer treatments.

Advantages and Disadvantages of AAV

AAV offers the advantage of infecting various cell types and maintaining long-term gene expression, providing sustained therapeutic benefits [86]. Its ability to deliver therapeutic genes for prolonged periods is particularly valuable for chronic conditions and cancers, reducing the need for repeated treatments and minimizing patient exposure to viral vectors [82, 87]. The advantage of AAV's ability to deliver a gene that continues to function and produce necessary proteins for an extended period lies in its potential for sustained therapeutic effects [86]. However, a major disadvantage of AAV is its limited gene-carrying capacity of approximately 4.8 kilobases (kb), restricting its ability to deliver larger genes [86]. Many therapeutic genes exceed this size limit, necessitating alternative strategies like splitting genes across multiple vectors or using other viral vectors with larger capacities [84], [88]. These methods can be more complex, reduce delivery efficiency, and sometimes lead to lower therapeutic outcomes, making AAV's size constraint a significant challenge in gene therapy for larger genes [89].

Clinical Trials and Success Rates of AAV

Based on research conducted by Naoto Sato et al. [90], AAV has been shown to inhibit tumor cells in cervical cancer. The experiment was conducted using five-year-old SCID mice. These mice were inoculated with cervical cancer cells subcutaneously, meaning the cancer cells were injected into the mice. Then, AAV containing a vector was injected into the area surrounding the tumor cells. Tumor size changes were recorded after the AAV injection, indicating that AAV could reduce tumor cell size and inhibit cancer cell growth by inducing apoptosis. The use of AAV in this study did not produce any effects, as indicated by the absence of weight loss or abnormalities at the site injected with AAV [90]. The absence of side effects is due to the proven safety of gene therapy using AAV [82]. One reason for this is that AAV is non-toxic when injected into the body, as it does not attack healthy cells and minimizes the risk of side effects or damage to normal body tissues [91].

However, in a study by Ya Feng Lv et al. [91], which tested the effectiveness of AAV using mice by subcutaneously introducing breast cancer tumor cells and then injecting AAV into tumor-bearing mice, the results showed that AAV could infect breast cancer cells and inhibit tumor growth by delivering apoptotic genes. This research indicated that AAV might pose a risk of liver cell damage, potentially caused by the "suicide gene" it carries [92]

Limitation

While this literature review provides a comprehensive comparison of viral vectors and highlights Adenovirus (AdV) as a promising candidate for gene therapy, certain limitations are inherent to the nature of the review. Differences in study designs, target cell types, and research objectives across the sources may influence the consistency of the comparisons. Additionally, the availability of data may be influenced by publication trends, with studies showing positive outcomes for AdV being more frequently reported. Although every effort was made to include relevant and recent literature, some studies may have been unintentionally missed due to language or access limitations. These considerations do not diminish the value of the findings but rather emphasize

the need for continued research and systematic approaches in future evaluations.

In conclusion, the findings of this review suggest that Adenovirus (AdV) remains the most promising viral vector for gene therapy, particularly due to its high transduction efficiency and capacity for carrying larger genes. This is consistent with previous evidence highlighting AdV's clinical progression to phase III trials. However, the continued emergence of novel vector engineering techniques and increasing interest in alternative vectors, such as Lentivirus and AAV with enhanced tropism or immune evasion properties, indicate that the field is rapidly evolving. Future research should focus on optimizing vector safety, improving tumor specificity, and evaluating long-term outcomes in diverse patient populations to fully realize the clinical potential of virotherapy.

Author Contribution Statement

FNZ, AAK, AOP, and DBD contributed to the literature investigation and critical analysis of the reviewed studies. NHS, PAAD, RN, and MA supervised the project and provided academic input throughout the manuscript development. FNZ and AAK were primarily responsible for drafting and revising the manuscript. All authors reviewed and approved the final version of the manuscript for submission..

Acknowledgements

This article was supported by the 2025 PNBP Grant. The work was conducted as part of an undergraduate thesis project and has been approved by the respective academic institution. We thank all to our gratitude to our academic supervisor, Dr. Muh. Ade Artasasta, S.Si., from the Biotechnology Study Program, Department of Applied Sciences, Faculty of Mathematics and Natural Sciences, Universitas Negeri Malang, and to the authors for their contributions.

Data Availability

This article is a review and does not include any original research data. All data and sources referenced in the manuscript are publicly available and properly cited.

Registered Research Database

This research does not fall into the category of clinical trials, guideline preparation, or meta-analysis that require registration in special registration datasets such as ClinicalTrials.gov or PROSPERO. Therefore, this study is not registered in any registration database. However, all research has been conducted based on scientific methodology guidelines from related research by related academic institutions.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

References

1. Bayat Mokhtari R, Homayouni TS, Baluch N, Morgatskaya E, Kumar S, Das B, et al. Combination therapy in combating cancer. Oncotarget. 2017;8(23):38022-43. https://doi.org/10.18632/oncotarget.16723.
2. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer chemotherapy and beyond: Current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2023;10(4):1367-401. https://doi.org/10.1016/j.gendis.2022.02.007.
3. Liu Y-Q, Wang X-L, He D-H, Cheng Y-X. Protection against chemotherapy- and radiotherapy-induced side effects: A review based on the mechanisms and therapeutic opportunities of phytochemicals. Phytomedicine. 2021;80:153402. https://doi.org/10.1016/j.phymed.2020.153402.
4. Katta B, Vijayakumar C, Dutta S, Dubashi B, Nelamangala Ramakrishnaiah VP. The incidence and severity of patient-reported side effects of chemotherapy in routine clinical care: A prospective observational study. Cureus. 2023;15(4):e38301. https://doi.org/10.7759/cureus.38301.
5. Wang K, Tepper JE. Radiation therapy-associated toxicity: Etiology, management, and prevention. CA Cancer J Clin. 2021;71(5):437-54. https://doi.org/10.3322/caac.21689.
6. Montaño M, Bravo Estupinan DM, Méndez-Guerrero O, Alarcón-Hernández E, Ibáñez Hernández M. Strategies for targeting gene therapy in cancer cells with tumor-specific promoters. Front Oncol. 2020;10. https://doi.org/10.3389/fonc.2020.605380.
7. Zu H, Gao D. Non-viral vectors in gene therapy: Recent development, challenges, and prospects. Aaps J. 2021;23(4):78. https://doi.org/10.1208/s12248-021-00608-7.
8. Mohd Abas MD, Mohd Asri MF, Yusafawi NAS, Rosman NAZ, Baharudin NAZ, Taher M, et al. Advancements of gene therapy in cancer treatment: A comprehensive review. Pathol Res Pract. 2024;261:155509. https://doi.org/10.1016/j.prp.2024.155509.
9. Zafar A, Khan M, Abu J, Naeem A. Revolutionizing cancer care strategies: Immunotherapy, gene therapy, and molecular targeted therapy. Mol Biol Rep. 2024;51. https://doi.org/10.1007/s11033-023-09096-8.
10. Martin-Liberal J, Ochoa de Olza M, Hierro C, Gros A, Rodon J, Tabernero J. The expanding role of immunotherapy. Cancer Treat Rev. 2017;54:74-86. https://doi.org/10.1016/j.ctrv.2017.01.008.
11. Finer M, Glorioso J. A brief account of viral vectors and their promise for gene therapy. Gene Ther. 2017;24(1):1-2. https://doi.org/10.1038/gt.2016.71.
12. Zhao Z, Anselmo A, Mitragotri S. Viral vector-based gene therapies in the clinic. Bioeng Transl Med. 2021;7. https://doi.org/10.1002/btm2.10258.
13. Bin Umair M, Nao Akusa F, Kashif H, Fatima S, Butt F, Azhar M, et al. Viruses as tools in gene therapy, vaccine development, and cancer treatment. Arch Virol. 2022;167:1387-404. https://doi.org/10.1007/s00705-022-05432-8.
14. Gallinaro A, Borghi M, Bona R, Grasso F, Calzoletti L, Palladino L, et al. Integrase defective lentiviral vector as a vaccine platform for delivering influenza antigens. Front Immunol. 2018;9:171. https://doi.org/10.3389/fimmu.2018.00171.
15. Lahey HG, Webber CJ, Golebiowski D, Izzo CM, Horn E, Taghian T, et al. Pronounced therapeutic benefit of a single bidirectional aav vector administered systemically in sandhoff mice. Mol Ther. 2020;28(10):2150-60. https://doi.org/10.1016/j.ymthe.2020.06.021.

16. 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 Dis. 2017;4(2):43-63. https://doi.org/10.1016/j.gendis.2017.04.001.
17. Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. Viral vector platforms within the gene therapy landscape. Signal Transduct Target Ther. 2021;6(1):53. https://doi.org/10.1038/s41392-021-00487-6.
18. Scheller EL, Krebsbach PH. Gene therapy: Design and prospects for craniofacial regeneration. J Dent Res. 2009;88(7):585-96. https://doi.org/10.1177/0022034509337480.
19. Pathak S. Gene therapy – principles and applications. Glob J Transfus Med. 2022;7:3. https://doi.org/10.4103/gjtm.gjtm_87_21.
20. Gonçalves GAR, Paiva RMA. Gene therapy: Advances, challenges and perspectives. Einstein (Sao Paulo). 2017;15(3):369-75. https://doi.org/10.1590/s1679-45082017rb4024.
21. Singh V, Khan N, Jayandharan GR. Vector engineering, strategies and targets in cancer gene therapy. Cancer Gene Ther. 2022;29(5):402-17. https://doi.org/10.1038/s41417-021-00331-7.
22. Senapati S, Mahanta AK, Kumar S, Maiti P. Controlled drug delivery vehicles for cancer treatment and their performance. Signal Transduct Target Ther. 2018;3:7. https://doi.org/10.1038/s41392-017-0004-3.
23. Chen Y, Keiser M, Davidson B. Viral vectors for gene transfer. Curr Protoc Mouse Biol. 2018;8:e58. https://doi.org/10.1002/cpmo.58.
24. Seyed-Khorrami SM, Azadi A, Rastegarvand N, Habibian A, Soleimanjahi H, Łos MJ. A promising future in cancer immunotherapy: Oncolytic viruses. Eur J Pharmacol. 2023;960:176063. https://doi.org/10.1016/j.ejphar.2023.176063.
25. Vargas JE, Chicaybam L, Stein RT, Tanuri A, Delgado-Cañedo A, Bonamino MH. Retroviral vectors and transposons for stable gene therapy: Advances, current challenges and perspectives. J Transl Med. 2016;14(1):288. https://doi.org/10.1186/s12967-016-1047-x.
26. Fukuhara H, Ino Y, Todo T. Oncolytic virus therapy: A new era of cancer treatment at dawn. Cancer Sci. 2016;107(10):1373-9. https://doi.org/10.1111/cas.13027.
27. Fus-Kujawa A, Prus P, Bajdak-Rusinek K, Teper P, Gawron K, Kowalczuk A, et al. An overview of methods and tools for transfection of eukaryotic cells in vitro. Front Bioeng Biotechnol. 2021;9:701031. https://doi.org/10.3389/fbioe.2021.701031
28. Williams-Fegredo T, Davies L, Knevelman C, Miskin J, Mitrophanous K, Rafiq QA. Auto-transduction in lentiviral vector bioprocessing: A quantitative assessment and a novel inhibition strategy. Biotechnol Bioeng. 2024;121(12):3728-41. https://doi.org/10.1002/bit.28834.
29. Radek C, Bernadin O, Drechsel K, Cordes N, Pfeifer R, Sträßer P, et al. Vectofusin-1 improves transduction of primary human cells with diverse retroviral and lentiviral pseudotypes, enabling robust, automated closed-system manufacturing. Hum Gene Ther. 2019;30. https://doi.org/10.1089/hum.2019.157.
30. Chernyi N, Gavrilova D, Saruhanyan M, Oloruntimehin S, Karabelsky A, Bezsonov E, et al. Recent advances in gene therapy for hemophilia: Projecting the perspectives. Biomolecules. 2024;14. https://doi.org/10.3390/biom14070854.
31. De Haan P, Van Diemen FR, Toscano MG. Viral gene delivery vectors: The next generation medicines for immune-related diseases. Hum Vaccin Immunother. 2021;17(1):14-21. https://doi.org/10.1080/21645515.2020.1757989.
32. Curty G, Marston JL, de Mulder Rougvie M, Leal FE, Nixon DF, Soares MA. Human endogenous retrovirus k in cancer: A potential biomarker and immunotherapeutic target. Viruses. 2020;12(7):726.
33. Mougel M, Akkawi C, Chamontin C, Feuillard J, Pessel-Vivares L, Socol M, et al. Nxf1 and crm1 nuclear export pathways orchestrate nuclear export, translation and packaging of murine leukaemia retrovirus unspliced rna. RNA Biol. 2020;17(4):528-38. https://doi.org/10.1080/15476286.2020.1713539.
34. Meissner ME, Talledge N, Mansky LM. Molecular biology and diversification of human retroviruses. Front Virol. 2022;2. https://doi.org/10.3389/fviro.2022.872599.
35. Krebs AS, Mendonça LM, Zhang P. Structural analysis of retrovirus assembly and maturation. Viruses. 2021;14(1). https://doi.org/10.3390/v14010054.
36. Rezaie J, Aslan C, Ahmadi M, Zolbanin NM, Kashanchi F, Jafari R. The versatile role of exosomes in human retroviral infections: From immunopathogenesis to clinical application. Cell Biosci. 2021;11(1):19. https://doi.org/10.1186/s13578-021-00537-0.
37. Wang J, Lu X, Zhang W, Liu GH. Endogenous retroviruses in development and health. Trends Microbiol. 2024;32(4):342-54. https://doi.org/10.1016/j.tim.2023.09.006.
38. Bushman FD. Retroviral insertional mutagenesis in humans: Evidence for four genetic mechanisms promoting expansion of cell clones. Mol Ther. 2020;28(2):352-6. https://doi.org/10.1016/j.ymthe.2019.12.009.
39. Passos DO, Li M, Craigie R, Lyumkis D. Retroviral integrase: Structure, mechanism, and inhibition. Enzymes. 2021;50:249-300. https://doi.org/10.1016/bs.enz.2021.06.007.
40. Grabski DF, Ratan A, Gray LR, Bekiranov S, Rekosh D, Hammarskjold M-L, et al. Human endogenous retrovirus-k mrna expression and genomic alignment data in hepatoblastoma. Data in Brief. 2020;31:105895. https://doi.org/10.1016/j.dib.2020.105895.
41. Domazet-Lošo T. Mrna vaccines: Why is the biology of retroposition ignored? Genes. 2022;13:719. https://doi.org/10.3390/genes13050719.
42. Hanson HM, Willkomm NA, Yang H, Mansky LM. Human retrovirus genomic rna packaging. Viruses. 2022;14(5). https://doi.org/10.3390/v14051094.
43. Geis FK, Goff SP. Silencing and transcriptional regulation of endogenous retroviruses: An overview. Viruses. 2020;12(8). https://doi.org/10.3390/v12080884.
44. Chen C, Akerstrom V, Baus J, Lan MS, Breslin MB. Comparative analysis of the transduction efficiency of five adeno associated virus serotypes and vsv-g pseudotype lentiviral vector in lung cancer cells. Virol J. 2013;10:86. https://doi.org/10.1186/1743-422x-10-86.
45. Kurachi M, Kurachi J, Chen Z, Johnson J, Khan O, Bengsch B, et al. Optimized retroviral transduction of mouse t cells for in vivo assessment of gene function. Nat Protoc. 2017;12(9):1980-98. https://doi.org/10.1038/nprot.2017.083.
46. Wang JY, Wang L. Car-t cell therapy: Where are we now, and where are we heading? Blood Sci. 2023;5(4):237-48. https://doi.org/10.1097/bs9.0000000000000173.
47. Grigor EJM, Fergusson D, Kekre N, Montroy J, Atkins H, Seftel MD, et al. Risks and benefits of chimeric antigen receptor t-cell (car-t) therapy in cancer: A systematic review and meta-analysis. Transfus Med Rev. 2019;33(2):98-110. https://doi.org/10.1016/j.tmrv.2019.01.005.
48. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. Three-year follow-up of kte-x19 in patients with relapsed/refractory mantle cell lymphoma, including

high-risk subgroups, in the zuma-2 study. J Clin Oncol. 2023;41(3):555-67. https://doi.org/10.1200/jco.21.02370.
49. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large b-cell lymphoma (zuma-1): A single-arm, multicentre, phase 1-2 trial. Lancet Oncol. 2019;20(1):31-42. https://doi.org/10.1016/s1470-2045(18)30864-7.
50. Schubert ML, Schmitt M, Wang L, Ramos CA, Jordan K, Müller-Tidow C, et al. Side-effect management of chimeric antigen receptor (car) t-cell therapy. Ann Oncol. 2021;32(1):34-48. https://doi.org/10.1016/j.annonc.2020.10.478.
51. Noorbakhsh J, Vazquez F, McFarland JM. Bridging the gap between cancer cell line models and tumours using gene expression data. Br J Cancer. 2021;125(3):311-2. https://doi.org/10.1038/s41416-021-01359-0.
52. Valiullina AK, Zmievskaya EA, Ganeeva IA, Zhuravleva MN, Garanina EE, Rizvanov AA, et al. Cytotoxic effect of car-t cells against modified mcf-7 breast cancer cell line. Mol Biol Res Commun. 2023;12(4):139-48. https://doi.org/10.22099/mbrc.2023.47125.1820.
53. Berg K, Schäfer VN, Bartnicki N, Eggenschwiler R, Cantz T, Stitz J. Rapid establishment of stable retroviral packaging cells and recombinant susceptible target cell lines employing novel transposon vectors derived from sleeping beauty. Virology. 2019;531:40-7. https://doi.org/10.1016/j.virol.2019.02.014.
54. Xiao B. Tumor growth inhibition effect of hil-6 on colon cancer cells transfected with the target gene by retroviral vector. World J Gastroenterol. 2000;6:89. https://doi.org/10.3748/wjg.v6.i1.89.
55. Ricobaraza A, Gonzalez-Aparicio M, Mora-Jimenez L, Lumbreras S, Hernandez-Alcoceba R. High-capacity adenoviral vectors: Expanding the scope of gene therapy. Int J Mol Sci. 2020;21(10). https://doi.org/10.3390/ijms21103643.
56. Kubo S, Takagi-Kimura M, Kasahara N. Efficient tumor transduction and antitumor efficacy in experimental human osteosarcoma using retroviral replicating vectors. Cancer Gene Ther. 2019;26(1-2):41-7. https://doi.org/10.1038/s41417-018-0037-y.
57. Lynch JP, 3rd, Kajon AE. Adenovirus: Epidemiology, global spread of novel serotypes, and advances in treatment and prevention. Semin Respir Crit Care Med. 2016;37(4):586-602. https://doi.org/10.1055/s-0036-1584923.
58. Gallardo J, Pérez-Illana M, Martín-González N, San Martín C. Adenovirus structure: What is new? Int J Mol Sci. 2021;22(10). https://doi.org/10.3390/ijms22105240.
59. Crenshaw BJ, Jones LB, Bell CR, Kumar S, Matthews QL. Perspective on adenoviruses: Epidemiology, pathogenicity, and gene therapy. Biomedicines. 2019;7(3). https://doi.org/10.3390/biomedicines7030061.
60. Kulanayake S, Tikoo SK. Adenovirus core proteins: Structure and function. Viruses. 2021;13(3). https://doi.org/10.3390/v13030388.
61. Baker A, Greenshields-Watson A, Coughlan L, Davies J, Uusi-Kerttula H, Cole D, et al. Diversity within the adenovirus fiber knob hypervariable loops influences primary receptor interactions. Nat Commun. 2019;10. https://doi.org/10.1038/s41467-019-08599-y.
62. Flatt JW, Butcher SJ. Adenovirus flow in host cell networks. Open Biol. 2019;9(2):190012. https://doi.org/doi:10.1098/rsob.190012.
63. Dai X, Wu L, Sun R, Zhou H. Atomic structures of minor proteins vi and vii in the human adenovirus. J Virol. 2017;91:JVI.00850-17. https://doi.org/10.1128/JVI.00850-17.
64. Condezo GN, San Martín C. Localization of adenovirus morphogenesis players, together with visualization of assembly intermediates and failed products, favor a model where assembly and packaging occur concurrently at the periphery of the replication center. PLoS Pathog. 2017;13(4):e1006320. https://doi.org/10.1371/journal.ppat.1006320.
65. Kuroki LM, Jin X, Dmitriev IP, Kashentseva EA, Powell MA, Mutch DG, et al. Adenovirus platform enhances transduction efficiency of human mesenchymal stem cells: An opportunity for cellular carriers of targeted trail-based tr3 biologics in ovarian cancer. PLOS ONE. 2017;12(12):e0190125. https://doi.org/10.1371/journal.pone.0190125.
66. Nagasato M, Rin Y, Yamamoto Y, Henmi M, Hiraoka N, Chiwaki F, et al. A tumor-targeting adenovirus with high gene-transduction efficiency for primary pancreatic cancer and ascites cells. Anticancer Res. 2017;37(7):3599-605. https://doi.org/10.21873/anticanres.11730.
67. Tseha ST. Role of adenoviruses in cancer therapy. Front Oncol. 2022;Volume 12 - 2022. https://doi.org/10.3389/fonc.2022.772659.
68. Kremer EJ. Pros and cons of adenovirus-based sars-cov-2 vaccines. Mol Ther. 2020;28(11):2303-4. https://doi.org/10.1016/j.ymthe.2020.10.002.
69. Syyam A, Nawaz A, Ijaz A, Sajjad U, Fazil A, Irfan S, et al. Adenovirus vector system: Construction, history and therapeutic applications. Biotechniques. 2022;73(6):297-305. https://doi.org/10.2144/btn-2022-0051.
70. Lu W, Fang Y, Meng X, Wang X, Liu W, Liu M, et al. Improving the transduction efficiency and antitumor effect of conditionally replicative adenovirus by application of 6-cyclohexyl methyl-β-d-maltoside. Molecules. 2023;28(2):528.
71. Rosewell Shaw A, Suzuki M. Recent advances in oncolytic adenovirus therapies for cancer. Curr Opin Virol. 2016;21:9-15. https://doi.org/10.1016/j.coviro.2016.06.009.
72. Salauddin M, Saha S, Hossain MG, Okuda K, Shimada M. Clinical application of adenovirus (adv): A comprehensive review. Viruses. 2024;16(7). https://doi.org/10.3390/v16071094.
73. Dong J, Kong L, Wang S, Xia M, Zhang Y, Wu J, et al. Oncolytic adenovirus encoding apolipoprotein a1 suppresses metastasis of triple-negative breast cancer in mice. J Exp Clin Cancer Res. 2024;43:102. https://doi.org/10.1186/s13046-024-03011-0.
74. Garcia-Carbonero R, Bazan-Peregrino M, Gil-Martín M, Álvarez R, Macarulla T, Riesco-Martinez MC, et al. Phase i, multicenter, open-label study of intravenous vcn-01 oncolytic adenovirus with or without nab-paclitaxel plus gemcitabine in patients with advanced solid tumors. J Immunother Cancer. 2022;10(3). https://doi.org/10.1136/jitc-2021-003255.
75. Kuz CA, McFarlin S, Qiu J. The expression and function of the small nonstructural proteins of adeno-associated viruses (aavs). Viruses. 2024;16(8). https://doi.org/10.3390/v16081215.
76. Naso MF, Tomkowicz B, Perry WL, 3rd, Strohl WR. Adeno-associated virus (aav) as a vector for gene therapy. BioDrugs. 2017;31(4):317-34. https://doi.org/10.1007/s40259-017-0234-5.
77. Wang JH, Gessler DJ, Zhan W, Gallagher TL, Gao G. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduct Target Ther. 2024;9(1):78. https://doi.org/10.1038/s41392-024-01780-w.
78. Wörner TP, Bennett A, Habka S, Snijder J, Friese O, Powers T, et al. Adeno-associated virus capsid assembly is divergent

and stochastic. Nat Commun. 2021;12(1):1642. https://doi.org/10.1038/s41467-021-21935-5.
79. Rodriguez-Estevez L, Asokan P, Borrás T. Transduction optimization of aav vectors for human gene therapy of glaucoma and their reversed cell entry characteristics. Gene Ther. 2020;27(3-4):127-42. https://doi.org/10.1038/s41434-019-0105-4.
80. Sutter SO, Lkharrazi A, Schraner EM, Michaelsen K, Meier AF, Marx J, et al. Adeno-associated virus type 2 (aav2) uncoating is a stepwise process and is linked to structural reorganization of the nucleolus. PLoS Pathog. 2022;18(7):e1010187. https://doi.org/10.1371/journal.ppat.1010187.
81. Liu B, Li Z, Huang S, Yan B, He S, Chen F, et al. Aav-containing exosomes as a novel vector for improved gene delivery to lung cancer cells. Front Cell Dev Biol. 2021;9:707607. https://doi.org/10.3389/fcell.2021.707607.
82. Santiago-Ortiz JL, Schaffer DV. Adeno-associated virus (aav) vectors in cancer gene therapy. J Control Release. 2016;240:287-301. https://doi.org/https://doi.org/10.1016/j.jconrel.2016.01.001.
83. Bieńkowska A, Ducher M, Orzechowska M, Słyk Ż, Ciepiela O, Jaworowski J, et al. Increased temperature-related adeno-associated virus vectors transduction of ovarian cancer cells - essential signatures of aav receptor and heat shock proteins. Exp Ther Med. 2019;18(6):4718-32. https://doi.org/10.3892/etm.2019.8112.
84. Liu D, Li T, Liu L, Che X, Li X, Liu C, et al. Adeno-associated virus therapies: Pioneering solutions for human genetic diseases. Cytokine Growth Factor Rev. 2024;80:109-20. https://doi.org/https://doi.org/10.1016/j.cytogfr.2024.09.003.
85. Mulcrone PL, Herzog RW, Xiao W. Adding recombinant aavs to the cancer therapeutics mix. Mol Ther Oncolytics. 2022;27:73-88. https://doi.org/10.1016/j.omto.2022.09.009.
86. Rapti K, Grimm D. Adeno-associated viruses (aav) and host immunity – a race between the hare and the hedgehog. Front Immunol. 2021;Volume 12 - 2021. https://doi.org/10.3389/fimmu.2021.753467.
87. Baryakova TH, Pogostin BH, Langer R, McHugh KJ. Overcoming barriers to patient adherence: The case for developing innovative drug delivery systems. Nat Rev Drug Discov. 2023;22(5):387-409. https://doi.org/10.1038/s41573-023-00670-0.
88. Au HKE, Isalan M, Mielcarek M. Gene therapy advances: A meta-analysis of aav usage in clinical settings. Front Med (Lausanne). 2021;8:809118. https://doi.org/10.3389/fmed.2021.809118.
89. Hudry E, Vandenberghe LH. Therapeutic aav gene transfer to the nervous system: A clinical reality. Neuron. 2019;101(5):839-62. https://doi.org/https://doi.org/10.1016/j.neuron.2019.02.017.
90. Sato N, Saga Y, Uchibori R, Tsukahara T, Urabe M, Kume A, et al. Eradication of cervical cancer in vivo by an aav vector that encodes shrna targeting human papillomavirus type 16 e6/e7. Int J Oncol. 2018;52(3):687-96. https://doi.org/10.3892/ijo.2018.4245.
91. Lv Y-f, Zhang H, Cui Z, Ma C-j, Li Y-l, Lu H, et al. Gene delivery to breast cancer by incorporated epcam targeted darpins into aav2. BMC Cancer. 2023;23. https://doi.org/10.1186/s12885-023-11705-5.
92. Ertl HCJ. Immunogenicity and toxicity of aav gene therapy. Front Immunol. 2022;13:975803. https://doi.org/10.3389/fimmu.2022.975803.
93. Imtiaz S, Ferdous UT, Nizela A, Hasan A, Shakoor A, Zia AW, et al. Mechanistic study of cancer drug delivery: Current techniques, limitations, and future prospects. Eur J Med Chem. 2025;290:117535. https://doi.org/https://doi.org/10.1016/j.ejmech.2025.117535.
94. Yudaeva A, Kostyusheva A, Kachanov A, Brezgin S, Ponomareva N, Parodi A, et al. Clinical and translational landscape of viral gene therapies. Cells. 2024;13(22). https://doi.org/10.3390/cells13221916.
95. Chameettachal A, Mustafa F, Rizvi TA. Understanding retroviral life cycle and its genomic rna packaging. J Mol Biol. 2023;435(3):167924. https://doi.org/10.1016/j.jmb.2022.167924
96. Singh S, Kumar R, Agrawal B. Adenoviral vector-based vaccines and gene therapies: Current status and future prospects. 2018. p. 1-38.
97. Lopez-Gordo E, Chamberlain K, Riyad JM, Kohlbrenner E, Weber T. Natural adeno-associated virus serotypes and engineered adeno-associated virus capsid variants: Tropism differences and mechanistic insights. Viruses. 2024;16(3). https://doi.org/10.3390/v16030442.
98. Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019;18(5):358-78. https://doi.org/10.1038/s41573-019-0012-9.
99. Schambach A, Morgan M. Retroviral vectors for cancer gene therapy. 2016. p. 17-35.
100. Asamoah G. Hiv/aids as a developmental problem in cameroon: Issues, impacts & way forward. 2019.
101. Budzik KM, Nace RA, Ikeda Y, Russell SJ. Oncolytic foamy virus - generation and properties of a nonpathogenic replicating retroviral vector system that targets chronically proliferating cancer cells. J Virol. 2021;95(10). https://doi.org/10.1128/jvi.00015-21.
102. Wörner TP, Snijder J, Friese O, Powers T, Heck AJR. Assessment of genome packaging in aavs using orbitrap-based charge-detection mass spectrometry. Mol Ther Methods Clin Dev. 2022;24:40-7. https://doi.org/10.1016/j.omtm.2021.11.013.
103. Berry GE, Asokan A. Cellular transduction mechanisms of adeno-associated viral vectors. Curr Opin Virol. 2016;21:54-60. https://doi.org/10.1016/j.coviro.2016.08.001.