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REVIEW

Viruses as tools in gene therapy, vaccine development, and cancer treatment

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Abstract

Using viruses to our advantage has been a huge leap for humanity. Their ability to mediate horizontal gene transfer has made them useful tools for gene therapy, vaccine development, and cancer treatment. Adenoviruses, adeno-associated viruses, retroviruses, lentiviruses, alphaviruses, and herpesviruses are a few of the most common candidates for use as therapeutic agents or efficient gene delivery systems. Efforts are being made to improve and perfect viral-vector-based therapies to overcome potential or reported drawbacks. Some preclinical trials of viral vector vaccines have yielded positive results, indicating their potential as prophylactic or therapeutic vaccine candidates. Utilization of the oncolytic activity of viruses is the future of cancer therapy, as patients will then be free from the harmful effects of chemo- or radiotherapy. This review discusses *in vitro* and *in vivo* studies showing the brilliant therapeutic potential of viruses.

Introduction

Viruses are the most abundant biological entities, with remarkable genetic diversity, substantial resilience to environmental changes, and significant ability to mutate and survive [68]. As effective agents of horizontal gene transfer, viruses have played an important role in influencing the immune system and genetic makeup of every cellular organism and have thus driven biological evolution [13]. The genetic and morphological diversity of viruses provides a large manipulable viral library that can be utilized for tailored applications in research and medicine [30].

Viruses are currently being employed in gene therapy, oncolysis, and vaccine development. Since viruses utilize the host's cellular machinery for their replication, they have evolved to overcome or evade the host's immune response

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and effectively deliver their nucleic acid to the infected cells. These features can be manipulated to allow them be used as gene delivery agents in gene therapy. Novel viral vectors that are human-friendly, non-immunogenic, and non-pathogenic and can transport large genes for stable and long-term expression are being developed to transfer therapeutic genes efficiently [41]. Self-amplifying RNA viral vectors have been shown to elicit strong humoral and cellular immunity in animal models as well [85].

In October 2015, the FDA approved the first oncolytic virus therapy, T-VEC, for the treatment of melanoma. It is a modified herpes simplex virus that acts on cancer cells and promotes their lysis [130]. Oncolytic viruses target dendritic cells, make the tumor immunogenic, and stimulate the immune response against cancerous cells [84]. They regulate the microenvironment of cancerous cells such that T cell therapies can be used to treat solid tumors [45].

With the increasing world population and constant evolution of infectious agents, there is a dire need to develop more-efficient and novel immunization methods for which vaccines can be produced on a large scale. Viral-vector-based vaccines eliminate the need for additional adjuvants and induce a strong immune response [157].

In this review, we present an account of the major classes of viruses, both DNA viruses, including adenoviruses, adeno-associated viruses (AAVs), poxviruses, and herpesviruses, and RNA viruses, including lentiviruses, retroviruses, alphaviruses, measles virus, and Newcastle disease virus, that have shown great promise for current and future medical treatments and related applications (Table 1).

DNA viruses

Adenoviruses

Adenoviruses (Ads), are the largest non-enveloped viruses (90–100 nm) and are efficient vehicles for gene delivery. They contain a linear, non-segmented, double-stranded DNA genome of 26 to 45 kbp in their icosahedral capsid [126]. Adenoviruses can deliver transgenes of more than 8 kbp, and the viral genome resides in the nucleus episomally when injected by an adenovector, as it cannot integrate into the host genome. The genome of the adenovirus is divided into early units (E1 to E4), whose genes are responsible for the expression of non-structural proteins, and late units (L1 to L5), whose genes encode the structural components of the virion [112]. The first-generation adenovirus vector generates a strong immune response and has a capacity of around 8.2 kb for the insertion of a transgene because it lacks the E1 and E3 regions. Second-generation adenovectors are made by eliminating all of the early genes. Third-generation adenovectors, which are also known as "gutless" adenovectors, high-capacity adenoviruses (HC-Ad), or helper-dependent adenoviruses (HD-Ad), retain only the ITRs and the packaging signal (Ψ), which is essential for the final assembly of the virus [74, 83]. Adenoviral vectors have been extensively studied for their ability to carry transgenes for gene therapy. An adenovector encoding hypoxia-inducible factor 1 alpha (AdHIF-1α) has been shown to have an antiapoptotic and neuroprotective effect on ischemia and reperfusion in rats and is thought to inhibit apoptosis in nerve cells [175]. A helper-dependent HDAd5/35^{++} adenovector expressing CRISPR/Cas9 was generated for potential hematopoietic stem cell (HSC) gene therapy of sickle cell disease and β-thalassemia via re-activation of fetal γ-globin expression (HDAd-globin-CRISPR). This construct caused an increase in γ-globin expression in HSCs isolated from transplanted mice after genome editing [76]. An adenovirus vector Ad-E4-122aT was used in neonatal mice with hemophilia B and found to be a promising gene delivery vector for treating this disease [52].

The current emphasis in research is to utilize adenoviral vectors for development of novel vaccines. A single intranasal dose of chimpanzee adenovirus (simian Ad36)-based SARS-CoV-2 vaccine encoding the S protein (ChAd-SARS-CoV-2-S) was shown to induce the production of neutralizing antibodies and T cell responses and to limit infection in the respiratory tract after challenge with SARS-CoV-2 [44]. Another COVID-19 vaccine made using a recombinant adenovirus type 5 (Ad5) vector expressing the S protein of SARS-CoV-2 was shown to be safe and immunogenic 28 days post-vaccination in a phase 1 trial [178] A single shot of a replication-defective human Ad5 encoding the S protein of SARS-CoV-2 (Ad5-nCoV) protected mice and ferrets completely against SARS-CoV-2 infection, suggesting that mucosal vaccination may provide sufficient protection, and this can be investigated further in human clinical trials [168]. A single dose of replication-competent, highly attenuated Ad26 vector expressing mosaic HIV-1 Env (rcAd26.MOS1.HIV-Env, "rcAd26") was found to be poorly immunogenic, suggesting that less-attenuated adenoviral vector HIV-1 vaccines should be used for oral administration [136]. The reactogenicity and immune response of a replication-defective recombinant chimpanzee Ad3-vectored Ebola virus vaccine (cAd3-EBO) was found to be dose-dependent in a phase 1 clinical trial [73]. A monovalent, recombinant, chimpanzee Ad3-vectored Zaire Ebola glycoprotein vaccine (ChAd3-EBO-Z) was found to be immunogenic and well tolerated in children aged 1 to 17 years in a phase 2 clinical trial [147]. Induction of humoral immunity by a replication-deficient human Ad5 vector expressing an empty foot-and-mouth disease virus (FMDV) capsid (AdtFMD) was studied, and the data revealed the short span of anti-FMDV- antibody-secreting cells (ASCs) and important performance characteristics of needle-free vaccination for FMDV [133].

Table 1 Characteristics of the commonly used viral vectors

Viral system	Genome	Genome size	Insert capacity	Enveloped	Vector genome form	Transgene expression	Duration of gene expression	Host range	Immunogenicity	Advantages	Drawbacks	Examples	References
Adenovirus	dsDNA	36 kb	8-36 kb	No	Episomal	High	Transient	Relatively broad	High	High titers, efficient transduction of most types of cells and tissues	A potent inflammatory response is mediated by the capsid	HAd5, HAd26, HAd35	[74, 80, 82, 83]
Adeno-associated virus	ssDNA	8.5 kb	<4 kb	No	Episomal (>90%), site specific integration (<10%)	Medium	Transient or stable	Relatively broad	Low	Safe delivery of transgenes, non-pathogenic, non-inflammatory	Packaging capacity is small, helper AdV is required for replication, hard to produce pure viral stock	AAV 2, 3, 5, 6, 8, 9	[74, 80, 82, 83]
Retrovirus	ssRNA	7-11 kb	8 kb	Yes	Random chromosomal integration	Medium	Stable	Restricted, dividing cells only	Low	Persistent gene transfer in dividing cells	Transduces dividing cells only, chance of induction of oncogenesis during integration	MMSV, MSCV, MMLV	[74, 80, 82–84]
Lentivirus	ssRNA	8 kb	8 kb	Yes	Chromosomal integration	High	Stable	Broad	Low	Low cytotoxicity, inducible expression, persistent gene transfer in most tissues	Integration might induce oncogenesis in some applications	HIV-1, HIV-2, EIAV	[74, 80, 82–84]

Table 1 (continued)

Viral system	Genome	Genome size	Insert capacity	Enveloped	Vector genome form	Transgene expression	Duration of gene expression	Host range	Immunogenicity	Advantages	Drawbacks	Examples	References
Herpesvirus	dsDNA	150 kb	>30 kb	Yes	Episomal	High	Transient	Broad	High	Packaging capacity is large, strong tropism for neural cells	Inflammatory, gene expression is transient in non-neural cells, no expression during latent infection	HSV, HSV-1	[74, 80, 82, 83]
Alphavirus	ssRNA	12 kb	8 kb	Yes	Cytosomal	High	Transient and extreme	Broad	Low	No integration, does not elicit anti-vector immunity, targets dendritic cells	Safety concerns regarding VEE, hard to produce	SFV, SIN, VEE, M1	[80, 82–84, 121]
Poxvirus	dsDNA	130-230 kb	>30 kb	Yes	Episomal	High	Transient	Broad	Low	Well suited as an oncolytic vector, particularly apt as attenuated recombinant vaccine, multiple sites for transgene insertion	Potentially cytotoxic, heterologous promoters difficult to use, complicated generation of recombinants	VV	[80, 82, 83, 156]
Picornavirus	ssRNA	6.7-10.1 kb	6 kb	No	Episomal	High	Stable	Broad	High	High titers, non-genotoxic effect, non-coding oncogenes and easy manipulation in cDNA	Inherent genetic instability	Coxsackievirus, enteroviruses, rhinoviruses	[82–84, 94]

Table 1 (continued)

Viral system	Genome	Genome size	Insert capacity	Enveloped	Vector genome form	Transgene expression	Duration of gene expression	Host range	Immuno-genicity	Advantages	Drawbacks	Examples	References
Newcastle disease virus	ssRNA	15.2 kb	6 kb	Yes	Episomal	High	Stable	Restricted	High	Simple genome, easily programmable, improved oncolytic vector, replication in cytoplasm	Restricted to the avian respiratory tract, sensitive to interferon	Lasota strain, R2B	[9, 40, 82–84]
Measles virus	ssRNA	16 kb	6 kb	Yes	Cytosomal	High	Transient	Relatively broad	High	Oncolytic stain, self-amplifying RNA replicon, persistent immunity, infects dendritic cells, macrophages, no integration, genetic stability	Pre-existing immunity in individuals	MV-Edm	[82–84, 103, 121]

Compared to the humoral responses induced by an inactivated influenza vaccine, the humoral responses induced by an adenovirus-vectored vaccine against the conserved stalk domain mediated cross-protection against heterosubtypic influenza viruses [63]. The efficacy of ChAdOx1, a replication-deficient simian adenovirus vaccine vector, for Middle East respiratory syndrome coronavirus (MERS-CoV), was shown to be highly immunogenic and to confer protection against lethal viral challenge [102].

Virotherapy using oncolytic adenoviruses is an effective anticancer strategy. Adenoviruses are excellent vectors in terms of manipulability and tolerance of transgenes. The potency and duration of anti-tumor activity of chimeric antigen receptor (CAR) T cells was increased using an adenovirus producing a cytokine, a checkpoint blockade, and a bispecific tumor-targeted T cell engager (BiTE) molecule, and this also ensured the dual targeting of two tumor antigens and significantly improved tumor control and survival [115]. Tumor growth was significantly inhibited by infection with a photoactivatable oncolytic adenovirus (paOAd), followed by blue light irradiation *in vitro* and *in vivo*. In addition, paOAd also showed a therapeutic effect on cancer stem cells [42]. Expression of p14 FAST from adenovirus can induce widespread syncytium formation, reduce the tumor growth rate, and improve vector efficacy for cancer treatment [28]. An oncolytic adenovirus, Delta-24-RGDOX, expressing the immune co-stimulator OX40 ligand (OX40L) showed tumor-specific activation of lymphocytes and proliferation of $CD8^+$ T cells specific for tumor-associated antigens, resulting in immunity [57].

Adeno-associated virus

Adeno-associated virus, a member of the family *Parvoviridae* is a non-enveloped ssDNA virus. The small size and nonpathogenic nature of AAV make it an ideal candidate for gene transfer, with an insert capacity of ~4.8 kilobases [129]. In primates, twelve different serotypes of AAV have been identified based on their capsid components. Most of them are being used in gene therapy [105, 129]. The use of adeno-associated viruses as vectors is not limited only to research. They are also being successfully employed as gene transfer systems for clinical purposes. To be used as a gene expression vector, the viral genome is engineered in a way that the viral DNA between the two ITRs is excised and the gene of interest is inserted in that region [129]. In one study, a novel variant of AAV called AAV-inner ear (AAV-ie) was designed and shown to be highly efficient for transduction of cochlear supporting cells and has been successfully employed for gene therapy of cochlea in a mouse model [145]. Another study showed that AAV-ANF, a variant of the AAV9 vector that contains the atrial natriuretic factor (ANF) promoter, is an efficient gene transfer vehicle for atrial-specific gene therapy [107]. Gene therapy using a recombinant AAV vector containing human rhodopsin replacement complementary DNA along with shRNA to silence mutated rhodopsin was used to treat retinitis pigmentosa in a canine model [23]. In another study, an engineered variant of AAV with better uncoating ability was shown to be effective as a carrier system for gene therapy of dendritic cells because it stimulated a stronger humoral response and induced a better adaptive response by producing anti-capsid $CD8^+$ T cells [123]. Moreover, a study in which the AAV2/4 RPE65.RPE65 vector was used for gene therapy of Leber congenital amaurosis showed good tolerance of this vector in all nine patients tested, but the efficacy level differed among them (https://doi.org/10.1016/j.ymthe.2017.09.014). The AAV vector plays a therapeutic role in treatment of some neuromuscular disorders as well. It has been reported that a single dose of AAV9 vector containing cDNA encoding the survival motor neuron 1 (SMN1) protein provided an efficient gene replacement therapy for the treatment of spinal muscular atrophy (https://doi.org/10.1056/NEJMoa1706198). In another study, two AAV vectors were used simultaneously to deliver the CRISPR/Cas9 system for the treatment of Duchenne muscular dystrophy in a mouse model. Guide RNAs of CRISPR were inserted into scAAV, while Cas9 was inserted into ssAAV. This delivery system harnessing two AAV vectors was able to restore the expression of dystrophin and also decreased the severity of the disease (https://doi.org/10.1126/sciadv.aay6812). In hamsters that show deficiency in apolipoprotein C2, gene therapy using a recombinant AAV8 vector carrying human apolipoprotein C2 gene has been shown to significantly reduce the levels of triglycerides in the blood (https://doi.org/10.1016/j.omtm.2020.07.011).

The AAV vector is also playing a role in combating infectious diseases. Recently, it has been employed in the development of an efficient antiviral therapy against orthopoxviruses *in vitro* for successful and safe delivery of the CRISPR/Cas 9 system [131]. Interestingly, a single dose of vaccine that was developed against SARS-CoV-2 using engineered AAV with the AAVrh32.33 capsid was shown to induce a strong immune response in mice and non-human primates, producing a high titer of neutralizing antibodies along with the production of memory T cells [177]. AAV-vector-based gene transfer of monoclonal antibodies has been shown to provide complete protection against Ebola virus in a mouse model [155]. This vector has also shown efficacy by inducing a strong immune response including broadly neutralizing antibodies and non-neutralizing antibodies against the envelope protein of HIV-1 [116],van den [154]. Additionally, a single dose of AAV vector encoding an anti-SIV antibody has been shown to provide strong and long-lasting protection against simian immunodeficiency virus (SIV) in monkeys [93]. With regard to cancer treatment, AAV2-shE6E7,

which encodes an shRNA targeting the E6 and E7 proteins of the human papillomavirus (HPV), has demonstrated the potential to eliminate cervical cancer both *in vivo* and *in vitro* [127]. The use of AAVP particles, which are a hybrid of AAV and phage, for target-specific delivery of transgenes into tumor cells has also been reported [119, 143].

Recently, AAV-based gene therapy of X-linked myotubular myopathy has resulted in the death of two patients [149]. The safety of AAV vectors is still being debated, and further research is required to address their efficacy and safety concerns.

Herpesviruses

Herpes simplex virus is a double-stranded DNA virus with icosahedral symmetry [78]. It is being used for gene therapy because it has a large genome that comprises almost 152 kbp and has 50 genes. By deletion in the immediate early (IE) genes, the virus can accommodate almost 30 kb of exogenous DNA and transfer it to host cells [35]. Transduction of the HSV-1 vector H24B-FXNlac carrying a reduced version of the human genomic loci FXN into fetal rat dorsal root ganglia neurons resulted in long-term expression of the human FXN transgene, allowing treatment of Friedreich's ataxia neuropathy [158]. Injection of the HSV-based vector JΔN16 into the hippocampus of a mouse resulted in robust transgene expression, and injection of JΔN16 into different parts of the brain of a mouse also resulted in transgene expression and helped in the treatment of various neurological diseases, such as Parkinson's disease [159]. Nasal application of an HSV-based vector expressing the human proenkephalin gene (SHPE) targeted the trigeminal ganglia and gave positive results in treating post-TBI (traumatic brain injury) craniofacial neuropathic pain in a rat model [96]. A recent suicide gene therapy with HSV-TK, which activates ganciclovir (GCV) by phosphorylation and targets tumor cells, gave promising results when treating mice with non-small-cell lung cancer xenografts [56].

Intramuscular administration of a single dose of HSV1716, an oncolytic virus, showed promising results in children with relapsed/refractory non-CNS solid tumors [138]. The use of rRp450, an attenuated HSV-1 vector that is used in the treatment of medulloblastoma and atypical teratoid/rhabdoid tumors (AT/RT), was associated with improved results in orthotopic xenograft pediatric brain tumor models, and 40% and 25% of mice with the BT-12 and BT-16 cell line, respectively, of AT/RT showed an increase in survival, whereas 41.6% and 27.3% with the D283med cell line and the D425med cell line, respectively, showed a decline in tumor cells [140]. The oHSV vector KNTc-gD:GDNFΔ38 infects breast cancer cells through high expression of GFRα1 in the MCF7 flank tumor model of nude mice and showed a decrease in tumor cells [43]. Three mouse models were used to study the anti-tumor effect of G47Δ, which is a third-generation oncolytic HSV. The three models with MNK-45, MNK-74, and 44As3 tumors injected intratumorally with G47Δ showed a significant decrease in the growth of the cancer cells, and various human gastric cancers were inhibited [141].

Recombinant rhCMV with antigens from SIV induces immunity against SIV with good efficacy. This rhCMV induces a wide range of T cells that are able to recognize MHC 1 and 2 epitopes and provide protection against this particular disease [7]. The HSV-d106 recombinant vector is being used as a vaccine vector against SARS-CoV-1. The S proteins expressed by d106-SARS-CoV1S localize to the surface of infected cells and promote the fusion of 293T cells expressing ACE2, the receptor for SARS. CoV-1 cells infected with d106-SARS-CoV-1S showed production of binding and neutralizing antibodies [70]. A replication-defective HSV recombinant vector has been used for vaccine development against West Nile virus. The recombinant d106-WNV expresses the prM and E proteins, leading to the formation of extracellular VLPs. This recombinant vector also induces the production of neutralizing anti-WNV IgG antibodies in immunized mice [148]. Cells of BALB/c mice vaccinated with HSV [VP6C] amplicon vector showed the presence of the VP6 protein and specific anti-RVC antibodies against group 6 rotavirus [125].

Poxviruses

Poxviruses are large enveloped DNA viruses that replicate in the cytoplasm. The oncolytic properties of these viruses make them promising candidates for treating cancer [118]. Removing the FIL gene from the poxvirus vaccinia virus greatly enhances its oncolytic activity and safety, as has been observed in a glioblastoma cell line [113]. Its ability to spread in a systemic manner in the bloodstream helps in treating metastasis, as shown in preclinical models [71]. Myxoma virus can also kill cancer cells in humans as well as in mice [118]. A myxoma virus that expresses tumor necrosis factor, called murine LIGHT, which is preloaded in mesenchymal stem cells derived from adipose tissue, is effective for treating murine pancreatic adenocarcinoma [55]. Five antiviral and three pro-viral RNA helicases have been identified that strongly affect the efficiency of myxoma virus replication in several types of human cancer cells [117]. Poxviruses are also being used as viral vectors for melanoma-based gene therapy applications in the treatment of pancreatic, prostate, and colon cancer [49]. In cats with vaccine-associated sarcomas, poxviruses have been injected postoperatively, and a lower rate of tumor reappearance was observed [87].

Poxviruses have importance as vaccines candidates, as they encode an essential element, i.e., nuclear factor kappa

light chain enhancer. By inducing certain modifications, they can be optimized to be used as vaccines [139]. Synthetically modified vaccinia vectors co-expressing the spike and nucleocapsid proteins of SARS-CoV-2 induced a strong antigen-specific immune response in mice and were therefore used as a candidate for a multi-antigenic SARS-CoV-2 vaccine [20]. In laboratory experiments, oronasal or tropical exposure of bats and mice with a recombinant raccoon poxvirus vaccine protected them against rabies [135]. In phase 2 clinical trials, intramuscular injection with a recombinant attenuated poxvirus expressing cytomegalovirus antigens is being used to prevent CMV in patients who received transplants [3]. A modified vaccinia virus Ankara expressing the viral glycoprotein and the VP40 protein to form VLPs showed immunogenicity against Ebola virus [72].

Recombinant poxviruses to be used as vectors were developed by targeted insertion inactivation of the viral thymidine kinase gene and insertion of a heterologous gene in the thymidine kinase locus of the genome [62]. Modified vaccinia viruses have been shown to be immunogenic against cancer and several infectious diseases and are thus potentially effective vector systems. In clinical trials, promising results have been obtained against Ebola virus and influenza virus infections [86]. Modified vaccinia virus Ankara has been applied to the mucosal surface of the respiratory tract to provide defensive immunity in the lungs to combat SARS-CoV-2 [37]. The safety, immunogenicity, and tolerability of a modified vaccinia virus glycoprotein expressing the MERS-CoV spike were assessed for Middle East respiratory syndrome by using it as a vaccine candidate that in healthy adults [64]. In a phase 1 clinical study, a replication-deficient modified vaccinia virus Ankara and a chimpanzee adenovirus expressing conserved antigens of influenza virus were administered to patients. The results showed these modified viruses to be safe, and in response to influenza antigens, they also boosted T cell levels [24]. In a phase 2 clinical study, a multivalent respiratory syncytial virus vaccine was developed in which a poxvirus with RSV antigens and surface proteins induced a broad immune response with no adverse effects, with antibodies remaining above baseline levels for 6 months [61].

RNA viruses

Lentiviruses

Lentiviruses (LVs) are one of the three families of retroviruses. They are associated with diseases of the human immune system which can become chronic and affect the nervous system [146]. Due to their ability to tolerate large inserts and their efficient transduction ability, lentiviruses have been employed to transfer the complete dystrophin gene in Duchenne muscular dystrophy cells, resulting in the successful restoration of dystrophin production *ex vivo* [25]. Moreover, at the clinical stage, the lentivirus equine infectious anemia virus has been shown to be a safe and effective vector for long-term expression of angiostatin and endostatin for gene therapy of neovascular age-related macular degeneration-based vision loss [17]. Autologous hematopoietic stem cells modified by γ-globin LV and reduced-intensity conditioning transplant results showed it to be a promising method for the treatment of sickle cell anemia [89]. Clinical trials on humans who received hematopoietic stem cells as well as lentiviral-based gene therapy against chronic granulomatous disease showed that they had no new disease-related infections, and some patients were able to discontinue antibiotic prophylaxis [65]. *In vitro* and *in vivo* lentivirus-based RNA silencing of PD-L1 signaling in a pancreatic cancer cell line and in SCID-hu mice with pancreatic cancer showed improved results after dendritic cell (DC) immunization. An *in vitro* investigation showed a cytotoxic T-cell-based antitumor response, while *in vivo*, lung metastasis and tumor growth in mice were inhibited and survival rates increased [164]. In one study, an integrase-defective lentiviral vector (IDLV) was engineered to express genes for the influenza virus nucleoprotein (NP) and hemagglutinin (HA), and this was used to inoculate CB6F1 mice. A single dose of multi-antigen IDLV efficiently induced production of antiviral antibodies after 24 weeks. An H1N1 subunit vaccine was used as a positive control [39]. Receptor-targeted LVs have higher gene transfer rates than VSV LVs, and it was reported by Jamali et al. that vectofusin-1 gene delivery was considerably enhanced by CD4 and CD8 LVs [54]. A lentiviral vector-based vaccine eliciting neutralizing antibodies against the spike glycoprotein of SARS-CoV-2 was developed, and marked prophylactic effects of LV-based vaccination against SARS-CoV-2 were seen in preclinical trials. The study also showed intranasal immunization to be effective against COVID-19 [69]. In another study, an HIV vaccine using lentiviral-vector-based dendritic cells expressing CD40 ligand (CD40L), soluble programmed cell death (PD-1) dimer, and the HIV-1 SL9 epitope induced antigen-specific T cell proliferation and memory differentiation in humanized mice, and upon challenge with HIV-1, the viral load was suppressed by 2 logs for 6 weeks [108]. Phagocytosis-shielded LVs with a large amount of CD47 on the virion surface showed enhanced transduction efficacy, increased hepatocyte gene transfer, and selective targeting to spleen and liver without symptoms of toxicity [97]. Gene delivery to human T cells via CD4- and CD8-targeted lentiviral vectors was enhanced with vectofusin-1 transduction enhancer. Vectofusin-1 improved gene delivery of CD4 and CD8-LV without affecting the killing potential of CAR T cells, and the selectivity of the target cell was also maintained [53]. Using a cancer immunotherapy vaccine based on an integrase-defective lentiviral vector with IDLVs delivering

ovalbumin (OVA) as a non-self antigen, tumor growth was eliminated after a single immunisation, indicating that non-self tumor-associated antigens delivered by IDLVs have a robust potential for countering the growth of various tumors [99]. A recent study also showed a 46% **??**restoration of the chloride response**??** after the cystic fibrosis transmembrane conductance regulator (CFTR)-knockout rats were treated with airway delivery of a lentiviral vector with the CFTR gene [120].

Other Retroviruses

Retroviruses are enveloped viruses containing two copies of the non-segmented ssRNA genome [84]. Retrovirus-based vectors have been shown to be a reliable vector system to deliver transgenes and are suitable for long-term gene therapy applications. [82]. A codon-optimized UNC-13D expression cassette delivered by a retrovirus into a T-cell culture derived from familial hemophagocytic lymphohistiocytosis-3 (FHL-3) in a patient restored expression of the functional Munc13-4 protein and lessened disease severity [29]. The A2UCOE.EFS and CBX3.EFS vectors of RV exhibited the highest levels of transgene expression in patient-specific pluripotent stem cell gene therapy and are therefore suitable for treatment for monogenetic disorders [47]. Toca511, a tumor-selective replicating retroviral vector that encodes cytokine deaminase, has been used in prodrug gene therapy in high-grade glioma patients and has shown promising results [46]. Severe combined immunodeficiency (SCID) caused by adenosine deaminase (ADA) deficiency due to a defect in the ADA gene has been treated with the help of gamma retrovirus gene therapy by transducing $CD34^+$ cells with a gamma retrovirus encoding the human ADA cDNA sequence [36]. Retroviruses are also highly effective for killing glioma and other cancer cells [19]. Replication of the MoMLV-VSV-G vector was shown to be cytotoxic to human rhabdomyosarcoma cells and different prostate cancer cells due to syncytium formation [58]. The retroviral vector MoMLV-10A1, which encodes an R-peptide-truncated 10A1 envelope glycoprotein, has also been shown to be effective against human rhabdomyosarcoma cells [75]. Oncolytic foamy virus (oFV), which is a non-pathogenic, complex retrovirus, has shown promising results for slowing tumor growth in intraperitoneal ovarian cancer xenografts [14]. Toca511 and Toca 5C clinical trials showed effective results in patients with recurrent high-grade glioma, especially in metastatic colorectal cancer [174]. A non-replicating baculovirus expressing a human endogenous retrovirus envelope gene has been used in the development of vaccines against MERS-CoV and SARS-CoV-2 and has been shown to provide complete protection against both viruses [21]. HIV pseudotyped ncRNA VLPs have been shown to induce strong cellular and humoral immune responses [114]. Moreover, the introduction of the host proteins into retrovirus-derived VLPs induced the production of antibodies against hepatitis C virus (HCV) in mice [134]. Intramuscular immunization of mice and pigs with Ac mPERV-C5/C6 induced strong humoral and cellular responses against porcine reproductive and respiratory syndrome virus [22].

Alphaviruses

Alphaviruses, with their positive-sense single-stranded self-amplifying RNA, a broad spectrum of hosts, and significant transgene expression levels, are attractive candidates for exploitation in gene therapy and vaccine-based applications [81, 85, 137]. The high potency of alphavirus-vector-based vaccines makes them very desirable [153]. A Venezuelan equine encephalitis virus (VEEV)-based vaccine against Zika virus (ZIKV) proved effective, and viremia was not detected in mice after challenge with ZIKV [33]. In another study, the VEE TC-83 vaccine was used as construct multivalent virus-like particle vectors (VLPVs) in which the glycoprotein genes of Lassa virus (LASV) were replaced with VEEV structural genes. The VLVP vaccine stimulated dendritic and T cells and was shown to be protective [165]. Similarly, VEEV VLVPs were used to develop vaccines against two mammalian arenaviruses, Machupo virus and Junin virus, and guinea pigs were shown to be protected against these viruses [60]. A novel vaccine, V4020, which was prepared from VEEV, made mice resistant to WT-VEEV, with a high titer of antibodies. Macaques vaccinated with V4020 also showed resistance to VEEV infection after aerosol challenge [150, 151]. VEEV TC83 was used to produce RNA replicons for a SARS-CoV-2 vaccine, and a strong antibody response was observed, with antibodies being present for at least 70 days [32]. VEEV was used as a vector in a study to make **??**self-amplifying RNA**??** (saRNA) as a vaccine strategy for SARS-CoV-2. Animals immunized with lipid-nanoparticle-encapsulated saRNA expressing the spike protein of SARS-CoV-2 showed high antibody titers, and on re-stimulation with the viral peptides, strong cellular responses were observed [95].

In another study, Indian HIV-1C env/gag/polRT genes expressed in virus-like replicon particles based on Semliki Forest virus showed significant immunogenic potential [2]. SFV-based DNA-launched self-replicating RNA replicon (DREP) Ebola vaccines elicited a cellular and humoral immune response that was specific for Ebola virus [110]. Sindbis virus was employed to develop a vaccine against influenza A virus, and the E2S1-M2e vaccine, when given via the intranasal route, induced glycoprotein-M2e-specific antibodies, and the mice were protected from lethal challenge with the virus [67].

In 2017, the SVF-VA7 strain was observed to destroy various human prostate cancer cell lines. The infectivity and killing ability of this oncolytic alphavirus were independent of the hormone response status of the cell lines, and a nonmalignant cell line showed resistance to the virus [92]. An SFV-based DREP, developed to target cancers induced by papillomaviruses with the HPV oncogenes E6 and E7 showed anti-tumor activity, and after 108 days, 85% of the mice tested were free of tumors [153]. The M1 alphavirus showed the selective killing of muscle-invasive bladder cancer cells by inducing apoptosis without affecting normal cells [50]. SFV-AM6-124T, which is resistant to IFN-1, along with anti-PD1, increased $CD8^+$ cell counts and infiltration of immune cells in GL261 gliomas was also enhanced [91].

The HPV16-derived antigens E6 and E7, encoded by replicon particles of Semliki Forest virus that were replication-incompetent, were used in the Vvax001 vaccine, whose phase I clinical trials took place in 2020. It was observed to be safe and well-tolerated, eliciting a response with $CD4^+$ and $CD8^+$ T cells against E7 and E6. The vaccine showed a positive immune response in all 12 participants in the trial [66].

Newcastle disease virus

Newcastle disease virus (NDV), also called avian avulavirus 1, is a spherical-shaped and enveloped zoonotic virus that belongs to the family *Paramyxoviridae*. Below the viral envelope, the nucleocapsid encloses the helical, non-segmented, negative-sense, single-stranded RNA genome of the virus [40]. NDV is an ideal vector for therapeutic use due to its ability to replicate in the host cytoplasm, thereby eliminating the risk of foreign gene incorporation into the host genome [9]. This virus has been used as a therapeutic agent in oncolysate vaccines, oncolytic and immunogenic vectors, signaling vectors, and gene delivery agents [128, 172].

Various studies have been conducted to improve or enhance the existing oncolytic properties of the virus [128]. An engineered vesicular stomatitis virus-NDV chimera (rVSV-NDV) efficiently and selectively initiated immunogenic apoptosis in hepatocellular carcinoma (HCC) [1]. Another study showed effective suppression of HCC upon exposure to a recombinant oncolytic NDV vector containing tumor necrosis factor-related apoptosis-inducing ligand (TRIAL) [169]. Expression of NDV-vectored p53 protein (rNDV-p53) in malignant glioma cells resulted in inhibition of growth, induction of apoptosis, and stimulation of a tumor-specific cytotoxic T-cell response against the glioma [34].

When NDV infects tumor cells, it attracts both innate and adaptive immune cells to the site, thus initiating immune responses against the cancer cell [16]. Various studies have used genetically engineered NDV to express immunostimulatory agents such as cytokines to combat and treat cancer [128]. An *in vivo* study of a recombinant NDV tumor vaccine (rNDV-TV) revealed the ability of the vaccine, mediated by natural killer cells, to induce degradation of WEHI164 fibrosarcoma [144]. In another study, macrophage inflammatory protein 3α (MIP-3α) incorporated in rNDV (rNDV-MIP-3α) significantly suppressed B16 and CT26 tumors by oncolysis and a tumor-specific immune response [51]. Immunization with an autologous recombinant NDV vaccine expressing IL-12 (rNDV-IL12) demonstrated the strong biological anti-tumor activity of IL-12 against murine melanoma *in vitro* and *in vivo* [172]. The rNDV-IL12 vaccine also showed selective cytotoxicity against human breast cancer cells in a BALB/c mouse model [98]. When tested in HT29 colon cancer cells, rNDV-IL12 induced apoptosis as well as an immune response [104]. Likewise, IL-24 incorporated into the rNDV induced apoptosis and T-cell-mediated immunity against murine melanoma [173]. In a novel approach, a combined therapeutic and preventive anti-cancer vaccine was designed to coexpress IL-7 and IL-15. Exposure of the recombinant vaccine to B16 murine melanoma degraded the tumor and stimulated the production of memory cells for prolonged protection against cancer [171].

Recombinant-NDV-vectored antibody-mediated inhibition of immunity regulators has shown great potential for treating cancer [16]. One study showed that an rNDV-vectored anti-CTLA4 single-chain variable fragment (scFv) enhanced radiotherapy-mediated myeloma oncolysis in a mouse model [161]. In another study, six different rNDV vaccines containing checkpoint inhibitor antibodies (anti-PD1 and anti-PDL1), super-antagonists (anti-CD28), and antibody-fused cytokines (anti-CD24-murine IL-12, antiPD1-mIL-12, and antiPDL1-mIL12) caused a reduction in murine myeloma, with reduced cytotoxic effects [160].

An rNDV strain expressing the immunogenic gp160 envelope protein of simian immunodeficiency virus (SIV) induced specific humoral and mucosal responses to SIV when tested in a guinea pig model [90]. Another study demonstrated the stimulation of host immunity against poliomyelitis virus by injection with rNDV expressing the poliovirus precursor capsid protein and the associated protease [162]. Similarly, induction of immunity against Ebola virus (EBOV) was also reported when an rNDV vaccine expressing EBOV glycoprotein was administered to guinea pigs [176]. Two rNDV strains expressing Japanese encephalitis virus (JEV) envelope protein and non-structural protein 1 were found to stimulate host immunity against JEV [106]. A modified NDV vector for expression of the full-length spike (S) glycoprotein of SARS-CoV-2 conferred an immune response [122]. Another group

expressed wild-type and membrane-anchored S glycoprotein using an NDV vector and reported an immune response in the mice [142].

Picornavirus

The picornaviruses are a large and important family of small, non-enveloped viruses with a single-stranded, positive-sense RNA genome. The family *Picornaviridae* has 47 genera and 110 species [12, 167]. Their small size, lack of a genotoxic effect, lack of oncogenes, and easy manipulation as cDNA makes them suitable as vaccines and vectors in cancer gene therapy [94].

Inactivated and oral live-attenuated vaccines (IPV and OPV) against poliovirus have been used successfully worldwide, but they carry a finite risk of causing a dangerous infection. Therefore, recombinant virus-like particles (VLPs) have been developed and have been found to be a safe and sustainable alternative [6]. Formalin-inactivated coxsackievirus A5 used as a vaccine showed good efficacy against multivalent hand, foot, and mouth disease HFMD in a mouse model [59]. In another study, the A6, A10, and A16 strains of coxsackieviruses (CA6, CA10, and CA16) were also found to be effective vaccines against HFMD after inactivation with formalin or β-propiolactone (BPL), with BPL-treated vaccines yielding better results [77]. Another novel vaccine candidate was developed recently by introducing codon-deoptimized viral protein 1 (CD-VP1) together with high-fidelity 3D polymerase (3D-HF) into enterovirus A71 (EV-A71). This vaccine induced an efficient immune response against neurological as well as HFDM-causing pathogens [152].

The most commonly used immunotherapeutic and oncolytic virus of the family *Picornaviridae* is coxsackievirus. The use of strain CAVATAK (CVA21) together with ipilimumab has shown promising results against advanced melanoma in patients receiving anti-PD1 blockade therapy in phase I clinical trials [27]. In phase II, the combination of CVA21 and ipilimumab was given to melanoma patients without any previous therapy, and again it provided successful treatment without any serious side effects [26]. CAVATAK with a subtherapeutic dose of mitomycin C has been shown to be effective against non-muscle-invasive bladder cancer (NMIBC), and after treatment with pembrolizumab, CAVATAK showed effective oncolytic activity against advanced melanoma [4, 132]. Recently, an infectious synthetic RNA (iRNA) based on coxsackievirus A21 (R-CVA21) exhibited an efficient oncolytic effect in an animal model [31]. A novel picornavirus plasmid was designed in which the P2A site was modified to express interleukin-12 (IL-12p70) to improve its therapeutic efficacy against tumor cells [15].

A chimera of poliovirus and rhinovirus (PVSRIPO) has been developed that was found to mediate the anti-tumor effect of CD8T cells and activated dendritic cells and thereby increase the survival rate in rodent tumor models [100]. Another picornavirus, Seneca Valley virus (SVV) 001, has the ability to target tumor cells in various cancers with minimal toxicity. In phase I clinical trials, SVV-001 in an attenuated form was given intravenously to 36 patients with small-cell lung carcinoma (SCLC), neuroendocrine tumors, or carcinoid tumors, and the results were positive at a dose of 10^7 to 10^{11} virus particles per kilogram (VP/kg). In phase II, 40 patients with ED-SCLC (extensive disease) were given 10^{11} VP/kg, and the results were the same, i.e. antiviral antibodies were produced after 14 days without any significant symptoms [88].

Measles virus

Measles virus is an enveloped, non-segmented single-stranded negative-sense RNA virus [8]. A vaccine based on a modified measles virus strain, MeV-Stealth, has been used to treat ovarian cancer in mice by causing lysis of cancer cells expressing CD46 [101]. Measles vaccines encoding interleukin 12 and interleukin 15 agonists were tested in tumor models *in vitro*. A construct expressing IL12 showed robust viral gene expression and activation of immune cells, resulting in stronger anti-tumor activity [5]. For treatment of colon cancer in a rat model, MeVac FmIL-12, a measles virus encoding IL12 fusion proteins, was found to enhance anti-tumor immunity both *in vitro* and *in vivo* [163]. PEI-GOS-PEG-FA was used for coating of measles virus to form a viral GOS complex, and in mice, this resulted in increased viral replication in the tumor mass and an enhanced antitumor response [170]. An engineered measles virus Edmonston strain expressing human sodium iodide symporter or carcinoembryonic antigen was used as oncolytic viral therapy, as it provoked oncolytic effects in human hepatocellular carcinoma cell lines [10]. A recombinant measles virus, i.e., SLAM-blind measles virus, showed antitumor activity, and it is also a promising candidate for the treatment of metastatic and nectin-4-positive triple negative breast cancer, as was demonstrated in xenografted mouse model [38]. In patients with multiple myeloma, an enhanced tumor-antigen-specific T-cell response was observed after oncolytic measles virus therapy [111].

A measles-virus-based recombinant vaccine expressing the spike protein of SARS-CoV-2 was developed and shown to induce high levels of neutralizing antibodies in animals, and it was found to be protective against SARS-CoV-2 in hamsters [79]. Mice were immunized with a measles virus vaccine containing the spike glycoprotein gene of SARS-CoV-2 at two different positions in the MeV genome. It was observed that the variant with lower protein

expression induced an increased Th1-biased antibody and T-cell response [48]. A measles virus encoding a soluble form of the E protein and the prM protein of Zika virus (ZIKV) conferred protection against ZIKV infection in an allogenic mouse pregnancy model [109]. A recombinant measles virus expressing the HPV capsid protein was used as a vaccine candidate for prevention of cervical cancer, and transgenic mice immunized with this virus exhibited a strong humoral immune response [18]. In a mouse model, a robust and multifunctional T-cell response was generated by immunization with a live attenuated bivalent vaccine derived from measles virus targeting Middle East respiratory syndrome coronavirus [11].

A one-cycle measles virus vector was developed by substituting the gene for the viral attachment protein with genes encoding four reprogramming factors (OCT4, SOX2, KLF4 and c-Myc), and this induced pluripotent stem cell reprogramming and was used as a platform for delivery of multiple genes [166]. A measles-virus-vectored vaccine has been used to prevent chikungunya fever in cynomolgus macaques. This vaccine was shown to induce neutralizing antibodies and to be well tolerated [124].

Conclusion

All of the aforementioned viruses have the potential to be applied in clinical settings for vaccine, gene therapy, and oncolytic strategies after appropriate safety testing. Despite their potential benefits, some of the viral vectors have drawbacks that have to be overcome. A large amount of research has been done to make adenoviral vectors more efficient for therapeutic use, although concerns such as a lack of specificity, the immunodominance of adenoviral antigens over the vaccine transgene antigen, and immune modulation by viral antigens still exist. Nevertheless, some approaches based on adenoviral vectors have been approved for gene therapy and oncotherapy. Similarly AAV, due to its very low immunogenicity and low risk of proliferation, is a promising viral vector that has been shown to be useful for long-term expression of genes. However, recent studies have raised safety concerns regarding AAV that need to be addressed. Herpes simplex virus, with its latent infection cycle, ability to be attenuated and capacity to carry large genes, has considerable potential as a vector, but it is a human pathogen, and when its genes are "turned off", shutdown of the transgene can also occur. Clinical and pre-clinical investigations on oncolytic poxviruses have indicated that they are extremely safe and can potentially be used to treat a variety of cancers that are currently incurable. In clinical trials, vaccines against Ebola virus and influenza virus have yielded promising results. When using poxviruses as a vector, difficulty in making recombinant constructs, the transient nature of transgene expression, and possible cytotoxicity are some of the concerns that arise. Compared to other mRNAs, the immune response produced by the self-amplifying RNA of alphaviruses is more robust. saRNA vaccines based on alphaviruses have the potential to protect against diseases such as SARS-CoV-2, and are being tested in clinical trials. The main concern regarding picornavirus-based vectors, i.e., genetic instability, has been resolved by developing genetically stable expression vectors. The use of a combination of two or more picornaviral vectors such as PVSRIPO is being considered for building engineered vectors with increased stability and anti-tumor and immunogenic properties. Measles virus is a potential oncolytic platform because of its selectivity towards tumor cells and its capacity to retain its oncolytic characteristics even when it is modified. Furthermore, because it is naturally lymphotropic, it has potential for use in the development of HIV vaccines. Similarly, Newcastle disease virus is potentially useful for cancer therapy.

Although studies have suggested that the benefits of using these viruses for vaccine development, gene therapy, and oncolysis outweigh the associated risks, more research is needed to rule out potential threats before human trials can be initiated.

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