Therapeutic Implementation of Oncolytic Viruses for Cancer Immunotherapy: Review of Challenges and Current Clinical Trials

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Abstract

The development of cancer therapeutics has evolved from general targets with radiation and chemotherapy and shifted toward treatments with a more specific mechanism of action such as small molecule kinase inhibitors, monoclonal antibodies against tumor antigens, or checkpoint inhibitors. Recently, oncolytic viruses (OVs) have come to the forefront as a viable option for cancer immunotherapy, especially for “cold” tumors, which are known to inhabit an immunologically suppressive tumor microenvironment. Desired characteristics of viruses are selected through genetic attenuation of uncontrolled virulence, and some genes are replaced with ones that enhance conditional viral replication within tumor cells. Treatment with OVs must overcome various hurdles such as premature viral suppression by the host’s immune system and the dense stromal barrier. Currently, clinical studies investigate the efficacy of OVs in conjunction with various anti-cancer therapeutics, including radiotherapy, chemotherapy, immune checkpoint inhibitors, and monoclonal antibodies. Thus, future research should explore how cancer therapeutics work synergistically with certain OVs in order to create more effective combination therapies and improve patient outcomes.

Keywords

Oncolytic virus; Cancer; vaccinia virus; Adenovirus; Herpes simplex virus; Vaccinia virus; Newcastle disease virus; Poliovirus; Tumor stroma
Introduction

Cancer continues to be one of leading causes of death and remains a major threat to human health. The World Health Organization predicts the rate of cancer incidence and mortality will continue to rise in the next 20 years [1]. The effects of traditional therapeutic modalities such as surgical resection, chemotherapy, radiation therapy, and recently developed immunotherapy are not optimal despite recent improvements. Thus, there is a critical need for novel anti-tumoral strategies.

Vaccines have become an important milestone in the development of the field of immunology and success in healthcare. The basic premise lies with inoculation of an attenuated form or noninfectious portion of the infectious organism into the body to elicit an immune response. Vaccines grant protective immunity to the body by program the immune system to recognize and target these foreign invaders. When exposed to the nonimpaired version of a virus, the immune system is then able to respond quickly and efficiently to subdue the virus, thus preventing major infection [2]. As a result, vaccine development has been critical in drastically reducing the number of deaths due to smallpox, yellow fever [3], measles, mumps, rubella, and varicella [4]. Certain viruses have been recognized for the ability to target tumor cells. These oncolytic viruses (OVs) tend to use live and infectious viruses and have become a topic of interest in the arena of cancer therapeutics because of their ability to induce selective cell death and specific anti-tumor immunity. In this review, we summarize and integrate what has been published in the literature in terms of the wide diversity of OVs, discuss the challenges in oncolytic viral therapy, and suggest how modification and implementation of OVs in conjunction with traditional cancer therapies may enhance the overall success of adjuvant treatments.

Characteristic of Oncolytic Virus

OVs are a type of cancer therapy where viruses are selected for their oncolytic capacity. Often, these viruses are attenuated through alterations in the viral genome that allow for reduced cytotoxicity toward non-cancerous cells and conditional replication in cancer cells. Alternatively, OVs may also be selected for through multiple passages in tumor tissues. Key viral genes needed for virulence are substituted with genes that encode proteins to specifically target tumor cells. Thus, this strategy prevents viral targeting of nonmalignant tissues and restricts viral replication to only within tumor cells [5,6]. Moreover, use of engineered viruses in virotherapy often comes with a question of potential insertional mutagenesis where the viral genome integrates itself into the host’s genome [7]. However, OVs undergo multiple preclinical studies which assess for efficacy and safety before application in humans as demonstrated by Duerner et al.’s (2008) study of conditionally replication-competent murine leukaemia virus [8]. Despite the low possibility of genomic integration and detrimental impact in the clinical outcome, concrete long term safety data regarding administration of these engineered viruses in humans is needed as more therapeutics are implementing these viruses in both oncology and non-oncology clinics [7].

This selective elimination of cancer cells often depends on the viral strain, cancer type, tumor microenvironment (TME), and host immune system. OVs are intended to
preferentially target cancer cells by exploiting unique extracellular surface markers on cancer cell, thus gaining entry into the cell. Commonly overexpressed surface markers in tumor cells are CD46, CD155, and integrin α2β1, which serve as receptors for measles virus, poliovirus, and echovirus respectively [9,10]. Cancer cells often have specific mutations (i.e., aberrations with in BCL-2, EGFR, PTEN, RAS, RB1, TP53, and WNT) that allow for unregulated cell proliferation. However, these mutations may also predispose the tumor cells to viral infection and subsequent cytotoxic elimination [11–13]. Nevertheless, normal cells often induce interferon (IFN) expression in response to viral infection. However, due to the inability of cancer cells to to induce type 1 IFN signaling [11], OVs are able to freely replicate within cancer cells, subsequently inducing oncolysis and release of viral progeny to continue the infection cycle. In addition, OVs may be armed to express immunostimulatory cytokines/chemokines (e.g., tumor necrotic factor (TNF), Interferon (IFN) α, and granulocyte-macrophage colony-stimulating factor (GM-CSF)), which allow the viruses to elicit a strong host immune response [3,4].

OV treatment begins with inoculation of the virus followed by viral replication which generates excessive virus-induced damage, compromising the integrity of cancer cells and results in oncolysis [11]. OV replication has also been found to promote strong anti-tumor immunity through the induction of immunogenic cell death (ICD), which releases tumor antigens (TA), damage-associated molecular patterns (DAMPs), OV-derived pathogen-associated molecular patterns (PAMPs), and inflammatory cytokines to activate and recruit both innate and adaptive immune cells [14,15]. PAMPs function to alert the immune system of the presence of pathogens [16], while DAMPs function to bring awareness to tissue trauma by binding to corresponding receptors on dendritic cells to induce T-cell activation and strongly influences the immune balance in the TME [17,18].

Furthermore, some OVs trigger the anti-tumor response without viral replication-mediated oncolysis. Binding of OVs to the tumor cell triggers activation of an antiviral immune response, where PAMPS trigger secretion of cytokine, and DAMPS to recruit immune cells to the area. Thus, this alternative pathway also promotes an anti-tumor immune response (Figure 1) [19,20]. Therefore, OVs are a feasible option to target notoriously non-immunogenic “cold” tumors, which are known to inhabit a TME that suppresses immune responses and T cell invasion by effectively stimulating both the innate and adaptive immune system. Many of these cold tumors are also non-responsive to current available immune checkpoint inhibitors and demonstrates in crucial need improvement in the efficacy of cancer immunotherapy [21,22].

Oncolytic Virus Strains

Various OVs with anti-tumor properties have been explored, including both DNA and RNA viruses (Table 1). It is important to note that not all DNA/RNA viruses are oncolytic viruses. The important factor that separates oncolytic viruses from other genetically altered viruses for treatment purposes is that OVs are able to replicate and induce cell lysis, hence their name. Genetically altered viruses such as certain adenovirus agents serve as viral vectors that simply deliver the gene(s) of interest, often tumor antigens, and are replication-defective (RD), which is a characteristic that aids in the safety of this treatment modality [23].
is commonly implemented in vaccine development and has proven to provide effective protection with no serious adverse events such as clinical infection or shedding of the virus into the surrounding environment. This has been demonstrated in RD-recombinant chimpanzee adenovirus type 3-vectored ebolavirus vaccine (cAd3-EBO) and many other studies using adenviral vectors as a vaccine platform [24].

From a biological perspective, DNA viruses demonstrate higher genome stability due to their high-fidelity DNA polymerases. Their larger genomes often allow for greater ability to incorporate larger transgene insertions without jeopardizing the capacity for viral infection and replication. Replication takes place in the nucleus. However, the large genome size impedes replication kinetics [25–27]. While DNA viruses can encode proteins that protect viral nucleic acid detection [27], these viruses are also able to elicit strong antiviral responses which can aid in anti-tumor immunity. The caveat remains that high neutralizing antibodies (nAbs) may limit viral replication, thus hindering viral spread [28]; however there have been reports that oncolytic viruses are able to replicate efficiently even in the presence of nAbs that target the backbone virus [29].

On the other hand, RNA viruses such as Newcastle disease virus (NDV), poliovirus, and reovirus have limited genomic packaging capacity but can be more immunogenic. Some viruses may encode proteases that cleave RNA virus sensors, which inhibits the antiviral response [27]. Moreover, replication of RNA viruses takes place in the cytoplasm and demonstrate rapid proliferation. Their high mutation rates introduces new genetic variation due to the low-fidelity RNA polymerase [25,27]. This allows for rapid evolution toward a beneficital oncolytic phenotype but also may cause divergence from this desired characteristic. The issue of genetic stability has also been proposed as a possible advantage for “personalized” targeted therapy, where multiple optimized virus variants can promote tumor clearance even in the presence of antiviral immunity [30]. Thus, the use of RNA viruses can be a double-edged sword, thus calling for a judicious design in the construction of OVs and study designs.

Common examples of oncolytic DNA virus include vaccinia virus (VV), adenoviruses, and herpes simplex virus (HSV). VV is a double-stranded DNA (dsDNA) virus that infects and replicates within the cytoplasm of mammalian cells [31]. There have been various vaccinia virus agents being studied. Pexa-Vec is an oncolytic VV with inactivated thymidine kinase (TK) gene that is replaced with a transgene that expresses human GM-CSF and β-galactosidase [32]. Pexa-Vec has been evaluated in the treatment of hepatocellular carcinoma (HCC) and colorectal cancer [31,33,34]. Moreover, GL-ONC1 (VV with Ruc-GFP, β-glucuronidase, and β-galactosidase transgene insertions), vvDD (VV with deletion of the vaccinia growth factor and TK genes), and TBio-6517 (VV that expresses Flt3 ligand, the cytokine IL-12, and an antibody targeting CTLA4) are under clinical investigation [35–37].

Adenovirus is a dsDNA virus. Onyx-015 (Iontucirev) was the first recombinant adenovirus to be tested in humans and features viral attenuation and conditional replication due to deletion of the E1B locus, which encodes for 55 kD E1B protein [38]. Onyx-015 has been discontinued midway through phase III trials looking at head and neck cancer in
China despite the possible success in meeting the primary endpoint based on the interim report of efficacy and safety [39]. Second-generation adenoviruses such as DNX-2401 (tasadenoturev) have demonstrated success in treating glioblastomas [40]. The 24 base pair deletion of the E1A gene prevents DNX-2401 from replicating in cells that maintain normal retinoblastoma (Rb) pathways and selectively targets cancer cells with Rb pathway abnormalities [41]. DNX-2401 has shown success in treating malignant gliomas and has been granted fast track orphan drug designation by the US Food and Drug Administration (FDA) in malignant glioma in 2014 [40]. Currently, combination with immune checkpoint inhibitors are being pursued. Additional examples of oncolytic adenoviruses under clinical investigation include enadenotucirev (chinerix Ad11p/Ad3 oncolytic adenovirus with a 25 bp deletion of E4 and 2444 bp deletion in E3ORF), LOAd703 (a serotype 5 adenovirus with serotype 35 fiber and knob and encodes trimerized membrane-bound CD40L and 4–1BBL), and ONCOS-102 (a modified sertotype 5 adenovirus with a serotype 3 knob, insertion of the GM-CSF transgene, and a 24 bp deletion of the Rb binding site of the E1A gene) [42–45].

Herpes simplex virus (HSV), specifically HSV-1 and HSV-2, is a dsDNA virus that naturally infects humans [46,47]. HSV oncolytic therapy has been applied to the treatment of melanomas, gliomas, and colorectal cancer [48,49]. HSV1716, a mutant that lacks the ICP34.5 neurovirulence gene, selectively targets and replicates in human glioblastoma cells [50]. NV1020 is a mutant HSV with deletions of a 15-kb region at the UL/S junction including the U₅₆ gene and further attenuation by a 700-bp deltion encompassing the TK gene and the U₅ 24 promotor [51,52]. Reinsertion of viral HSV-1 TK gene enables control of NV1020 infection with TK-converted produgs like acyclovir. Weekly hepatic arterial infusion of NV1020 was noted to stabilize the liver metastasis in 50% of patients with heavily treated colorectal cancer at the optimal biological dose of $1 \times 10^8$ plaque-forming unit (PFU) [49]. Other HSV-based OVs that are under clinical trials include G207 (an HSV-1 strain with deletion of the neurovirulent $\gamma$134.5 gene and insertion of $\beta$-galactosidase to inactiave U₅₉ gene), ONCR-177 (an HSV-1 agent with a mutant UL37 gene, tissue-specific miRNA attenuation, and insertion of five transgenes for IL-12, FLT3LG, CCL2, and antagonists against PD-1 and CTLA-4), OH2 (genetically modified HSV-2 which expresses GM-CSF), and RP1 (an HSV-1 agent that expresses GM-CSF) [53–57].

NDV is a single-stranded RNA (ssRNA) virus that naturally infects avian hosts (poultry) [58,59]. One of the most studied strains of NDV is MTH-68/H, which has been applied to treatment of epithelial tumors as well as high-grade glioma [48, 60, 61]. Another NDV agent, LaSota, is a lentogenic strain of lower pathogenicity. LaSota has been studied in vitro using HPV E6/E7 expressing TC-1 cells that serves as a cervical cancer model and showed that the tumor cells had suppressed growth by OV induced apoptosis [61].

Poliovirus is a ssRNA virus that naturally targets neurons, which makes this an effective vehicle for glioma-targeted oncolytic therapies. Poliovirus infection is limited to human and old-world primates due to viral binding with the poliovirus receptor Nectin-like molecule 5 (Nect-5) or CD155 in order to enter host cells [62,63]. Recombinant virus PVSRIPO is an attenuated chimera created from non-pathogenic strains of rhinovirus and type 1 poliovirus vaccine and has been studied in malignant glioma and melanoma [64,65]. The poliovirus internal ribosomal entry site (IRES) has been replace with that of rhinovirus [66].
of the poliovirus IRES attenuates neurovirulence and selects for conditional replication in tumor cells, specifically binding to CD155 which has been found to be highly upregulated in many cancer types [63,67].

Respiratory enteric orphan virus (reovirus) is a nonenveloped, double-stranded RNA (dsRNA) virus that is able to infect a wide range of mammalian hosts [68], including bats, humans, minks, and pigs [69]. Reovirus is mostly nonpathogenic in humans and has demonstrated preferential replication within cancer cells that express a constitutively activated Ras pathway. However, the virus does not affect nonmalignant cells without Ras activation [70]. Pelareorep is a shortened form of reovirus that was given an orphan drug status in 2015 by the FDA and the European Medicine Agency (EMA) for the treatment of malignant gliomas, ovarian cancer, and pancreatic cancer, which are considered as Ras-activated tumors [71]. Since then, reovirus has also been used to treat melanomas, breast cancer, and head and neck squamous cell carcinoma [72–74].

Approved Oncolytic Viruses

The OV field is continuously gaining traction as a feasible option for immunotherapy, and intensive developmental pipelines have led to the approval of four OVs throughout the world. The first registered OV was ECHO-7 (trade name Rigvir), which was approved in Latvia in 2004 [75]. ECHO-7 is a type 7, group IV, enteric cytopathogenic human orphan (ECHO) virus that has been repeatedly passaged in human tumor tissue cultures and selected for enhanced selective replication within tumor cells [75,76]. ECHO-7 was approved for local treatment of skin and subcutaneous melanoma metastases and delivered via intramuscular injections. However, it has been shown to be effective in a variety of cancer types other than melanoma, including colorectal, gastric, and small cell lung cancers [77,78]. Pumpure et al. (2020) documented the treatment of a female patient diagnosed with stage IVA primary malignant melanoma of the cervix. The patient reported no side effects or adverse reactions, and the patient had a survival of 67 months and progression-free survival (PFS) of 57 months at the time of publication [79]. However, the State Agency of Medicines of Latvia suspended marketing authorization of Rigvir in 2019 due to poor quality control [80].

In 2005, the Chinese State Food and Drug Administration approved H101 (trade name Oncorine) for treatment of head and neck cancer [81]. H101 is a type 5 recombinant human adenovirus with deletions of the gene that encodes the 55-kDa E1B protein and the E3 region gene segment. E1B works to bind and inactivate p53, thus deletion of this gene allows for proper p53 tetramer formation and cell cycle checkpoint regulation [82]. The E3 region contains seven expressed open reading frames that function to inhibit host immunity to enhance viral dissemination [83]. H101 has been tested on multiple types of solid tumors including gastric carcinoma, HCC, and lung cancer [84–86]. Zhang et al. (2021) evaluated H101 treatment with or without chemotherapy on 95 patients who were diagnosed with advanced gastric cancer. The study demonstrated that H101 combination therapy yielded a more effective response compared to single agent H101 or chemotherapy with a median overall survival (OS) of 29 months and a median PFS of 14.8 months [86].

In 2015, talimogene laherparepvex (tradename T-VEC) was approved as the first oncolytic virus by the FDA for local treatment of unresectable, cutaneous, subcutaneous, and nodal
lesions of advanced melanoma or postoperative recurrent melanoma. T-VEC is a genetically modified herpes simplex 1 virus (HSV-1), where both copies of the gene that encodes infected cell protein 34.5 (ICP34.5), a peptide that enhances the virus’ neurovirulence [87], were deleted and replaced with a gene encoding GM-CSF. GM-CSF gene substitution induces secretion of the cytokine to recruit antigen presenting cells (APC) to the TME, and promote cytotoxic T lymphocytes (CD8+ T cells) responses to tumor-associated antigens (TAA). This modification is thought to improve viral replication in tumor cells that are defective in IFN pathways [88–91]. T-VEC has mainly been implemented in the treatment of melanomas. However, there has been some clinical trials focused on lymphomas as well [92,93]. Ramirez et al. (2021) looked at intralesional T-VEC treatment of 13 patients with primary cutaneous B cell lymphomas (pCBCL). The patients reported mild side-effects such as flu-like symptoms, including chills, fever, and shivering, but no patients developed suspected HSV-associated systemic infection. T-VEC treatment demonstrated enhanced recruitment of an early innate immune response composed of activated natural killer (NK) cells and monocytes, followed by increased CD8+ T cell populations and reduced regulatory T cell (Treg) populations. Overall, T-VEC treatment was found to be effective in treating pCBCL (complete response (CR) = 46.2%, partial response (PR) = 38.4%, and progressive disease = 15.4%) [88]. A phase Ib trial investigated the T-VEC treatment in combination with Ipilimumab, a CTLA-4 inhibitor, in 19 patients with stage IIIB-IVM1c melanoma that was not suitable for surgical resection. Puzanov et al. (2016) noted that the combination treatment was safe. A few patients developed grade 3/4 adverse events, but these findings did not lead to the discontinuation of T-VEC or ipilimumab. The treatment demonstrated promising results (CR = 22%, PR = 28%, stable disease (SD) = 22%). Probability of survival at 12 months and at 18 months was 72% and 67% respectively [94]. Harrington et al. (2016) carried out a phase III OPTiM trial on the response rate of intratumoral injection of T-VEC compared to subcutaneous injection of GM-CSF in 249 patients with stage IIIB/C or IVM1a melanoma. OV treatment (Durable response rate (DRR) = 25.2%, overall response rate (ORR) = 40.5%) was determined to be more beneficial compared to GM-CSF treatment (DRR = 25.2%, ORR = 2.3%) Median OS of T-VEC versus GM-CSF treatment is 41.1 and 21.5 months respectively. Both therapeutic arms were well tolerated with patients reporting mild adverse events such as chills, fatigue, and influenza-like illness [95]. Thus, the data shows encouraging results suggesting that more in-depth research to confirm these results is warranted.

In 2021, teserpaturev (G47Δ; trade name DELTACT) was conditionally approved for malignant glioma in Japan. Teserpaturev is an HSV-1 with deletion of the both copies of the γ34.5 gene, and deletion of the α47 gene with the US11 promoter. The lacZ gene as inserted to inactivate the ICP6 gene [96]. The γ34.5 gene functions to impede host cell-induced shutdown of protein synthesis in response to viral infection. Thus, deletion of this gene allows for viral replication in cancer cells as malignant cells often lack the ability to inactivate protein synthesis [97]. Deletion of the α47 gene removes viral inhibition of host cell transporters associated with antigen presentation, leading to enhanced anti-tumor immune activation [98]. Lastly, inactivation of the ICP6 gene induces selective viral replication in actively dividing cells since ICP6 encodes the large subunit of ribonucleotide reductase that is needed for viral DNA replication [99]. Uchihashi et al. (2021) investigated
teserpaturev treatment of oral squamous cell carcinoma in a murine model. Teserpaturev was found to inhibit growth of primary lesions and prolonged the survival of athymic nude and immunocompetent mice that injected with tongue cancer cells. Injected teserpaturev was found to immediately disseminate into cervical lymph nodes to effectively suppress lymph node metastases [100].

**Challenges of Oncolytic Virus Treatment**

Implementation of OVs requires careful evaluation as there are multiple factors that must be taken into account. Different methods of inoculation have benefits and drawbacks with viral therapy. Moreover, the tumor extracellular matrix (ECM) must be accounted for as an important factor as well as the tumor stroma. Cell populations including cancer-associated fibroblasts (CAFs) and tumor-associated macrophages (TAMs) can dramatically hinder oncolytic virotherapy efficacy.

**Oncolytic Virus Administration**

OVs can be administered either through direct inoculation into the tumor bulk or systemic injection, which includes intravenous (IV) or intraarterial (IA) injections [101]. There are benefits and challenges with both methods of administration. Direct intratumoral (IT) inoculation has been the most successful, as shown with FDA approved fast tracking of T-VEC. Direct IT inoculation maximizes the concentration of virus at the site of the lesion and thus induces a strong immunological response. However, this method is limited by tumor accessibility. Deep-seated tumors or those that are located in sensitive locations restrict the applicability and feasibility of IT inoculation as such invasive procedures carry a risk of injuries and complications. Moreover, other limitations include poor intratumoral retention due to viral dissemination into the bloodstream, limited viral dispersion in tumor tissues, and adverse inflammatory responses [102].

In contrast, systemic therapy utilizes the body’s vascular system to circulate OVs throughout the body similar to the delivery of chemotherapy or other anti-cancer agents. Likewise, there are a few hypothesized disadvantages associated with indirect inoculation. The first area of concern lies with systemic toxicity, whether the dosage of OVs may result in unanticipated off-tumor tissue or organ damage. Another major concern is immune clearance or the neutralization of OVs by the B cell generated antibodies, which interferes with internalization of the virus and dramatically abates the viral titer that ultimately reaches the tumor site [101,103].

This brings up the issue with seropositivity to the backbone virus, which is especially important viruses that are highly prevalent in the community. For example, there are multiple reports confirming high prevalence of seropositivity against human adenovirus (hAdV) infections throughout the world, including the United States, Australia, Japan, and the Philippines. Ye et al. (2018) looked at the prevalence of nAbs to HAdV type 4 and type 7 in a group of volunteers from Hunan Province, China. The seropositivity rates for HAdV4 and HAdV7 nAbs were 58.4 and 63.8% respectively [104]. Thus, it can be predicted that a large portion of worldwide populations in areas with a history of HAdV infection contain high seropositivity for HAdV nAbs. The issue remains that seropositivity limits
viral replication [105]. Neutralizing antibodies would bind to the OVs and inhibit cellular receptor binding [103,106]. Thus, decision about the choice of viral strain and the mode of administration should be made with careful consideration to preexisting immune responses.

Most of the literature agrees that suppression of humoral immunity is essential for systemic administrated oncolytic virotherapy [107]. The IFN pathway, specifically IFN-α, antagonizes OVs by reducing viral replication and stymying virus-mediated apoptosis [108]. Since cancers cells often lack a type 1 IFN response, these cell are more permissive to OV infection and replication [59].

Attempts have been made to protect OVs from the innate and adaptive immune system, specifically the humoral response with the use of IFN response inhibitors to enhance viral replication and efficacy of oncolysis. However, there have been safety concerns regarding the use of IFN antagonists. Saren et al. (2017) noted that treatment of glioblastoma bearing mice with Semliki Forest virus equipped with vaccinia virus-encoded type 1 IFN decoy receptor B18R controlled tumor growth but also induced severe neurotoxicity as the virus disseminated and replicated in healthy brain tissue [109].

Another method to protect OVs involves the use of genetically engineered protective coatings composed of chemical polymers, cell-derived nanovesicles, and liposomes that serve as a more direct method of overcoming the humoral immune response [110–112]. These protective coatings reduce immune recognition of the virus, thus limiting the production of nAbs against the OVs. The addition of tumor-targeting ligands can also help the OVs hone in on the tumor. The major concern with protective coatings is the practicality of the design. Protection of OVs increase the viral titer that reaches the tumor; however, the coatings may undermines the ligand-receptor interactions between OVs and tumor cell receptors resulting in reduced internalization of OVs. Moreover, additional drawbacks include issues with high production costs and limitations with large-scale transport of OVs [107].

Another feasible method is the use of carriers, either patient-derived cellular carriers (i.e., OV-infected cells that are injected back into the patient) or engineer carriers (i.e., nanoparticles). A wide range of cell types can be used as cellular carriers: endothelial cells, mesenchymal stromal cells, T-cells, and even tumor cells. However, there are safety concerns using certain cell types. Even though the patient’s own tumor cells are attractive from an immunologic standpoint, tumor cells or transformed cells should be studied with proper safety measurements. Furthermore, mesenchymal stem cells or neuronal stem cells demonstrate tumor tropism, allowing delivery of OVs throughout the body. However, such cell types are known to evade the immune system by allowing immune escape of tumor cells. The use of biodegradable nanoparticles is also gaining traction for compact delivery of viral antigens and the wide selection of nonmetal and metal-based compositions to maximize delivery of OVs [107]. Liposomal nanoparticles have demonstrated a high degree of biocompatibility with the host’s body and can be rapidly degraded by macrophages, making them a favorable candidate as a OV carrier [113].

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Challenges with Tumor Structure

Moreover, physical barriers such as the tumor stroma may prevent chemotherapy, tumor infiltrative effector cells, and OVs from effectively approaching tumor cells [114,115]. The tumor stroma is composed of non-tumor cells and structural components of the tumor tissue. Tumor cells are able to secrete cytokines to suppress certain anti-tumor functions of immune cells, while the stromal cells construct the desmoplastic stroma barrier, which physically impedes immune infiltration [116]. The stroma encapsulates the dense ECM, CAFs, TAMs, and tumor vasculature; all of which reinforce tumor resistance against the host’s immune system [117–119].

The ECM is generated by CAFs and poses the greatest barrier as it composes most of a tumor’s mass, creates an impenetrable barrier around the tumor, and undermines immune invasion and anti-tumor drug efficacy [119]. The denseness of the ECM also creates a paucity of oxygen and nutrients, which tumor cells exploit to induce activation of metabolic stress-related signaling pathways. Activation of these signaling pathways allows tumor cells to sculpt the TME to better suit their needs. For example, vascular endothelial cells (VECs) can dedifferentiate into tumor endothelial cells (TECs), which demonstrate enhanced proliferation, augmented migration capabilities, and facilitation of angiogenesis [120,121]. Another effect is the activation of drug efflux pumps and induction of senescence, both of which enhance tumor resistance against anti-cancer agents such as chemotherapy [119]. CAFs recruit myeloid-derived suppressor cells (MDSCs) and Tregs to create an immunosuppressive environment [122]. M2 TAMs have been shown to secrete TGF-β, which stimulates secretion and cross-linking of collagen, bolstering and fortifying the ECM [114,123].

Some studies have investigated methods to target the tumor stoma. For example, OVs expressing proteases such as matrix metallopeptidases (MMP)-9 can degrade ECM components. Sette et al. (2019) demonstrated that treatment of glioblastoma multiforme (GBM) with OV-derived HSV armed with MMP-9 increased viral invasion of GBM stem-like spheroids and improved survival of tumor-bearing nude mice [124]. In addition, OVs can be equipped with tissue inhibitor metalloproteinases 1–4 (TIMPs 1–4), which regulate proteolytic activity of MMPs and prevent rearrangement of the ECM [125]. Another method is to repolarize anti-inflammatory M2 TAMs into the pro-inflammatory M1 phenotype. M2 TAMs promote tumor proliferation through immune modulation and tolerance in addition to the recruitment of Tregs [126,127]. On the other hand, M1 TAMs secrete proinflammatory cytokines (e.g., IL-6, IL-12, and TNF-α) and reactive oxygen species (ROS) in order to enhance immune recruitment and function against malignant cells [128]. Rao et al. (2020) demonstrated the use of genetically engineered cell membrane-coated magnetic nanoparticles that triggers M2-M1 TAM repolarization demonstrated inhibition of tumor proliferation, reduced metastasis, and improved survival of mice with triple-negative breast cancer [129].

Overall, the complexity of the tumor stroma and the various components work in tandem to create an immunosuppressive environment and a physical barrier against not only tumor infiltrating cells but also anti-cancer agents. OVs can influence the TME to convert the pro-tumor TME into an anti-tumor environment, but there still is room for improvement for
the strategies described above. Figure 2 shows an overview of novel approaches in OV development and more research in targeting both the tumor and the surrounding stroma.

**Current Oncolytic Virus-focused Clinical Trials**

Given the compiled efforts in the field of oncolytic virotherapy, there are multiple ongoing clinical trials that investigate OVs in a variety of cancers, including breast, gastrointestinal, skin, and pancreatic cancers. The most common OV candidates include vaccinia virus, HSV, and adenovirus. A subset of ongoing clinical trials solely focus on determining patient response to OV single agent therapy, while the vast majority of trials use a combination approach (Figure 2), often pairing OV treatment with chemotherapy, monoclonal antibodies, or radiotherapy (Table 2, data was collected from clinicaltrials.gov in May 2022). However, there is a critical need for studies on accurate biomarkers to tailor and optimize therapeutic options that combine various treatments for specific patients as disease characteristic may differ across different patients.

Table 2 summarized the growing body of research that focuses on immune checkpoint inhibition (ICI) as a means to eradicate tumor cells as a combination partner with OVs [11,48]. Several monoclonal antibodies are developed to target immune checkpoints such as cytotoxic T-lymphocyte antigen 4 (CTLA-4), programmed death protein 1 (PD-1), and programmed death protein ligand 1 (PD-L1). PD-1 is essential in maintaining exhausted T cells and blocking PD-1 after the development of exhausted T cells can boost the T cell immune effector functions, which can disrupt tumor cell immune evasion [130]. ICI has changed the landscape of cancer care since the first FDA approval for anti-CTLA antibody ipilimumab in 2011. However, the majority of the patients do not benefit from ICIs as the overall response rate remains around 20–40% in most of studied regimens so far. Thus, overcoming primary resistance to ICI by offering an opportunity to induce both novel tumor antigen specific immune responses and innate immune responses while shifting the TME toward a pro-inflammatory state is appealing.

Ribas et al. (2017) studied the effect of oncolytic virotherapy, T-VEC, and pembrolizumab, an anti-PD-1 antibody, in patients with advanced melanoma. Previous studies have demonstrated that certain patients are resistant to PD-1 blockade due to the paucity of CD8+ T cells within the tumor lesion [131,132]. The use of T-VEC and anti-PD1 blockade combination elicited a strong immune response, increasing systemic circulation of CD4+ and CD8+ T cells, upregulated levels of T cell tumor infiltration, and reduced T cell exhaustion. Common T cell inhibitory markers include increased expression of CTLA4, PD-1, TIGIT, TIM3, and LAG3 [133]. The combination treatment demonstrated a reduction in tumor size with an overall response rate of 62%, and a CR of 33% in a phase 1b study (n=21) with low toxicity [22]. The phase 2 study (n=692) which was carried out in the same setting showed an acceptable safety profile but did not meet the PFS primary endpoint 14.3 (median; range = 10.3–22.1) months where the placebo and pembrolizumab arm showed PFS of 8.5 (median; range = 5.7–13.5, hazard ratio = 0.86; CI = 0.71–1.04, p = 0.13). The OS as a dual primary endpoint strategy is to be reported [134]. Overall, this strategy showed feasibility but requires further investigation into the most efficacious and synergistic
combination regimen along with predictive biomarkers to better select the patients who will most benefit from the treatment with enhanced anti-tumor activity while minimizing unnecessary adverse events.

Conclusions and Future Directions

OVs have come to the forefront of immunotherapy, offering a wide range of viruses as a backbone which can be genetically engineered to selectively target and replicate within tumor cells, while leaving normal cells unscathed. Ultimately, cell lysis releases various factors that attract immune cells toward the tumor while viral progeny infects neighboring tumor cells to continue the oncolytic cycle. The conditional replication of OVs make them an appealing therapeutic option. However, the administration of OVs must overcome various barriers such as viral neutralization by the humoral immune response and the hostile TME, which requires further investigation. Some studies have looked into using protective coatings or cellular carriers to overcome viral neutralization and enhance delivery of OVs to the tumor site. Lastly, a review of clinical trial registries for ongoing clinical trials in oncolytic virotherapy reflects a profound interest in the involved biomedical community especially in combination approaches with conventional cancer treatments such as surgery, chemotherapy, radiation, as well as novel immune modulators. Future studies need to verify the long-term safety and efficacy of incorporating OV therapy. Additionally, further research is needed to develop a strategy that can target cancer heterogeneity while ensuring proper receptor binding for viral entry in the setting of rapidly evolving cancer cells which may need to involve precision medicine to offer a more personalized approach for patients.

In summary, oncolytic virotherapy has secured its role to support cancer immunotherapy as the fourth pillar of cancer treatment, and research will continue to expand on the utilities of OV as an important element in multimodality approaches.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer J Clinicians. 2021; 71: 209–249.
2. Pulendran B, Davis MM. The science and medicine of human immunology. Science. 2020; 369.
3. Monath TP. Yellow fever vaccine. Expert Rev Vaccines. 2005; 4: 553–74. [PubMed: 16117712]
4. Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. Ann Med. 2018; 50: 110–120. [PubMed: 29172780]
5. Raman SS, Hecht JR, Chan E. Talimogene laherparepvec: review of its mechanism of action and clinical efficacy and safety. Immunotherapy. 2019; 11: 705–723. [PubMed: 31045464]
6. Hemminki O, Dos Santos JM, Hemminki A. Oncolytic viruses for cancer immunotherapy. J Hematol Oncol. 2020; 13: 84. [PubMed: 32600470]
7. Goswami R, Subramanian G, Silayeva L, Newkirk I, Doctor D, Chawla K, et al. Gene Therapy Leaves a Vicious Cycle. Front Oncol. 2019; 9: 297. [PubMed: 31069169]
8. Duerner LJ, Schwantes A, Schneider IC, Cichutek K, Buchholz CJ. Cell entry targeting restricts biodistribution of replication-competent retroviruses to tumour tissue. Gene Ther. 2008; 15: 1500–1510. [PubMed: 18509380]
9. Anderson BD, Nakamura T, Russell SJ, Peng KW. High CD46 receptor density determines preferential killing of tumor cells by oncolytic measles virus. Cancer Res. 2004; 64: 4919–4926. [PubMed: 15256464]
10. He Y, Mueller S, Chipman PR, Bator CM, Peng X, Bowman WD, et al. Complexes of poliovirus serotypes with their common cellular receptor, CD155. J Virol. 2003; 77: 4827–4835. [PubMed: 12663789]
11. Rahman MM, McFadden G. Oncolytic Viruses: Newest Frontier for Cancer Immunotherapy. Cancers (Basel). 2021; 13.
12. Borrego-Diaz E, Mathew R, Hawkinson D, Esfandyari T, Liu Z, Lee PW, et al. Pro-oncogenic cell signaling machinery as a target for oncolytic viruses. Curr Pharm Biotechnol. 2012; 13: 1742–1749. [PubMed: 21740363]
13. Matveeva OV, Chumakov PM. Defects in interferon pathways as potential biomarkers of sensitivity to oncolytic viruses. Rev Med Virol. 2018; 28: e2008. [PubMed: 30209859]
14. De Munch J, Binks A, McNeish IA, Aerts JL. Oncolytic virus-induced cell death and immunity: a match made in heaven? J Leukoc Biol. 2017; 102: 631–643. [PubMed: 28720686]
15. Galluzzi L, Kepp O, Kroemer G. Immunogenic cell death in cancer therapy. Annu Rev Immunol. 2013; 31: 51–72. [PubMed: 23157435]
16. Tang D, Kang R, Coyne CB, Zeh HJ, Lotze MT. PAMPs and DAMPs: signal 0s that spur autophagy and immunity. Immunol Rev. 2012; 249: 158–175. [PubMed: 22889221]
17. Krysko DV, Garg AD, Kaczmarek A, Krysko O, Agostinis P, Vandenabeele P. Immunogenic cell death and DAMPs in cancer therapy. Nat Rev Cancer. 2012; 12: 860–875. [PubMed: 23151605]
18. Hua L, Wakimoto H. Oncolytic herpes simplex virus therapy for malignant glioma: current approaches to successful clinical application. Expert Opin Biol Ther. 2019; 19: 845–854. [PubMed: 31046478]
19. Davola ME, Mossman KL. Oncolytic viruses: how “lytic” must they be for therapeutic efficacy? Oncoimmunology. 2019; 8: e1581528.
20. Li Z, Jiang Z, Zhang Y, Huang X, Liu Q. Efficacy and safety of oncolytic viruses in randomized controlled trials: A systematic review and Meta-Analysis. Cancers (Basel). 2020; 12.
21. Bonaventura P, Shekarian T, Alcazer V, Valladeau-Guilemond J, Valsesia-Wittmann S, Amigorena S, et al. Cold Tumors: A Therapeutic Challenge for Immunotherapy. Front Immunol. 2019; 10: 168. [PubMed: 30800125]
22. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves Anti-PD-1 immunotherapy. Cell. 2017; 170: 1109–1119. [PubMed: 28886381]
23. Barry M Single-cycle adenovirus vectors in the current vaccine landscape. Expert Rev Vaccines. 2018; 17: 163–173. [PubMed: 29251011]
24. Ledgerwood JE, Sullivan NJ, Graham BS. Chimpanzee adenovirus vector ebola Vaccine--Preliminary report. N Engl J Med. 2015; 373: 776. [PubMed: 26287857]
25. Harrington K, Freeman DJ, Kelly B, Harper J, Soria JC. Optimizing oncolytic virotherapy in cancer treatment. Nat Rev Drug Discov. 2019; 18: 689–706. [PubMed: 31292532]
26. Lawler SE, Speranza MC, Cho CF, Chiocca EA. Oncolytic Viruses in Cancer Treatment: A Review. JAMA Oncol. 2017; 3: 841–849. [PubMed: 27441411]
27. Beachboard DC, Horner SM. Innate immune evasion strategies of DNA and RNA viruses. Curr Opin Microbiol. 2016; 32: 113–119. [PubMed: 27288760]
28. Vemula SV, Mittal SK. Production of adenovirus vectors and their use as a delivery system for influenza vaccines. Expert Opin Biol Ther. 2010; 10: 1469–87. [PubMed: 20822477]
29. Bommarmeddy PK, Patel A, Hossain S, Kaufman HL. Talimogene laherparepvec (T-VEC) and other oncolytic viruses for the treatment of melanoma. Am J Clin Dermatol 2017; 18: 1–15. [PubMed: 27988837]
30. Zainutdinov SS, Kochneva GV, Netesov SV, Chumakov PM, Matveeva OV. Directed evolution as a tool for the selection of oncolytic RNA viruses with desired phenotypes. Oncolytic Virother. 2019; 8: 9–26. [PubMed: 31372363]

31. Guo ZS, Lu B, Guo Z, Giehl E, Feist M, Dai E, et al. Vaccinia virus-mediated cancer immunotherapy: cancer vaccines and oncolytics. J Immunother Cancer. 2019; 7: 6. [PubMed: 30626434]

32. Kim JH, Oh JY, Park BH, Lee DE, Kim JS, Park HE, et al. Systemic armed oncolytic and immunologic therapy for cancer with JX-594, a targeted poxvirus expressing GM-CSF. Mol Ther. 2006; 14: 361–370. [PubMed: 16905462]

33. Park SH, Breitbach CJ, Lee J, Park JO, Lim HY, Kang WK, et al. Phase 1b Trial of Biweekly Intravenous Pexa-Vec (JX-594), an Oncolytic and Immunotherapeutic Vaccinia Virus in Colorectal Cancer. Mol Ther. 2015; 23: 1532–1540. [PubMed: 26073886]

34. B MCM, Xie C, Steinberg SM, Fioraventi S, Walker M, Mabry-Hrones D, Wood BJ, et al. A phase I/I study of Pexa-Vec oncolytic virus in combination with immune checkpoint inhibition in refractory colorectal cancer. J Clinic Oncol. 2020; 38: 117–117.

35. Lauer UM, Schell M, Beil J, Berchtold S, Koppenhöfer U, Glatzle J, et al. Phase I Study of Oncolytic Vaccinia Virus GL-ONC1 in Patients with Peritoneal Carcinomatosis. Clin Cancer Res. 2018; 24: 4388–4398. [PubMed: 29773661]

36. Downs-Canner S, Guo ZS, Ravindranathan R, Breitbach CJ, O’Malley ME, Jones HL, et al. Phase 1 Study of intravenous oncolytic poxvirus (vvDD) in patients with advanced solid cancers. Mol Ther. 2016; 24: 1492–501. [PubMed: 27203445]

37. TBio-6517.

38. Kirn D Clinical research results with dl1520 (Onyx-015), a replication-selective adenovirus for the treatment of cancer: what have we learned? Gene Ther. 2001; 8: 89–98. [PubMed: 11313778]

39. Xia ZJ, Chang JH, Zhang L, Jiang WQ, Guan ZZ, Liu JW, et al. Phase III randomized clinical trial of intratumoral injection of E1B gene-deleted adenovirus (H101) combined with cisplatin-based chemotherapy in treating squamous cell cancer of head and neck or esophagus. Ai Zheng. 2004; 23: 1666–70. [PubMed: 15601557]

40. Philbrick B, Adamson DC. DNX-2401: an investigational drug for the treatment of recurrent glioblastoma. Expert Opin Investig Drugs. 2019; 28: 1041–1049.

41. Lang FF, Conrad C, Gomez-Manzano C, Yung WKA, Sawaya R, Weinberg JS, et al. Phase I Study of DNX-2401 (Delta-24-RGD) Oncolytic Adenovirus: Replication and Immunotherapeutic Effects in Recurrent Malignant Glioma. J Clin Oncol. 2018; 36: 1419–1427. [PubMed: 29432077]

42. Chia SL, Lei J, Ferguson DJP, Dyer A, Fisher KD, Seymour LW. Group B adenovirus enadenotucirev infects polarised colorectal cancer cells efficiently from the basolateral surface expected to be encountered during intravenous delivery to treat disseminated cancer. Virology. 2017; 505: 162–171. [PubMed: 28260622]

43. Eriksson E, Milenova I, Wenthe J, Stahle M, Leja-Jarblad J, Ullenlagh G, et al. Shaping the tumor stroma and sparking immune activation by CD40 and 4–1BB signaling induced by an armed oncolytic virus. Clin Cancer Res. 2017; 23: 5846–5857. [PubMed: 28536305]

44. Wenthe J, Naseri S, Labani-Motlagh A, Emblad G, Wikström KI, Eriksson E, et al. Boosting CAR T-cell responses in lymphoma by simultaneous targeting of CD40 and 4–1BB signaling induced by an armed oncolytic virus. Clin Cancer Res. 2017; 23: 5846–5857. [PubMed: 28536305]

45. Ranki T, Pesonen S, Hemminki A, Partanen K, Kairemo K, Alanko T, et al. Phase I study with ONCOS-102 for the treatment of solid tumors - an evaluation of clinical response and exploratory analyses of immune markers. J Immunother Cancer. 2016; 4: 7.

46. Agarwalla PK, Aghi MK. Oncolytic herpes simplex virus engineering and preparation. Methods Mol Biol. 2012; 797: 1–19. [PubMed: 33666760]

47. Carpenter AB, Carpenter AM, Aiken R, Hanft S. Oncolytic virus in gliomas: a review of human clinical investigations. Ann Oncol. 2021; 32: 968–982. [PubMed: 33771666]

48. Geevergheese SK, Geller DA, de Haan HA, Hörer M, Knoll AE, Mescheder A, et al. Phase I/I study of oncolytic herpes simplex virus NV1020 in patients with extensively pretreated refractory
colorectal cancer metastatic to the liver. Hum Gene Ther. 2010; 21: 1119–1128. [PubMed: 20486770]

50. Dolan A, McKie E, MacLean AR, McGeoch DJ. Status of the ICP34.5 gene in herpes simplex virus type 1 strain 17. J Gen Virol. 1992; 73: 971–973. [PubMed: 13218822]

51. Roizman B. The function of herpes simplex virus genes: a primer for genetic engineering of novel vectors. Proceedings of the National Academy of Sciences of the United States of America. 1996; 93: 11307–11312. [PubMed: 8876131]

52. Kelly KJ, Wong J, Fong Y. Herpes simplex virus NV1020 as a novel and promising therapy for hepatic malignancy. Expert opinion on investigational drugs. 2008; 17: 1105–1113. [PubMed: 18549346]

53. Bernstock JD, Bag AK, Fiveash J, Kachurak K, Elsayed G, Chagoya G, et al. Design and rationale for First-in-Human phase 1 immunovirotherapy clinical trial of oncolytic HSV G207 to treat malignant pediatric cerebellar brain tumors. Hum Gene Ther. 2020; 31: 1132–1139. [PubMed: 32657154]

54. Haines BB, Denslow A, Grzesik P, Lee JS, Hewett J, et al. ONCR-177, an oncolytic HSV-1 designed to potentiate systemic antitumor immunity. Cancer Immunol Res. 2021; 9: 291–308. [PubMed: 33355229]

55. Zhang B, Huang J, Tang J, Hu S, Luo S, Luo Z, et al. Intratumoral OH2, an oncolytic herpes simplex virus 2, in patients with advanced solid tumors: a multicenter, phase I/II clinical trial. J Immunother Cancer. 2021; 9.

56. Wang Y, Zhou X, Wu Z, Hu H, Jin J, Hu Y, et al. Preclinical safety evaluation of oncolytic herpes simplex virus type 2. Hum Gene Ther. 2019; 30: 651–660. [PubMed: 30499341]

57. Zawit M, Swami U, Awada H, Arnouk J, Milhem M, Zakharia Y. Current status of intravesional agents in treatment of malignant melanoma. Ann Transl Med. 2021; 9: 1038. [PubMed: 34277383]

58. Tayeb S, Zakay-Rones Z, Panet A. Therapeutic potential of oncolytic Newcastle disease virus: a critical review. Oncolytic Virotther. 2015; 4: 49–62. [PubMed: 27512670]

59. Freeman AL, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther. 2006; 13: 221–228. [PubMed: 16257582]

60. Csatary LK, Moss RW, Beuth J, Torocsik B, Szederenyi J, Bakacs T. Beneficial treatment of patients with advanced cancer using a Newcastle disease virus (MTH-68/H). Anticancer Res. 1999; 19: 635–638. [PubMed: 10216468]

61. Keshavarz M, Nejad ASM, Esghaie M, Bokharaei-Salim F, Dianat-Moghadam H, Keyvani H, et al. Oncolytic Newcastle disease virus reduces growth of cervical cancer cell by inducing apoptosis. Saudi J Biol Sci. 2020; 27: 47–52. [PubMed: 3189816]

62. Goetz C, Dobrikova E, Shveygert M, Dobrikov M, Gromeier M. Oncolytic poliovirus against malignant glioma. Future Virol. 2011; 6: 1045–1058. [PubMed: 21984883]

63. Takai Y, Miyoshi J, Ikegami H, Ogita H. Neclins and nectin-like molecules: roles in contact inhibition of cell movement and proliferation. Nat Rev Mol Cell Biol. 2008; 9: 603–615. [PubMed: 18648374]

64. Desjardins A, Gromeier M, Herndon JE, Beaubier N, Bolognesi DP, Friedman AH, et al. Recurrent Glioblastoma Treated with Recombinant Poliovirus. N Engl J Med. 2018; 379: 150–161. [PubMed: 29943666]

65. Beasley GM, Nair SK, Farrow NE, Landa K, Selim MA, Wiggs CA, et al. Phase I trial of intratumoral PVSRIPO in patients with unresectable, treatment-refractory melanoma. J Immunotherapy Cancer. 2021; 9: e002203.

66. Gromeier M, Nair SK. Recombinant poliovirus for cancer immunotherapy. Annu Rev Med. 2018; 69: 289–299. [PubMed: 29414253]

67. Merrill MK, Bernhardt G, Sampson JH, Wikstrand CJ, Bignier DD, Gromeier M. Poliovirus receptor CD155-targeted oncolysis of glioma. Neuro Oncol. 2004; 6: 208–217. [PubMed: 15279713]

68. Snyder AJ, Wang J, Danthi P. Components of the reovirus capsid differentially contribute to stability. J Virol. 2019; 93.
69. Lelli D, Moreno A, Steyer A, Naglic T, Chiapponi C, Prosperi A, et al. Detection and Characterization of a Novel Reassortant Mammalian Orthoreovirus in Bats in Europe. Viruses. 2015; 7: 5844–5854. [PubMed: 26569289]

70. Maitra R, Ghalib MH, Goel S. Reovirus: a targeted therapeutic - progress and potential. Mol Cancer Res. 2012; 10: 1514–1525. [PubMed: 23038811]

71. Kicielinski KP, Chiocca EA, Yu JS, Gill GM, Coffey M, Markert JM. Phase 1 clinical trial of intratumoral reovirus infusion for the treatment of recurrent malignant gliomas in adults. Mol Ther. 2014; 22: 1056–1062. [PubMed: 24553100]

72. Thompson B Oncolytics Biotech(®) Inc.: REOLYSIN® for melanoma therapy. Melanoma Manag. 2015; 2: 105–107. [PubMed: 30190838]

73. Carew JS, Espitia CM, Zhao W, Kelly KR, Coffey M, Freeman JW, et al. Reolysin is a novel reovirus-based agent that induces endoplasmic reticular stress-mediated apoptosis in pancreatic cancer. Cell Death Dis. 2013; 4: e728. [PubMed: 23868061]

74. Jaime-Ramirez AC, Yu JG, Caserta E, Yoo JY, Zhang J, Lee TJ, et al. Reolysin and Histone Deacetylase Inhibition in the Treatment of Head and Neck Squamous Cell Carcinoma. Mol Ther Oncolytics. 2017; 5: 87–96. [PubMed: 28812060]

75. Donina S, Strele I, Proboka G, Auzins J, Alberts P, Jonsson B, et al. Adapted ECHO-7 virus Rigvir immunotherapy (oncolytic virotherapy) prolongs survival in melanoma patients after surgical excision of the tumour in a retrospective study. Melanoma Res. 2015; 25: 421–426. [PubMed: 26193376]

76. Alberts P, Tilgase A, Rasa A, Bandere K, Venskus D. The advent of oncolytic virotherapy in oncology: The Rigvir® story. Eur J Pharmacol. 2018; 837: 117–126. [PubMed: 30179611]

77. Alberts P, Olmane E, Brokane L, Krastina Z, Romanovska M, Kupcs K, et al. Long-term treatment with the oncolytic ECHO-7 virus Rigvir of a melanoma stage IV M1c patient, a small cell lung cancer stage IIIA patient, and a histiocytic sarcoma stage IV patient-three case reports. Apmis. 2016; 124: 896–904. [PubMed: 27457663]

78. Tilgase A, Olmane E, Nazarovs J, Brokane L, Erdmanis R, Rasa A, et al. Multimodality treatment of a colorectal cancer stage IV patient with FOLFOX-4, Bevacizumab, Rigvir Oncolytic Virus, and Surgery. Case Rep Gastroenterol. 2018; 12: 457–465. [PubMed: 30283278]

79. Pumpure E, Drucka E, Kigitovica D, Meskauskas R, Isajevs S, Nemoiro I, et al. Management of a primary malignant melanoma of uterine cervix stage IVA patient with radical surgery and adjuvant oncolytic virus Rigvir(®) therapy: A case report. Clin Case Rep. 2020; 8: 1538–1543. [PubMed: 32884791]

80. RIGVIR MARKETING AUTHORISATION SUSPENDED; INFORMATION FOR CURRENT PATIENTS. 2019 [cited 2022 February 28]; On 30 May 2019, the State Agency of Medicines (Agency) suspended marketing authorisation of the medicinal product “Rigvir solution for injections” (hereinafter – Rigvir).]

81. Garber K China approves world’s first oncolytic virus therapy for cancer treatment. J Natl Cancer Inst. 2006; 98: 298–300. [PubMed: 16507823]

82. Dobner T, Horikoshi N, Rubenwolf S, Shenk T. Blockage by adenovirus E4orf6 of transcriptional activation by the p53 tumor suppressor. Science. 1996; 272: 1470–1473. [PubMed: 8633237]

83. Benedict CA, Norris PS, Prigozy TI, Bodmer JL, Mahr JA, Garnett CT, et al. Three adenovirus E3 proteins cooperate to evade apoptosis by tumor necrosis factor-related apoptosis-inducing ligand receptor-1 and -2. J Biol Chem. 2001; 276: 3270–3278. [PubMed: 11050095]

84. Lei J, Li QH, Yang JL, Liu F, Wang L, Xu WM, et al. The antitumor effects of oncolytic adenovirus H101 against lung cancer. Int J Oncol. 2015; 47: 555–562. [PubMed: 26081001]

85. He CB, Lao XM, Lin XJ. Transarterial chemoembolization combined with recombinant human adenovirus type 5 H101 prolongs overall survival of patients with intermediate to advanced hepatocellular carcinoma: a prognostic nomogram study. Chin J Cancer. 2017; 36: 59. [PubMed: 28728568]

86. Zhang R, Cui Y, Guan X, Jiang X. A recombinant human adenovirus type 5 (H101) combined with chemotherapy for advanced gastric carcinoma: A retrospective cohort study. Front Oncol. 2021; 11: 752504. [PubMed: 34956877]
87. Liu BL, Robinson M, Han ZQ, Branston RH, English C, Reay P, et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther. 2003; 10: 292–303. [PubMed: 12595888]

88. Ramelyte E, Tastanova A, Balazs Z, Ignatova D, Turko P, Menzel U, et al. Oncolytic virotherapy-mediated anti-tumor response: a single-cell perspective. Cancer Cell. 2021; 39: 394–406.e4. [PubMed: 33482123]

89. Heinzerling L, Künzi V, Oberholzer PA, Kundig T, Naim H, Dummer R. Oncolytic measles virus in cutaneous T-cell lymphomas mounts antitumor immune responses in vivo and targets interferon-resistant tumor cells. Blood. 2005; 106: 2287–94. [PubMed: 15961518]

90. Johnson DB, Puzanov I, Kelley MC. Talimogene laherparepvec (T-VEC) for the treatment of advanced melanoma. Immunotherapy. 2015; 7: 611–619. [PubMed: 26098919]

91. Hercus TR, Thomas D, Guthridge MA, Ekert PG, King-Scott J, Parker MW, et al. The granulocyte-macrophage colony-stimulating factor receptor: linking its structure to cell signaling and its role in disease. Blood. 2009; 114: 1289–1298. [PubMed: 19436055]

92. Willemze R, Cerroni L, Kempf W, Berti E, Facchetti F, Swerdlow SH, et al. The 2018 update of the WHO-EORTC classification for primary cutaneous lymphomas. Blood. 2019; 133: 1703–1714. [PubMed: 30635287]

93. Ressler JM, Karasek M, Koch L, Silmbrod R, Mangana J, Latifyan S, et al. Real-life use of talimogene laherparepvec (T-VEC) in melanoma patients in centers in Austria, Switzerland and Germany. J Immunother Cancer. 2021; 9.

94. Puzanov I, Milhem MM, Minor D, Hamid O, Li A, Chen L, et al. Talimogene laherparepvec in combination with ipilimumab in previously untreated, Unresectable stage IIIB-IV melanoma. J Clin Oncol. 2016; 34: 2619–2626. [PubMed: 27298410]

95. Harrington KJ, Andtbacka RH, Collichio F, Downey G, Chen L, Szabo Z, et al. Efficacy and safety of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in patients with stage IIIIB/C and IV M1a melanoma: subanalysis of the Phase III OPTIM trial. Onco Targets Ther. 2016; 9: 7081–7093. [PubMed: 27895500]

96. Todo T, Martuzza RL, Rabkin SD, Johnson PA. Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc Natl Acad Sci U S A. 2001; 98: 6396–6401. [PubMed: 11353831]

97. Cassady KA, Gross M, Roizman B. The second-site mutation in the herpes simplex virus recombinants lacking the gamma134.5 genes precludes shutoff of protein synthesis by blocking the phosphorylation of eIF-2alpha. J Virol. 1998; 72: 7005–7011. [PubMed: 9696792]

98. York IA, Roop C, Andrews DW, Riddell SR, Graham FL, Johnson DC. A cytosolic herpes simplex virus protein inhibits antigen presentation to CD8+ T lymphocytes. Cell. 1994; 77: 525–35. [PubMed: 8187174]

99. Goldstein DJ, Weller SK. Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: Isolation and characterization of an ICP6 lacZ insertion mutant. J Virol. 1988; 62: 196–205. [PubMed: 2824847]

100. Uchihashi T, Nakahara H, Fukuhara H, Iwai M, Ito H, Sugauchi A, et al. Oncolytic herpes virus G47Δ injected into tongue cancer swiftly traffics in lymphatics and suppresses metastasis. Mol Ther Oncolytics. 2021; 22: 388–398. [PubMed: 34553027]

101. Raja J, Ludwig JM, Gettinger SN, Schalper KA, Kim HS, Oncolytic virus immunotherapy: future prospects for oncology. J Immunother Cancer. 2018; 6: 140. [PubMed: 30514385]

102. Hong J, Yun CO. Overcoming the limitations of locally administered oncolytic virotherapy. BMC Biomed Eng. 2019; 1: 17. [PubMed: 32903299]

103. Murin CD, Wilson IA, Ward AB. Antibody responses to viral infections: a structural perspective across three different enveloped viruses. Nat Microbiol. 2019; 4: 734–747. [PubMed: 30886356]

104. Ye X, Xiao L, Zheng X, Wang J, Shu T, Feng Y, et al. Seroprevalence of neutralizing antibodies to human adenovirus type 4 and 7 in healthy populations from southern China. Front Microbiol. 2018; 9: 3040. [PubMed: 30619131]

105. Muñoz-Alía M, Nace RA, Tischer A, Zhang L, Bah ES, Auton M, et al. MeV-Stealth: A CD46-specific oncolytic measles virus resistant to neutralization by measles-immune human serum. PLoS Pathog. 2021; 17: e1009283. [PubMed: 33534834]
106. Smith JG, Cassany A, Gerace L, Ralston R, Nemerow GR. Neutralizing antibody blocks adenovirus infection by arresting microtubule-dependent cytoplasmic transport. J Virol. 2008; 82: 6492–6500. [PubMed: 18448546]

107. Shin DH, Nguyen T, Ozpolat B, Lang F, Alonso M, Gomez-Manzano C, et al. Current strategies to circumvent the antiviral immunity to optimize cancer virotherapy. J Immunother Cancer. 2021; 9.

108. Ying L, Cheng H, Xiong XW, Yuan L, Peng ZH, Wen ZW, et al. Interferon alpha antagonizes the anti-hepatoma activity of the oncolytic virus M1 by stimulating anti-viral immunity. Oncotarget. 2017; 8: 24694–24705. [PubMed: 28449666]

109. Saren T, Ramachandran M, Martikainen M, Yu D. Insertion of the Type-I IFN decoy receptor B18R in a miRNA-Tagged semliki forest virus improves oncolytic capacity but results in neurotoxicity. Mol Oncol. 2017; 7: 67–75. [PubMed: 29159280]

110. Xia M, Luo D, Dong J, Zheng M, Meng G, Wu J, et al. Graphene oxide arms oncolytic measles virus for improved effectiveness of cancer therapy. J Exp Clin Cancer Res. 2019; 38: 408. [PubMed: 31533779]

111. Wang Y, Huang H, Zou H, Tian X, Hu J, Qiu P, et al. Liposome encapsulation of oncolytic virus M1 to reduce immunogenicity and immune clearance in vivo. Mol Pharm. 2019; 16: 779–785. [PubMed: 30604617]

112. Lv P, Liu X, Chen X, Liu C, Zhang Y, Chu C, et al. Genetically engineered cell membrane nanovesicles for oncolytic adenovirus delivery: A versatile platform for cancer virotherapy. Nano Lett. 2019; 19: 2993–3001. [PubMed: 30964695]

113. Ozcan G, Ozpolat B, Coleman RL, Sood AK, Lopez-Berestein G. Preclinical and clinical development of siRNA-based therapeutics. Adv Drug Deliv Rev. 2015; 87: 87–108. [PubMed: 25666164]

114. Valkenburg KC, de Groot AE, Pienta KJ. Targeting the tumour stroma to improve cancer therapy. Nat Rev Clin Oncol. 2018; 15: 366–381. [PubMed: 29651130]

115. Everts A, Bergeman M, McFadden G, Kemp V. Simultaneous tumor and stroma targeting by oncolytic viruses. Biomedicines. 2020; 8.

116. Zheng M, Huang J, Tong A, Yang H. Oncolytic viruses for cancer therapy: Barriers and recent advances. Mol Ther Oncolytics. 2019; 15: 234–247. [PubMed: 31872046]

117. Vaha-Koskela M, Hinkkanen A. Tumor restrictions to oncolytic virus. Biomedicines. 2014; 2: 163–194. [PubMed: 28548066]

118. Santi A, Kugeratski FG, Zanivan S. Cancer associated fibroblasts: The architects of stroma remodeling. Proteomics. 2018. 18: e1700167. [PubMed: 29280568]

119. Henke E, Nandigama R, Ergun S. Extracellular matrix in the tumor microenvironment and its impact on cancer therapy. Front Mol Biosci. 2019; 6: 160. [PubMed: 32118030]

120. Choi H, Moon A. Crosstalk between cancer cells and endothelial cells: implications for tumor progression and intervention. Arch Pharm Res. 2018; 41: 711–724. [PubMed: 29961196]

121. Maishi N, Hida K. Tumor endothelial cells accelerate tumor metastasis. Cancer Sci. 2017; 108: 1921–1926. [PubMed: 28763139]

122. Monteran L, Erez N. The dark side of fibroblasts: Cancer-Associated fibroblasts as mediators of immunosuppression in the tumor microenvironment. Front Immunol. 2019; 10: 1835. [PubMed: 31428105]

123. Liu Z, Kuang W, Zhou Q, Zhang Y. TGF-β1 secreted by M2 phenotype macrophages enhances the stemness and migration of glioma cells via the SMAD2/3 signalling pathway. Int J Mol Med. 2018; 42: 3395–3403. [PubMed: 30320350]

124. Sette P, Amankulor N, Li A, Marzulli M, Leromni D, Zhang M, et al. GBM-Targeted oHSV armed with matrix metalloproteinase 9 enhances anti-tumor activity and animal survival. Mol Ther Oncolytics. 2019; 15: 214–222. [PubMed: 31890868]

125. Fingleton B Matrix metalloproteinases: roles in cancer and metastasis. Front Biosci. 2006; 11: 479–491. [PubMed: 16146745]

126. Mills CD. M1 and M2 Macrophages: Oracles of health and disease. Crit Rev Immunol. 2012; 32: 463–488. [PubMed: 23428224]
127. Kulkarni A, Chandrasekar V, Natarajan SK, Ramesh A, Pandey P, Nirgud J, et al. A designer self-assembled supramolecule amplifies macrophage immune responses against aggressive cancer. Nat Biomed Eng. 2018; 2: 589–599. [PubMed: 30956894]

128. Zheng X, Turkowski K, Mora J, Brüne B, Seeger W, Weigert A, et al. Redirecting tumor-associated macrophages to become tumoricidal effectors as a novel strategy for cancer therapy. Oncotarget. 2017; 8: 48436–48452. [PubMed: 28467800]

129. Rao L, Zhao SK, Wen C, Tian R, Lin L, Cai B, et al. Activating Macrophage-Mediated cancer immunotherapy by genetically edited nanoparticles. Adv Mater. 2020; 32: e2004853. [PubMed: 33089578]

130. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. Nat Rev Immunol. 2017; 18: 153–167. [PubMed: 28990585]

131. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature. 2014; 515: 563–567. [PubMed: 25428504]

132. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014; 515: 568–571. [PubMed: 25428505]

133. Miggelbrink AM, Jackson JD, Lorrey SJ, Srinivasan ES, Waibl-Polania J, Wilkinson DS, et al. CD4 T-Cell exhaustion: Does it exist and what are its roles in cancer? Clin Cancer Res. 2021; 27: 5742–5752. [PubMed: 34127507]

134. Ribas A, Chesney J, Long GV, Kirkwood JM, Dummer R, Puzanov I, et al. MASTERKEY-265: A phase III, randomized, placebo (Pbo)-controlled study of talimogene laherparepvec (T) plus pembrolizumab (P) for unresectable stage IIIIB–IVM1c melanoma (MEL). Annals of Oncology. 2021; 32: S868–S869.
Figure 1.
Overview of the pathway in oncolytic virotherapy. Inoculation introduces the oncolytic virus (OV) to the tumor. OVs bind to specific extracellular surface markers that are solely expressed on tumor cells, gaining entry into the cell. Hijacking the host machinery, the OVs rapidly replicate and induce oncolysis, releasing viral progeny, pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), chemokines, and cytokines. Released viral progeny continue the oncolytic cycle by binding to neighboring tumor cells, while the other factors work to recruit various types of immune cells (e.g., CD4+ T cells, CD8+ T cells, and NK cells) to the tumor, allowing for tumor infiltration and enhanced eradication of malignant cells. Alternatively, some oncolytic viruses do not induce oncolysis, rather these viruses induce secretion of DAMPS and pro-inflammatory cytokines to recruit immune cells to target the tumor cells. Created with BioRender.com.
Figure 2:
Strategies to Overcome Cancer Resistance to Immunotherapy using Oncolytic Viruses. Mechanisms of resistance to currently available immune checkpoint inhibitor therapies and other immunotherapy remain multifactorial. Oncolytic viruses have potential to help overcome primary or secondary resistance to immunotherapy independently or in combination with other immune modulatory agents.
### Table 1:
Comparison between DNA and RNA virus characteristics.

| Characteristics                        | DNA Viruses                        | RNA Viruses                        |
|----------------------------------------|------------------------------------|------------------------------------|
| Greater genomic stability              | Genomic instability                |                                    |
| High-fidelity DNA polymerases          | Low-fidelity RNA polymerase        |                                    |
| Larger genomes                         | Smaller genomes                    |                                    |
| Greater genomic packaging capacity     | Limited genomic packaging capacity |                                    |
| May or may not replicate in presence of neutralizing antibodies | Mechanisms to block DNA virus sensing adaptors | Mechanisms to block RNA virus sensing adaptors |
| Longer replication duration            |                                    |                                    |
| Nuclear Replication                    | Shorter replication duration       |                                    |
| Cytoplasmic replication                |                                    |                                    |
| Mechanisms to block DNA virus sensing adaptors | Rapid evolution |                                    |
| Examples                               |                                    |                                    |
| Adenovirus                             | Echovirus                          |                                    |
| Herpes Simplex virus                   | Measles virus                      |                                    |
| Parvovirus                             | Newcastle disease virus            |                                    |
| Vaccinia virus                         | Poliovirus                         |                                    |
| Reovirus                               |                                    |                                    |
| Seneca Valley Virus                    |                                    |                                    |
| Vesicular Stomatitis Virus             |                                    |                                    |
Table 2: Select ongoing clinical trials involving oncolytic virus and other anti-cancer therapies.

| Identifier   | Cancer Type          | Phase | Oncolytic Virus | Injection | Cotreatment                                      |
|--------------|----------------------|-------|-----------------|-----------|--------------------------------------------------|
| NCT02705196  | Pancreatic cancer    | I/II  | ADV             | Intratumoral | Nucleoside, anti-PD1 Ab, antimicrotubule agent |
| NCT03004183  | NSCLC & breast cancer| II    | ADV/HSV         | Intratumoral | Nucleoside, radiation, & anti-PD1 Ab            |
| NCT03916510  | Rectal cancer        | I     | ADV             | Intravenous | Radiotherapy & antimetabolite                    |
| NCT05051096  | FG neoplasms         | NA    | ADV             | Intratumoral | Radiotherapy                                     |
| NCT05234905  | FG neoplasms         | II    | ADV             | Intratumoral | anti-PD1 Ab                                     |
| NCT03252808  | Pancreatic cancer    | I     | HSV             | Intratumoral | Nucleoside & antimicrotubule agent              |
| NCT03663712  | Ovarian cancer       | I     | HSV             | Intraperitoneal | NA                                               |
| NCT03865255  | GIC                  | I     | HSV             | Intratumoral | TOP1 inhibitor & anti-PD1 Ab                    |
| NCT04050406  | SCSC                 | II    | HSV             | Intratumoral | anti-PD1 Ab                                     |
| NCT0418311   | Breast cancer        | I     | HSV             | Intratumoral | anti-PD1 Ab, anti-CTLA4 Ab                      |
| NCT04349436  | Carcinoma            | I     | HSV             | Intratumoral | NA                                               |
| NCT04755543  | GIC                  | I     | HSV             | Intravenous | anti-PD1 Ab, alkylating agent, antimetabolites   |
| NCT05232136  | Bladder cancer       | I     | HSV             | Intravesical | NA                                               |
| NCT05235074  | CNS tumors           | I     | HSV             | Intratumoral | NA                                               |
| NCT03043391  | Glioma               | I     | Poliovirus      | Intratumoral | NA                                               |
| NCT03564782  | Breast cancer        | I     | Poliovirus      | Intratumoral | NA                                               |
| NCT04445444  | Breast cancer        | II    | Reovirus        | Intravenous | anti-PD-L1 Ab                                  |
| NCT02997156  | Advanced cancer      | I     | VV              | Intratumoral | anti-CTLA4 Ab                                  |
| NCT03206073  | CRC                  | I     | VV              | Intravenous | anti-CTLA4 Ab, anti-PD-L1 Ab                    |
| NCT03954067  | Advanced cancer      | I     | VV              | Intratumoral | w/wo anti-PD1 Ab                               |
| NCT04787003  | Advanced cancer      | I     | VV              | Intratumoral | w/wo anti-PD1 Ab, anti-PD-L1 Ab                 |

Abbreviations: adenovirus (ADV), non-small cell lung cancer (NSCLC), herpes simplex virus (HSV), female genital (FG), gastrointestinal cancer (GIC), Topoisomerase 1 (TOP1), squamous cell skin cancer (SCSC), central nervous system (CNS), colorectal cancer (CRC), and Vaccinia virus (VV)