Chapter

Viral Vectors in Gene Therapy and Clinical Applications

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Abstract

Developments in gene therapy, coupled with advances in genome sequencing and a greater understanding of DNA sequences, have given rise to an exciting area of research. The use of viral vectors in gene therapy has become a very promising and fast-emerging technology over the past few decades. Despite previous setbacks, the approval of viral vector therapies worldwide, with many in late-stage clinical trials has led to a significant increase in research in this area of gene therapy. Retroviral, adenoviral, adeno-associated viral, and lentiviral vectors are all key vectors currently being researched and used in clinical trials. There are many challenges with the use of viral vectors that are yet to be overcome including cost of production, the immune response, and the ability to precisely regulate the expression of the transgene. However, with increased numbers of clinical trials showing efficacy, safety, and growing financial investment, the future use of viral vectors in gene therapy is increasingly promising.

Keywords: gene therapy, viral vector, clinical trials, approved therapies, vector production

1. Introduction

Gene therapy, defined as the delivery of specific genes to a target cell to treat a disorder, is a promising molecular technology that has quickly become a prominent area of research. Clinical disorders that could be treated using gene therapy include severe combined immunodeficiency (SCID), haemophilia, retinitis pigmentosa, diabetes, and various types of cancers [1–3]. With our increasing understanding of gene function and interactions, as well as the greater availability of genome sequencing, our knowledge of how DNA sequences can be used to treat or cure diseases caused by genetic dysfunction has developed greatly.

The delivery of specific genetic material into a host cell requires the use of a vector, or vehicle, for the transfer of a transgene to a specific cell type, by either viral or non-viral means. Techniques for the delivery of non-viral vectors include electroporation, lipofection, and microRNA, which are all useful gene therapy methods as they carry decreased biological risk, offer reduced immunogenicity, and cost less in both money and time to produce when compared to viral vectors [4]. However, the ability

of a non-viral vector to enter a cell by transfection is not as efficient as viral vectors, accordingly, research over past decades has been more focused on the use of viral vectors and this is the focus of this review [5].

Common viruses that have been used as vectors include adenovirus, adeno-associated virus, retrovirus, and lentivirus [6–9]. While there have been limitations associated with the use of these viruses, further research, and enhancements in their construction will likely permit their use in a clinical setting. In fact, there are currently several clinical trials using viral vectors in gene therapy for various conditions worldwide [10]. The successful use of viral vectors in late-stage clinical trials and laboratory settings has facilitated growing investment from venture-capital firms and increasing acquisitions of gene therapy start-ups from pharmaceutical companies [11]. The increasing focus on, and investment in viral vectors in gene therapy is a very promising sign for their future use.

This chapter provides a summary of different viral vectors currently being investigated for use in gene therapy. It also provides a review of the different clinical applications of these viral vectors and addresses the advantages and limitations of their use. Successes observed using these vectors and the limitations that this area is currently facing are also discussed.

2. Viral vectors

Viruses have evolved structural characteristics that allow them to efficiently enter a host cell and replicate effectively [12]. We are positioned to exploit these features to produce safe vectors for clinical use, while still maintaining the ability of a virus, carrying a transgene, to enter a host cell. This offers tremendous potential for very impactful therapies for a range of diseases. A viral vector is broadly made-up of three different components, which will vary depending on the type of virus from which it is derived [13]. These essential components include an envelope, the desired transgene (which is encapsulated by the envelope), and a regulatory cassette consisting of a group of genes that control the expression of the transgene. The incorporation of all of these components to form a vector system is outlined in **Figure 1**.

Viral vectors have been used in clinical trials over the past four decades with various levels of success. In 1999, a clinical trial participant died after receiving an adenoviral vector to treat partial ornithine transcarbamylase (OTC) deficiency. The patient suffered a systemic pro-inflammatory response, causing multiple organ system failures [14]. In another clinical trial, success was observed when a patient with X-linked severe combined immunodeficiency (SCID X1) was treated by retrovirus-mediated gene transfer to CD34 bone marrow cells [15]. However, in other patients in the trial, this treatment triggered the development of leukaemia [16]. These negative outcomes reduced both funding and confidence in gene therapy, especially adenoviral and retroviral-based vector systems. Despite this, research has continued to better understand the safety and efficacy of viral vectors to make them a viable clinical option. The viral vectors that have been most intensively researched are retroviral, adenoviral, adeno-associated, and lentiviral vectors.

2.1 Retroviral vectors

Retroviruses possess two copies of single-stranded RNA, coding for the viral proteins; group antigens (gag), DNA polymerase (pol), and the viral envelope (env).

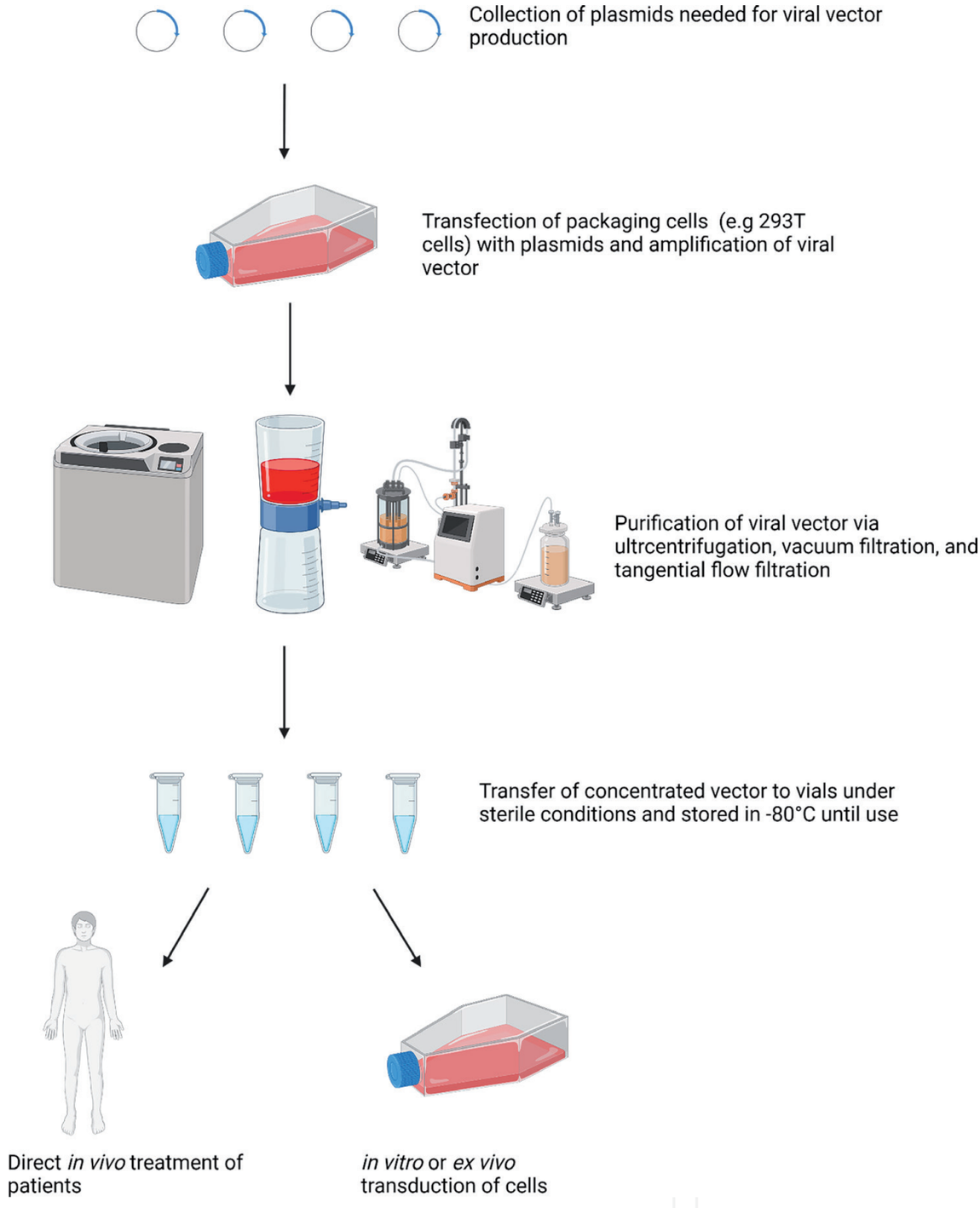

Figure 1.
Production of viral vectors for both in-vivo and in-vitro applications. Plasmid number and packaging cells may differ depending on the type of viral vector being produced. Image created with BioRender (Biorender.com).

The RNA strands are encapsulated by a glycoprotein envelope which allows this virus to enter a target cell. Once internalised, the viral genome integrates within the host DNA, forming a provirus [8]. Viral proteins are then able to be transcribed and translated, after which they exit the cell. Due to their ability to effectively enter a target cell, retroviral vectors are one of the most widely used viral vectors in gene therapy. Retroviral vectors are developed from a disabled murine virus and can only transduce dividing cells [17]. Retroviral vectors have been beneficial in gene therapy as they can integrate into the host cell genome, allowing for sustained gene expression. However, the production of viral proteins poses the risk of insertional mutagenesis occurring,

potentially leading to tumour development. This was evident in 2003 when this type of vector was used in a clinical trial for the treatment of (SCID)-X1 disease in which four participants developed leukaemia 3 years after treatment [16]. This was due to the activation of a cellular oncogene during retroviral-vector integration. This raised concerns surrounding the biosafety of this vector and caused a re-evaluation of the use of retroviral vectors in gene therapy, thereby shifting the focus to alternative viral vector systems.

2.2 Adenoviral vectors

Adenoviruses are non-enveloped, double-stranded DNA viruses which are members of the Adenoviridae family [18]. There are at least 47 human adenovirus types, which commonly cause conjunctival and respiratory diseases [6]. Human adenoviruses are ubiquitous in the environment; therefore most people will have immunity to the virus. Infection is usually only mild, but in immunosuppressed individuals, it can be severe. Unlike retroviral vectors, adenoviral vectors can transfer genes to both dividing and non-dividing cells and possess a relatively large cassette capacity (8 kB). They can also be produced in high titres and deliver genes at a high multiplicity of infection [17, 19]. Due to these properties, they have been one of the most common viral vectors used in *in-vivo* experiments and for gene therapy clinical trials. However, adenoviral vectors can elicit a strong inflammatory response due to past exposures generating immunological memory, which can significantly limit their clinical applicability [20]. Additionally, adenoviral vectors cannot integrate into the chromosome of the host, which means the expression of the transgene is episomal and therefore transient. Because of this limitation, adenoviral vectors are not commonly used for disorders that require sustained gene expression but are more frequently used to produce short-term gene expression. For example, adenoviral vectors have applications in cancer research to deliver a suicide gene to kill tumour cells [21].

2.3 Adeno-associated viral vectors

Adeno-associated viruses (AAV) are small, non-enveloped virions containing single-stranded DNA molecules. These viruses are members of the *Dependovirus* genus because they require co-infection with other viruses, and can transduce both dividing and non-dividing cells with long-term expression [22]. Adeno-associated viruses express the viral genes rep (replication), cap (capsid), and aap (assembly) viral genes, but these are removed when developing the AAV vectors, thereby, improving their safety profile [23]. The ability of AAV to enter a host cell and generate recombinant AAV molecules without the aid of viral proteins is a key component favouring their use and distinguishes them from other vector systems. The limited risk of the virus to cause disease and/or adverse events is the main reason why AAV has become an increasingly popular choice over recent decades. The site-specific nature of their integration further increases their safety profile as it limits potential oncogenic consequences. However, these vectors have a limited gene cargo capacity (4.8 kB), and many people have pre-existing antibodies against the variants of AAV, which may have an impact on gene transfer and expression levels [7]. Some serotypes of AAV are unable to reach expression levels high enough to be effective therapeutically, and this is a limitation that needs to be overcome for AAV to be utilised widely for clinical applications.

2.4 Lentiviral vectors

Lentiviruses are RNA viruses that are members of the Retroviridae family. Infection with lentiviruses can lead to many types of diseases, including neurological disorders, arthritis, and immunodeficiency. Lentiviruses have glycoproteins on their surface allowing them to gain entry into a variety of cell types [24]. Like retroviral vectors, they possess the viral genes gag, pol, and env, which allow survival and replication of the lentivirus, as well as the tat and rev genes, which enhance gene transcription and spread of the virus [25]. Being quite a virulent pathogen, fears of a replication-competent vector forming through the use of lentiviral vectors has reduced their applications in the past.

Lentiviral vectors can transduce both dividing and non-dividing cells, thereby making them an ideal choice for a range of gene delivery applications. Additionally, the lentiviral vectors do not elicit a strong immune response, therefore, these are a favourable option for clinical application. These vectors allow for long-term transgene expression as they integrate into the host genome, and insertion is less likely to occur in close proximity to proto-oncogenes, therefore, limiting the risk of insertional mutagenesis [26]. Most lentiviral vectors have been developed from the human immunodeficiency virus (HIV), which has led to some biosafety concerns.

To improve the safety profile of lentiviral vectors, the second-generation vectors have one packaging plasmid which encodes the gag, pol, rev, and tat genes, and the additional accessory virulence factors have been removed. Although the deletion of accessory factors represents a significant improvement to the original vector system, there is still a risk for the generation of a recombinant virus. To combat this, in the third-generation lentiviral vectors the packaging plasmid has been split further, with the gag and pol genes contained in one packaging plasmid, rev in another, and env in a third plasmid [27, 28]. By doing this, the chances of a recombinant virus forming are extremely low. The third-generation vectors are also self-inactivating due to deletions in the 3'LTR in the vector plasmid, thereby, preventing continuous virus replication. The use of a third-generation, self-inactivating lentiviral vector, as opposed to the second-generation vectors, significantly reduces the biosafety risk of viral replication and development of HIV through the removal of the long terminal repeat promoter [9].

3. Applications and clinical use of viral vectors

Over the past four decades, the number of clinical trials using viral vectors for gene therapy has grown significantly. Throughout this time, there have been many significant discoveries, as well as many setbacks. Despite these early obstacles, intensive research in this area has continued, and these efforts have led to the approval of many viral vector-based therapies, with many others currently undergoing late-stage clinical trials [10]. These therapies are predominately focused on treating different cancers, as well as a smaller number focused on the treatment of monogenic, cardiovascular, and infectious diseases. Over the past two decades, over 20 viral vector-based therapies have been approved, 7 of which are adenoviral, adeno-associated, and lentiviral vector-based therapies [29].

3.1 Approved viral-vector therapies

In the early 1990s, an adenoviral vector was approved for use in clinical trials, representing one of the first viral vectors to achieve such approval [30]. Since then,

some adenoviral vectors have been approved for widespread use. 'Gendicine' was the first approved viral vector technology, and was approved in 2003 by the China Food and Drug Administration (FDA) to treat patients with head and neck squamous cell carcinoma [31]. Gendicine is a recombinant adenovirus that expresses the tumour-suppressing protein, p53. As of 2020, 30,000 patients had been treated with Gendicine with significantly higher patient response rates observed when it was used in conjunction with chemotherapy, radiotherapy, and other conventional treatments. The clinical outcomes incorporating this viral system with traditional treatments were more efficacious than the use of traditional treatments alone [32]. Many cancerous tumours occur as a result of mutations to the p53 gene, therefore many clinical studies are currently in progress and the use of Gendicine is becoming increasingly widespread for the treatment of other types of cancers, including breast, liver, pancreas, and colorectal cancers [32]. Another adenoviral vector-based therapy, called Oncorine, was approved by the Chinese FDA in 2005 [33]. Oncorine is used to treat late-stage refractory nasopharyngeal cancer and has been very successful when used in conjunction with chemotherapy and radiotherapy. Due to a deletion in the E2B 55K regions, the vector can only infect and replicate in p53 deficient cells, leading to oncolysis of these cells [34].

The AAV vectors have not been intensively researched for as long as the adenoviral vectors, however, they have been extremely successful since their discovery in the 1960s [35]. There have been three AAV vector-based treatments approved, with two of them remaining on the market. Glybera is an AAV vector-based therapy, which was approved by the European Medical Agency in 2012. Glybera delivers lipoprotein lipase to patients who have lipoprotein lipase deficiency [36]. Although this treatment was able to effectively treat the disease, it was not economically viable to maintain it on the market because the incidence of this disorder is one in one million, and consequently it was discontinued in 2017 [37]. Luxturna is another AAV vector therapy that was granted approval by the FDA in the United States in 2017 [38]. It is prescribed for patients with an inherited retinal disease called Lebers congenital amaurosis, which causes progressive blindness. Luxturna is also a very expensive treatment ($425,000 per eye). However, because more people are affected by Lebers congenital amaurosis, the product has remained on the market [39]. Another AAV vector treatment that has been successful, despite being very expensive, is Zolgensma, which is used to treat patients with spinal muscular atrophy. The therapy works by delivering a motor neuron survival transgene to replace the non-functional gene in patients. It was approved in 2019 by the FDA and has seen patients improve to a point where they can walk unsupported, which had not been possible before the advent of this treatment [40].

Similar to AAV vectors, lentiviral vectors have not been researched for as long as other vector systems, but from the time of their first use in clinical trials in 2003 they have been very successful [41]. Kymriah was approved by the FDA in 2017 for the treatment of paediatric relapsed B-cell acute lymphoblastic leukaemia [42]. Kymriah was the first lentiviral vector-based gene therapy treatment and the first chimeric antigen receptor (CAR) T cell immunotherapy. This type of cancer therapy allows the genetic engineering of a patient's own T cells *ex-vivo* to enable them to recognise and eliminate CD19-positive cells. This has been an extremely successful treatment, with patients with lymphoblastic leukaemia achieving remission for a significant amount of time after treatment [43]. Yescarta is another lentiviral vector technology that uses CAR T cell immunotherapy to treat adults with relapsed B cell lymphoma [44]. Yescarta was approved by the FDA in 2017 and has been very effective in treating this disorder.

3.2 Viral vector therapies in clinical trials

There have been over 3000 approved, ongoing, or completed clinical trials involving the use of viral vectors for gene therapy in the past four decades [45]. The range of disorders being researched for treatment development has expanded with continued research success in the area of gene therapy. Clinical trials of gene therapy for many different types of cancers are currently in progress, including head and neck, lung, ovarian, breast, prostate, hepatocellular carcinoma, and melanoma. A number of monogenic diseases have also been investigated, including SCID-X1, ADA-SCID, mucopolysaccharidosis, and Fanconi anaemia, as well as infectious diseases such as HIV and most recently, COVID-19 [45]. Retroviral, adenoviral, adeno-associated, and lentiviral vectors make up over half of the 3000 clinical trials and as stated above have translated into approved therapeutic treatments that have become available on the market. Looking at the trends in the current clinical trial data, much can be deduced regarding the direction of the future of viral vector-based gene therapy (**Figure 2**).

During the 1990s, retroviral vectors were the most common viral vector used in clinical trials for several disorders, including different cancer types, monogenic diseases, and HIV (**Figure 2a**). Despite being the most popular choice of viral vector 30 years ago, the use of retroviral vectors has been steadily declining. This phenomenon is most likely attributed to its inability to be used in non-dividing cells and a significant risk of insertional oncogenesis, leading to cancerous cell formation [15]. Despite the completion of 536 clinical trials using a retroviral vector, this has not resulted in any retroviral vector-based gene therapy being currently available on the market [45]. One treatment, called Strimvelis, was on the market but has since been removed. Strimvelis was approved by the European Medicines Agency in 2016 as a treatment for ADA-SCID using a retroviral vector to deliver adenosine to a patient's cells by *ex-vivo* delivery [46]. However, the development of leukaemia in a patient in 2020 has been reported anecdotally by Orchard Therapeutics, which has since ceased treatment until the risk factors become better understood and can be mitigated. An observational clinical study is currently underway in Italy with 50 patients, which will be conducted for a minimum of 15 years [46]. In order for retroviral vectors to gain greater use in the future, much more research regarding the mechanism of insertional mutagenesis and ways to improve the safety profile is required.

Of all the viral vectors, adenoviral vectors have been most commonly used in clinical trials with 573 either approved, in progress or completed (**Figure 2b**). With two therapies currently on the market for cancer treatment and two more in late-stage clinical trials, adenoviral vector research and gene therapy approaches are demonstrating considerable success [45]. With 70% of the clinical trials being for cancer treatments, adenoviral vectors have become the most popular viral vector used in cancer gene therapy worldwide [45]. Adenoviral vectors are a popular choice for cancer treatment because of their high immunogenicity. While not beneficial in other contexts, the induction of a robust pro-inflammatory response is highly advantageous for cancer treatment [47]. However, like retroviral vectors, adenoviral vector use has declined in the past decade [10]. This may be because of their lack of translation to late-stage clinical trials, and an increase in the use of both adeno-associated and lentiviral vectors in gene therapy clinical trials.

Adeno-associated viral vectors have been used in a limited number of clinical trials, as compared to other vector systems, however, this has not limited their clinical success. The last decade has seen two AAV-vector-based therapies enter the market, as well as a

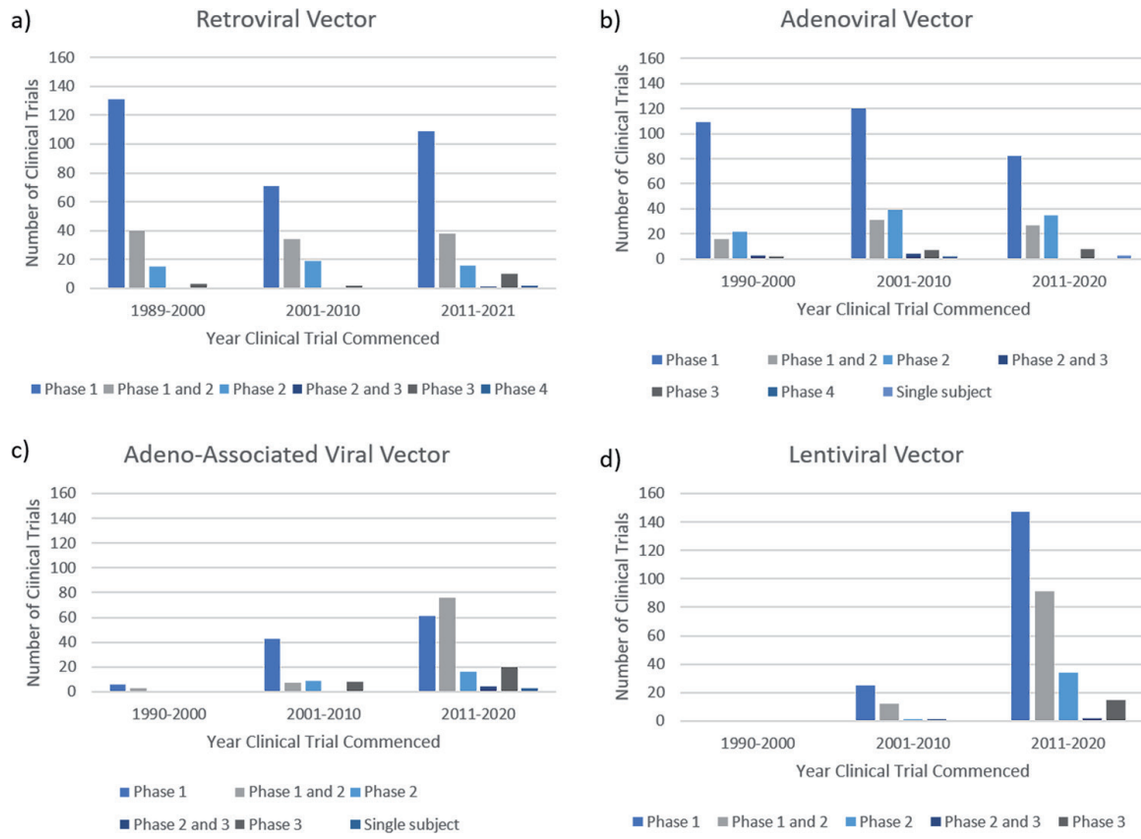

Figure 2.
In-vivo and ex-vivo clinical trials conducted from 1989 to 2021 involving retroviral, adenoviral, adeno-associated, and lentiviral vectors (a–d respectively). Data source from Wiley database on Gene Therapy Trials Worldwide. Available from: http://www.abedia.com/wiley/vectors.php.

sharp increase in phase I and phase II clinical trials (**Figure 2c**). Although many of the AAV vector phase I trials did not begin as early as the other vector trials, they now have the most phase III trials approved, ongoing, or completed as of 2021 (**Figure 2c**). AAV vectors have also shown clinical efficacy in a range of diseases, including antitrypsin deficiency, ocular diseases, and haemophilia [48–51]. Seeing the strides AAV vectors have made in only the past two decades, they appear to be a promising technology for future use. Another promising technology when reviewing clinical trial data is lentiviral vectors. Lentiviral vectors have had the greatest number of clinical trials approved, ongoing, or completed in the past decade despite having the smallest number before 2010 (**Figure 2d**). Some disorders that lentiviral vector use is primarily focused on include cancers, β-thalassemia, HIV, and Fanconi Anaemia. The benefits of using a lentiviral vector over a retroviral vector for transgene delivery is that they can transduce slow dividing or non-dividing cells and seem to have less affinity for integration into oncogenetic sites, especially the self-inactivating, third-generation lentiviral vectors [52, 53]. These lentiviral vectors with a strong promotor largely mitigate the risk of insertional mutagenesis, however, this risk is not eliminated completely. A self-inactivating lentiviral vector has been used in clinical trials for the treatment of HIV with a total of 65 patients treated with the vector and no adverse events reported for more than 8 years after vector infusion [41]. Analysing both the limited numbers of adverse events and the successful clinical trial data over the past three decades reveals that both AAV and lentiviral vectors are favourable gene therapy technologies for the future.

4. Concerns facing viral vector-based gene therapy

Despite their growing success in gene therapy clinical trials, there are still many issues that viral vector technology will need to overcome to be accepted as a widespread therapeutic option. Key areas of concern with the use of viral vectors are the induction of an immune response when delivered *in-vivo*, determining the optimal therapeutic dose required, the cost of production, and the precise regulation of transgene expression levels.

4.1 Immune response

The immunogenicity of a viral vector is measured both quantitatively by the magnitude of the immune response over time, and qualitatively by the types of immune responses that are initiated [54]. Many factors determine the immunogenicity of a vector, and it varies greatly depending on the structure of the viral vector system. It is crucial to understand the interaction of the vector with the immune system before entering clinical trials as the occurrence of a severe immune response upon injection can result in many severe complications, and, in some instances, death [55].

Adenoviral and adeno-associated viral vectors are of particular immunological concern. The prevalence of different adenovirus serotypes varies regionally. For example, serotype 5 (Ad5) has a 50% prevalence in America, but in Africa, this approximates 100% [56]. Despite such variations, adenoviruses are generally prevalent in the environment and are highly immunogenic, which can present concerns when administering vectors using the same serotype [57]. If a patient has already been exposed to the serotype used in the therapeutic vector, this is likely to cause a robust immune response characterised by a rapid influx of pre-existing neutralising antibodies to the injection site, thereby reducing the therapeutic dose and limiting the ability of the vector to exert its clinical effect, and causing safety concerns for the patient due to complement activation and resultant inflammation [55]. This is a similar situation for AAV as approximately 80% of the worldwide population has already been exposed to an AAV serotype [58]. Previous exposure to serotypes will prove to be a major hurdle to overcome in clinical trials for both adenoviral and AAV vectors. In some cases, however, a highly immunogenic vector can be beneficial for the treatment of certain disorders. Adenoviral vectors are the most common vector for cancer therapy mostly due to their highly immunogenic nature. Triggering an anti-tumour response through oncolytic adenovirus treatment has proven to show some success in treating cancerous tumours with two approved cancer treatments on the market [59].

Lentiviral vectors have a very favourable immunogenic profile, as compared to adenoviral vectors, and this is a notable reason why they have been a popular vector choice in the past decade. Lentiviral infection in humans is quite limited, and, therefore, only a small percentage of individuals will carry pre-existing antibodies to the virus. Additionally, in many lentiviral vector systems, the original viral envelope for the Vesicular Stomatitis Virus envelope glycoprotein (VSV-G) has been substituted [24]. Lentiviral vectors trigger long-lasting T-cell immunity, without causing an adverse vector-specific immunity or inflammatory reaction [60], thereby favouring clinical applications.

4.2 Cost of production

The cost to produce a viral vector is an important consideration if the end goal is the clinical application [61]. There are many costs to consider in the production of a viral vector system, including equipment, laboratory material, purification, storage, and the amount of labour needed. As exemplified by the AAV vector-based gene therapy, Glybera, if the product is too expensive to produce and the number of patients affected by the disorder is too low, it may not be economically feasible for the product to stay on the market. A major factor in the cost of production of a vector is the dosage required for one patient. For example, a low inoculation dose can offset a large production cost [62]. One way to lower the cost and time to produce a large amount of the vector is for the vector to have a high titre level. Adenoviral vectors are very efficient at gene transfer, so the titres for these vectors are very high, which is beneficial when produced on a large scale [61]. Adenoviral vectors are a very popular choice for vaccinations, and this aspect of their high titre capability is part of the reason for their popularity. Overall, the cost to produce viral vectors is a significant hurdle that will have to be overcome if they are to be used on a commercial scale. There is currently significant research dedicated to streamlining the process of vector production to lower the cost and time required for production and to allow production in low-resource areas. This discussion is beyond the scope of this review; however, it has been considered elsewhere [63–65].

4.3 Expression of the transgene

Another consideration for viral vector use is the delivery of the transgene and to what degree this process can be controlled. The ability to deliver a specific gene to a cell has proven to be a very effective therapeutic treatment, however, if this does not occur in a regulated manner, it can be detrimental to the patient, especially in cases of random integration. Transgene expression seems at times to be unpredictable, with research showing that in some instances genetic variation can influence expression [66]. Depending on the condition, the transgene will need to be expressed at different levels and potentially only in specific areas or cell types. To control the expression of transgenes and combat unpredictability, strategies such as the use of tissue-specific promoters and self-inactivation have been implemented. Tissue-specific promoters restrict the expression of the transgene to certain cell types only, thus limiting widespread expression. This is ideal when used therapeutically to target a specific cell or tissue type and avoid expression in non-target cells or tissues [67]. Furthermore, as seen in the third-generation lentiviral vectors, a self-inactivating mechanism has been incorporated. Modification in the 3′ long terminal repeat prevents continued expression after one round of integration, effectively allowing the amount of transgene expression to be controlled with the dose of the vector [28]. Despite these positive outcomes, additional research that will enable tightly regulated transgene expression is still required.

5. Conclusions

Viral vector-based gene therapy has made very encouraging strides over the past two decades, suggesting there is a positive future for this therapy in medicine. As reported by IQVIA, the first half of 2021 saw a record amount invested into

biopharmaceutical companies by venture capital firms, with cell and gene therapies attracting a significant amount of this investment [11]. The value of pharmaceutical mergers and acquisitions (M&A) in 2021 showed a stark increase from the year before many of which were viral vector and gene therapy-based deals [11, 68]. While these are promising statistics for the future of viral vector use, the concerns facing this method of gene therapy still stand and will require a considerable amount of research to overcome them. Moving forward, considering both the clinical trial data and the drawbacks of each viral vector, it seems lentiviral and adeno-associated viral vectors are the most favourable options to focus research on in the future. With limited adverse reactions and favourable immunogenic profiles, these viral vectors have the potential to be a key treatment in modern medicine.

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Conflict of interest

The authors declare no conflict of interest.

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