Oncolytic Virotherapy

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REVIEW

Virus–Receptor Interactions and Virus Neutralization: Insights for Oncolytic Virus Development

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Abstract: Oncolytic viruses (OVs) are replication competent agents that selectively target cancer cells. After penetrating the tumor cell, viruses replicate and eventually trigger cell lysis, releasing the new viral progeny, which at their turn will attack and kill neighbouring cells. The ability of OVs to self-amplify within the tumor while sparing normal cells can provide several advantages including the capacity to encode and locally produce therapeutic protein payloads, and to prime the host immune system. OVs targeting of cancer cells is mediated by host factors that are differentially expressed between normal tissue and tumors, including viral receptors and internalization factors. In this review article, we will discuss the evolution of oncolytic viruses that have reached the stage of clinical trials, their mechanisms of oncolysis, cellular receptors, strategies for targeting cancers, viral neutralization and developments to bypass virus neutralization.

Keywords: oncolytic viruses, virus-receptor interaction, virus neutralization

Introduction

Cancer remains one of the most prevalent non-communicable diseases worldwide.[1] While traditional cancer therapies including chemotherapy, radiotherapy, surgery and radiosurgery can result in a beneficial outcome, they often cause severe off-target cytotoxicity. The necessity to specifically aim at cancer cells, while sparing healthy cells, has encouraged the development of targeted cancer treatment paradigms. In recent years significant progress has been made in developing viruses as a therapeutic strategy against cancer.[2] Oncolytic viruses (OVs) are replication competent viral strains that specifically infect and lyse cancer cells. Many of the advantages of using OVs for cancer therapy arise from the fact they can be considered self-amplifying anti-cancer agents. Following tumor cell entry, OVs replicate and eventually trigger cell lysis, releasing new viral progeny, which in turn will invade and kill neighboring cells. The fact that viral amplification occurs within the tumour is likely to play an important role in tumor control through cell-cell spread.[3] Additionally, viruses released from lysed cells can be transported by the circulatory system to tumors residing remotely from the original site.

The first documented case of using viruses as a potential cancer treatment dates back to 1910s, when a patient diagnosed with cervical carcinoma experienced remission after vaccination with a live-attenuated rabies vaccine.[4] This incident prompted further clinical studies using rabies vaccine as an anti-cancer agent and exploitation of many other

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oncolytic viral strains such as Egypt 101 virus,[5] adenoidal-pharyngeal-conjunctival virus,[6] Newcastle disease virus[7,8] and mumps virus.[9] However, it should be noted that these initial trials were fraught with unethical practices. In recent years, there has been a resurgence of studies focused on possible roles for viruses in killing cancer cells. At the moment, more than 570 clinical trials using OVs are either active or recruiting patients,[10] while many other viruses are in pre-clinical trials. This interest was ignited in part by the approval of Talimogene Laherparepvec (T-VEC), a modified oncolytic herpes simplex virus-1[11] for clinical use in USA, Europe and Australia,[12] along with the clinical use of adenovirus derived Oncorine for head and neck cancers treatment in China[13] and native Echovirus 7 under the name of Rigvir[14] for the treatment of melanoma in several European countries.[15]

This review is focused on the evolution of oncolytic viruses that have reached the stage of clinical trials, their mechanisms of oncolysis and interactions with cellular receptors. In addition, limitations associated with oncovirotherapy such as antiviral immune response (viral neutralization) will be discussed along with recent developments towards overcoming such obstacles.

Mechanisms of Oncolysis

Most oncolytic viruses exert anti-tumor activity by penetrating the tumor cells, establishing a lytic cycle and ultimately causing the activation of cell death pathways. While some OVs have the natural capacity to infect specific tumors through receptor-mediated internalization,[14,16-18] most OVs have been engineered to enhance their tumor selectivity and to reduce virulence in normal cells.[12,13,19,20] Even though natural receptors responsible for oncolytic viral entry are expressed on non-malignant cells thereby allowing a successful infection,[21–23] OVs often require a defect in innate immunity to successfully infect and propagate, which is only present in tumor cells but not in healthy cells.[24] Alterations in transcriptional and cell signaling pathways, such as increased expression of B-cell lymphoma-extra-large (Bcl-xL) and activation of mitogen-activated protein kinases (MAPK) signaling can lead cancer cells to be more susceptible to OVs.[25,26] In addition to direct cell lysis, OV infection and subsequent cell lysis trigger the release of danger-associated molecular patterns (DAMPs) that contribute to a long-lasting adaptive antitumor immune response.[27–29] In fact, substantial effort has been made to develop OVs that encode transgenes designed to induce an immunogenic cell death (ICD) with the goal of priming the immune system against tumors.[30–33] ICD releases DAMPs, which are recognized by antigen-presenting cells (APCs) such as macrophages and dendritic cells in the tumor microenvironment to elicit an innate immune response.[34] As viral replication and tumor lysis progress, APCs produce cytokines, eventually recruiting other immune cells. The ultimate goal of this immune priming process is to activate T lymphocytes against specific tumor antigens in order to establish an adaptive immunity.[35] Evidence for OV-induced innate and adaptive immune responses comes from several clinical trials. For instance, increased abundance of CD8+ and CD4+ T cells has been reported in patients with advanced melanoma, who received T-VEC or coxsackievirus in separate clinical trials.[31,36-38] Patients with metastatic pancreatic adenocarcinoma showed increased B cells and natural killer cells when treated with a combination of reovirus and paclitaxel/carboplatin and these responses were linked to an increased disease control rate (DCR) in responding patients.[33] Furthermore, increased expression of anti–inflammatory cytokines such as interleukin (IL) 6, IL 10 and tumor necrosis factor-α (TNF-α) was reported in patients with refractory primary or metastatic liver cancer treated with poxvirus strain JX-594,[32] with some patients showing a durable objective response according to response evaluation criteria in solid tumors. In addition, OVs have also been reported to directly interfere with tumor perfusion. Engineered forms of adenoviruses[39] and vaccinia virus[40] have been shown to elicit antiangiogenic effects in mouse models. While not yet clinically documented, the possibility of a single OV to employ all three mechanisms of oncolysis in ongoing trials or in future developments holds promise.

Architecture of Oncolytic Viruses

The nature of the genome and the morphology of OVs are two essential factors that influence their amenability for cancer treatment development. First, oncolytic viruses pose different advantages for oncovirotherapy depending on their DNA or RNA genome. The structural stability of DNA combined with the precision of the DNA polymerase allows double-stranded DNA (dsDNA) viruses to encode a large number of proteins. The dsDNA viruses have the advantage of a stable genome that can be engineered to attenuate a pathogenic viral strain, to increase tumor specificity, to avoid anti-viral immune response or to code for proteins that can act synergistically with existing inflammatory host responses to establish a stronger anti-tumor immune response.[2] A classic example is T-VEC, which has been modified to attain reduced neurovirulence[41] and to stimulate the immune response.[42]

On the other hand, oncolytic RNA viruses possess the advantages of tumor specificity and a low off-target cytotoxicity dependent on the affinity to specific receptor usage. Paramyxoviruses and picornaviruses are two virus families that have been extensively studied for their oncolytic potential. Newcastle disease virus and measles virus from *Paramyxoviridae* family feature a negative-sense, non-segmented single-stranded RNA, which requires conversion into a positive-sense RNA before translation.[43] In picornaviruses, the positive-sense single-stranded RNA (ssRNA) genome acts as a messenger RNA (mRNA) and it is translated into the viral polyprotein shortly after penetrating the host cells. This provides a mechanism for oncolytic picornaviruses to replicate and propagate faster.[44,45] RNA viruses inherit a high error rate in genome replication and therefore are genetically unstable.[46] From an evolutionary point of view, such mechanisms allow them to outmaneuver the host's antiviral response due to viral diversity but can raise challenges for oncovirotherapy, especially when the virus is pathogenic in nature.

In addition to the classification of oncolytic viruses based on their genetic materials, they can be further sub-divided into two groups depending on virion architecture. Herpes simplex virus, vaccinia virus and rhabdoviruses fall into the enveloped DNA virus group, whereas adenovirus and reovirus belong to the non-enveloped DNA virus group. In the oncolytic RNA virus group, paramyxoviruses such as Newcastle disease virus and measles virus are enveloped, whereas picornaviruses are in the non-enveloped group. In terms of viral morphology, enveloped viruses are easily amenable to modification for use as OVs. Their morphology implies a relatively direct mode of infection in which the viral and cellular membranes fuse in order to deliver the nucleocapsid to the cytoplasmic space.[47] The mechanism is mediated by the presence of fusion proteins evolved to recognize specific cellular receptors (Figure 1). Triggered by special cues, either changes in pH or the binding of co-receptors, fusion proteins undergo major conformational changes to bring the two membranes in close proximity and eventually causing them to merge.[47] Non-enveloped virus architecture consists of a protein cage with icosahedral or helical symmetry harbouring the genome. Their mechanisms of cell entry are less understood, but it is known to involve the binding of a specific cellular receptor (Figure 1) that could trigger a signaling process leading to capsid endocytosis.[48] Alternatively, receptor binding could function just as an attachment strategy to be followed by entry.[49] The requirement of a specific receptor for tumor recognition and infection has been intensively investigated in picornaviruses and adenoviruses (Figure 1).

The virion size and morphology are two important factors controlling OVs applicability. In order to spread and elicit their antitumor effect, oncolytic viruses must be able to overcome numerous physical barriers in the tumor microenvironment such as tight cell-cell junctions, extracellular matrix deposits, stromal cells and interstitial fluid pressure.[50] Some oncolytic viruses, such as picornaviruses, have a small size (~30 nm) and can overcome such physical barriers.[51,52]

A serious obstacle for both enveloped and non-enveloped oncolytic viruses is to pass the genome across the cellular membrane. This can be done either by disrupting the membrane continuity or by using a channel formed by viral and/or cellular proteins.[48]

Strategies for Targeting Cancer Cells and Reducing off-Target Cytotoxicity

Selective targeting of tumors is of utmost importance and perhaps the most frequently discussed topic in the field of oncovirotherapy due to the use of human pathogenic viruses to treat cancers.[53] These strategies could either involve exploiting inherent properties of a wild-type oncolytic virus such as specific receptor/dis-regulated cellular mechanisms usage and/or manipulating specific viral genes and surface properties to render tumor specificity.

Utilizing the Natural Tropism of OVs

Natural tropism is the capacity of a population of viruses to exploit extracellular markers expressed in cancer cells or to utilize intracellular pathways or immune-avoidance mechanisms to target tumors. Receptors responsible for oncoviral permissivity in tumors often play an essential role in tumor growth and progression or in providing protection from antitumor immune mechanisms.[24] For instance, cluster of differentiation (CD) 155, CD46, and integrin α2β1 overexpressed in tumors, providing innate immunity for cancer cells, serve as entry ports for oncolytic poliovirus, measles virus and echovirus, respectively.[54–56] On the other hand, herpes virus entry mediator (HVEM) and nectin-1, anthrax toxin receptor 1 (ANTXR1), laminin receptor, intracellular adhesion molecule-1 (ICAM1) and decay-accelerating factor (DAF), which all have a functional role in tumor growth and progression, have been identified as the cellular receptors for herpes simplex virus, Seneca Valley virus-001, Sindbis virus and coxsackievirus.[17,57-61] In addition, numerous oncogenic pathways involved in carcinogenesis overlap with requirements for

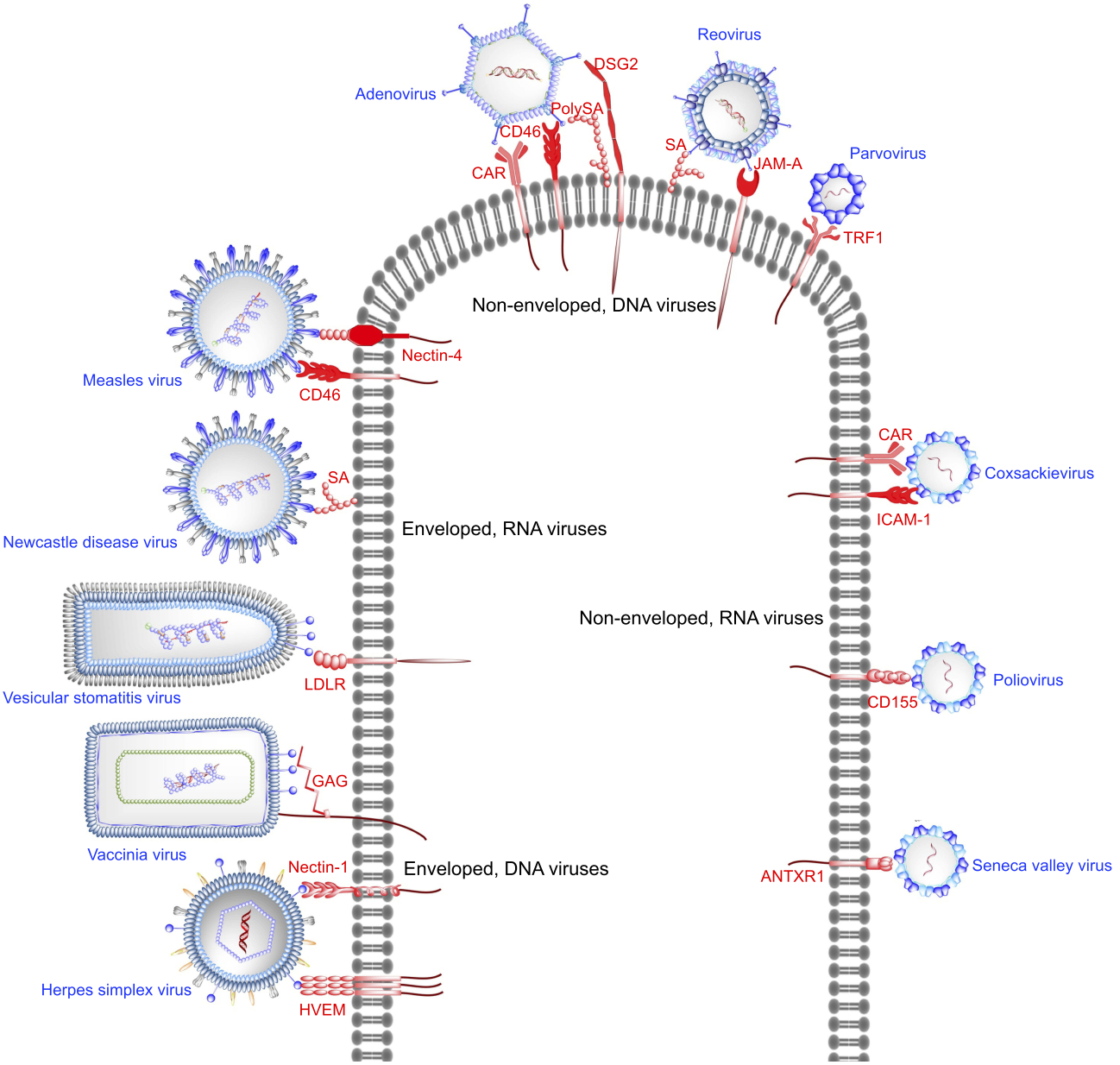

Figure 1 Targeting of receptors overexpressed in cancers with oncolytic viruses. Enveloped DNA viruses utilize their surface glycoproteins to bind receptors overexpressed in cancers. Herpes simplex virus glycoprotein D binds herpes virus entry mediator (HVEM) or nectin-1 prior to initiation of host membrane fusion by HSV glycoprotein B. Vaccinia virus and vesicular stomatitis virus bind cell surface glycosaminoglycans (GAGs) and low-density lipoprotein (LDLR) receptor, respectively. Enveloped, RNA viruses Newcastle disease virus and measles virus interact with cell surface sialic acid (SA) and CD46 or nectin-4, respectively, to facilitate entry into host cells. Sialic acid or poly-sialic acid (PolySA) serves as an attachment receptor for non-enveloped DNA viruses such as reovirus and human adenovirus. Junction adhesion molecule-A (JAM-A) acts as the entry receptor for reovirus, whereas coxsackievirus-adenovirus receptor (CAR), CD46, desmoglein-2 (DSG) have been shown to be the entry receptors for adenoviruses. Parvovirus (ssDNA) exploits cell surface transferrin receptor 1 as the entry receptor. Among the non-enveloped RNA viruses Seneca Valley virus, poliovirus, coxsackievirus bind anthrax toxin receptor-1 (ANTXR1), CD155, intercellular adhesion molecule-1 (ICAM-1) or CAR, respectively.

a successful infection and replication in some native oncolytic viruses. For example, tumor selectivity of Reolysin is dependent on a number of endogenous tumor factors such as RAS activation, downregulation of interferon (IFN) antiviral response and p53 pathway.[26,62,63] Furthermore, Newcastle disease virus has been shown to target cancer cells overexpressing an anti-apoptotic protein Bcl-xL,[25] while the high specificity of vesicular stomatitis virus for cancer cells is governed by its high sensitivity to type I IFNs, a system defective in most cancer types.[64]

Engineered Tropism

There are several strategies dictated by their viral architecture for modifying OVs to specifically target cancer

cells. For enveloped viruses, a direct method is the insertion of glycoproteins from other viruses that recognize the targeted receptor (Figure 2). For instance, it was shown that a modified lentiviral vector, that possesses E2 glycoprotein in the envelope, can target a P-glycoprotein in melanoma.[65] The neurotoxicity of vesicular stomatitis virus (VSV) has been abrogated by the substitution of its glycoprotein G with a glycoprotein variant of the lymphocytic choriomeningitis virus (LMCV-GP).[66] Similarly, coat proteins can be modified with peptide ligands or antibody fragments recognized by the desired receptors.[24,67] Adenovirus capsid fibers have been modified with an insertion of arginine-glycine-aspartic acid (RGD) moiety acting as the binding site of integrin receptors overexpressed in tumors (Figure 2).[68] An alternative retargeting approach that does not involve the modification of the virus is the use of bispecific soluble adapters designed to bind both the OV and any given targeted antigen on cell membrane, mimicking a bona fide virus-receptor engagement (Figure 2). This strategy was employed to redirect herpes simplex virus-1 binding from nectin-1 to epidermal growth factor receptor (EGFR) by using a soluble adaptor protein P-V528LH that harbors a gD-binding domain of nectin-1 fused to virus and a single-chain antibody with affinity to EGFR.[69]

Several genetic modifications have been introduced in some oncolytic viruses to warrant tumor selectivity and to monitor the biodistribution of such viruses after administration (Table 1). Mutations introduced into herpes simplex virus-1 (HSV-1), vaccinia virus (VV), adenovirus (HAdV)

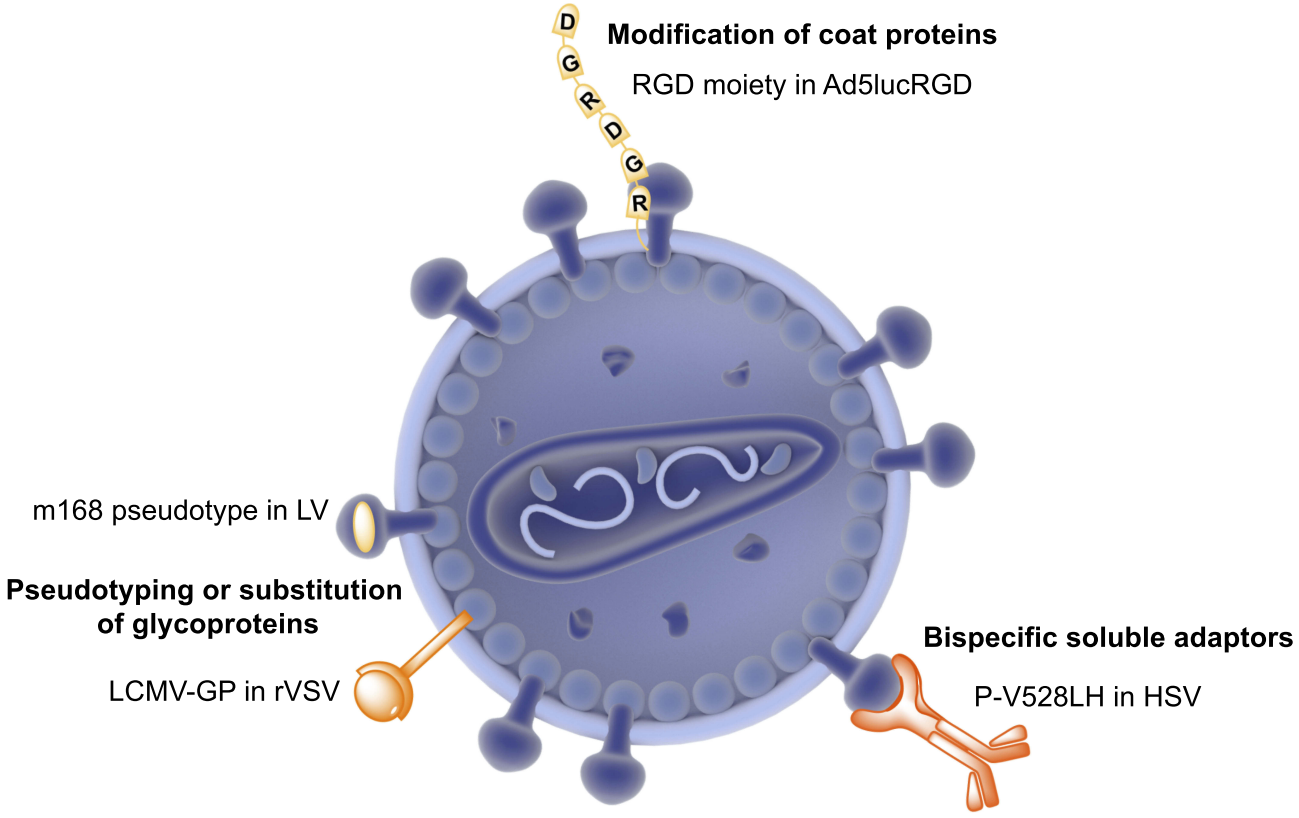

Figure 2 Strategies for retargeting cancers with oncolytic viruses. Oncolytic viral architecture can be modified primarily in three different ways to target cancer-specific receptors. Pseudotyping of lentiviral (LV) envelope glycoproteins with a variant of Sindbis virus envelope protein has enabled successful targeting of P-glycoprotein expressed on melanoma cells. Substitution of vesicular stomatitis virus envelope glycoprotein with a variant glycoprotein from lymphocytic choriomeningitis virus glycoprotein (LCMV-GP) has enhanced the tumor specificity of the recombinant vesicular stomatitis virus (rVSV). Recombinant adenovirus strains have been developed (Ad5lucRGD) by incorporating an RGD moiety required for interaction with integrin receptors overexpressed in cancers. Finally, bispecific soluble adaptors (P-528LH) have been used in the case of herpes simplex virus (HSV), that includes gD-binding domain of nectin-1 fused to virus and a single-chain antibody with affinity to epidermal growth factor receptor (EGFR).

Table 1 Modified Oncolytic Viruses in Clinical Trials

Oncolytic Virus	Modifications	Development Status	Examples for Phase Studies
Herpes simplex virus-1 (HSV-1)	Replacement of ICP34.5 and 47 genes with granulocyte-macrophage colony-stimulating factor (Talimogene Laherparepvec or T-VEC)	US FDA approved	Phase II/III • Patients with unresectable Stage IIIb, IIIc and IV melanoma (completed, NCT00769704 and NCT00289016) • In combination with pembrolizumab (MK-3475) for treatment of unresectable Stage IIIb-IVM1c melanoma (active, NCT02263508) • T-VEC as a neoadjuvant treatment after surgery against unresectable Stage IIIb-IVM1a melanoma (active, NCT02211131) • Treatment for unresectable recurrent breast cancer (active, NCT02658812)
Adenovirus (Ad)	Deletion of E1B55K and E3B genes (Oncorine or H101)	Approved in China	Phase III • Treatment of malignant pleural effusions in non-small cell lung carcinoma in combination with recombinant human endostatin injections (status unknown, NCT02579564) • Hepatic artery infusion chemotherapy in combination with Oncorine for the treatment of hepatocellular carcinoma (recruiting, NCT03780049)
	Deletion of E1B55K, E3B and E1ACR2 regions. Addition of Arginine-Glycine-Aspartic acid motif in capsid fibers (Delta-24-RGD)	Phase I/II clinical trials	Phase I/II • Safety study in patients with recurrent glioblastoma (completed, NCT01582516) • Treatment for recurrent glioblastoma and gliosarcoma, followed by administration of pembrolizumab (active, NCT02798406)
Vaccinia virus (VV)	Deletion of thymidine kinase gene, vaccinia growth factor gene and expressing granulocyte-macrophage colony-stimulating factor (JX594 or Pexa-Vec)	Phase II/III clinical trials	Phase II/III • Combination therapy with metronomic cyclophosphamide against advanced breast cancer and advanced soft-tissue sarcoma (recruiting, NCT02630368) • Treatment for patients with advanced hepatocellular carcinoma unresponsive to sorafenib (completed, NCT01387555) • In combination with Durvalumab and Tremelimumab for treatment of refractory colorectal cancer (recruiting, NCT03206073) • Treatment for hepatocellular carcinoma in conjunction with sorafenib administration (active, NCT02562755)
Vesicular stomatitis virus (VSV)	Expressing interferon-β and sodium iodide symporter (VSV–IFNβ-NIS)	Phase I clinical trials	Phase I • Treatment for refractory liver cancer or advanced solid tumors (active, NCT01628640) • As a monotherapy and in combination with avelumab against refractory solid tumors (recruiting, NCT02923466) • Combination therapy with pembrolizumab in refractory non-small cell lung cancer and head and neck squamous cell carcinoma (recruiting, NCT03647163)
	Expressing interferon-β and tyrosinase-related protein1 genes (VSV–IFNβ-TYRP1)	Phase I clinical trials	Phase I • Treatment for stage III–IV melanoma (recruiting, NCT03865212)

(*Continued*)

Table 1 (Continued).

Oncolytic Virus	Modifications	Development Status	Examples for Phase Studies
Measles virus (MV)	Encoding sodium iodide symporter (MV-NIS)	Phase II clinical trials	Phase II • Vaccine therapy for recurrent or refractory multiple myeloma with or without cyclophosphamide (active, NCT00450814) • In combination with cyclophosphamide for treating patients with relapsed/refractory myeloma (recruiting, NCT02192775) • MV-NIS infected mesenchymal stem cells in treating recurrent ovarian cancer (recruiting, NCT02068794) • Comparative study for the effectiveness of MV-NIS vs paclitaxel/topotecan hydrochloride/gemcitabine hydrochloride/pegylated liposomal doxorubicin hydrochloride in treating fallopian, ovarian and peritoneal cancer (recruiting, NCT02364713)
Poliovirus (PV)	Internal ribosome entry site (IRES) of poliovirus replaced with that of human rhinovirus 2 (PVSRIPO)	Phase II clinical trials	Phase II • Combination therapy of atezolizumab and PVSRIPO for treatment of patients with recurrent malignant glioma (not yet recruiting, NCT03973879) • Stand-alone treatment for patients with grade IV malignant glioma (recruiting, NCT02986178)

Notes: Clinical trials as registered at https://clinicaltrials.gov. Clinical trial status and National Clinical Trial (NCT) identifier number are given within parentheses at the end of each clinical trial description.

and poliovirus (PV) strains have been shown to restrict the replication of these viruses to cancer cells and to reduce the toxicity associated with wild-type strains. The selectivity of T-VEC, a modified HSV-1 strain, for tumors is regulated by inherently low expression of protein kinase R (PKR) in cancers, that otherwise serves as an upstream target in normal cells to phosphorylate eukaryotic translation initiation factor 2 (eIF2) to terminate host cell protein synthesis.[70] However, HSV infected cell protein (ICP) 34.5 has the capacity to reverse this mechanism by dephosphorylating eIF2.[71] In addition, HSV ICP47 can inhibit the transporter associated with antigen presentation (TAP), ultimately reducing the expression levels of the antigen-major histocompatibility complex (MHC) type I.[72] Therefore, both ICP34.5 and 47 genomic sites have been deleted in T-VEC and replaced with two copies of hematopoietic granulocyte-macrophage colony-stimulating factor (GM-CSF), that promotes the recruitment of dendritic cells and antigen-presenting cells (APC) to the tumor site.[73] The deletion of ICP47 also serves in translocating the herpes virus protein US11 to decrease the activity of PKR in cancer cells.[41]

Numerous HAdV strains have been genetically engineered to overcome healthy tissue damage and to selectively target tumors. ONYX-015, one of the first strains of genetically engineered HAdV, was designed to target p53 gene-deficient tumors. A deletion in E1B region prevents the expression of E1B55K protein, that inactivates p53-dependent apoptosis in normal cells.[74] In the absence of E1B55K protein, normal cells undergo p53-dependent apoptosis, thereby halting the viral life cycle. By contrast, ONYX-015 has the capacity to replicate in tumors with p53 deficiency, given the function of E1B55K can be compensated by other mechanisms in tumor cells. An in vivo study suggested that ONYX-015 can exert greater antitumor activity when combined with radiotherapy.[75] Furthermore, in a Phase II clinical trial in patients with recurrent squamous cell cancer of the head and neck, intratumoral administration of ONYX-015 in conjunction with 5-fluorouracil and cisplatin showed a more significant effect when compared to monotherapy.[76] H101, a successor of ONYX-015, has been further modified with a deletion in E3B gene. H101 was the first HAdV to be approved for cancer treatment in China in 2006 under the name of Oncorine.[13] Next

generation modified adenoviral strains harbor small deletions in E1A gene (E1ACR2 mutants) to suppress the release of E2F transcription factor by ablating the interactions between retinoblastoma protein (pRb) and E1A.[77] This modification further restricts adenoviral replication only in tumor cells with activated E2F expression.

In order to make vaccinia virus safer for cancer therapy, two deletions have been made: thymidine kinase gene (TK) and vaccinia growth factor gene (VGF).[78] Further attenuated viral strains have been developed by introducing mutations in F14.5L and A56R genes.[20] These genes are responsible for encoding a secretory signal peptide and hemagglutinin, respectively. JX-594, a TK-mutant, expressing GM-CSF has been tested in Phase I and Phase II clinical studies in hepatocellular carcinoma and liver cancer as stand-alone treatment or in combination with sorafenib. Collectively, these studies showed a safe yet profound anti-tumor response in JX-594 monotherapy and combination treatment groups in comparison to sorafenib alone.[79–81]

Several strategies have been tested in reducing PV neurotoxicity: (1) use of live-attenuated poliovirus vaccines,[82] (2) delivery of engineered PV genome deficient of P1 coding region (replicons), thereby preventing the formation of new viral progenies and spread,[83] (3) A133G mutation in cis-acting replication element (CRE) and relocation of CRE to a spacer region,[84] (4) replacement of internal ribosome entry site (IRES) of PV (PVSRIPO) with that of human rhinovirus 2 (HRV2).[85] Ribonucleoprotein complex formed in PVSRIPO is incompatible with HRV2 IRES-mediated translation in normal human central nervous system, therefore, rendering the neuronal incompetence of PVSRIPO.[86] PVSRIPO has completed a Phase I dose-finding clinical study in patients with grade IV malignant glioma with no neurotoxicity reported.[87]

Virus Neutralization

A major limitation of the extensive use of OVs in cancer treatment is the rapid neutralization by the immune system, which can restrain the viral spread and reduce the efficacy of repeat administrations.[88] Antiviral immune response could hinder viral infection or replication at several stages: 1) Neutralization, opsonization and sequestration prior to cell entry, 2) Inhibition of virus replication by induction of antiviral responses such as type I interferons in infected cells, and 3) Lysis of infected cells by innate immune cells prior to viral-induced lysis of cells. Many of the viruses used in cancer therapy are human pathogens and pre-existing antiviral antibodies obstruct the systemic delivery to the tumor, limiting the potential routes for viral delivery to intratumoral injection. Various pre-clinical and Phase I clinical studies have shown decreased oncolytic viral replication, viral clearance and reduced anti-tumor activity in immunocompetent hosts.[89–96]

Evidence for pre-existing immunity against oncolytic viruses has been well documented for vaccinia virus due to its use in eradicating the smallpox and also for reovirus, that is universally abundant in the environment.[97] In vaccinia virus, neutralizing antibodies have been shown to target H3L envelope protein, that plays an essential role in viral-host cell membrane fusion.[98] Structural insights into antibody neutralization of reovirus suggested that neutralizing antibodies sterically hinder the JAM-A receptor binding to reovirus.[99] Pre-clinical studies on prostate-specific attenuated replication competent adenovirus (ARCA) showed a decreased antitumor activity in the presence of pre-existing antibodies.[92] In the case of measles virus (MV), pre-existing antibodies act as a major limitation in treating cancers in previously vaccinated patients.[100] Therefore, MV oncovirotherapy may only be limited for patients with certain cancers such as advanced multiple myeloma, where the immunosuppressed patients have a low level of anti-measles antibodies.[101] Furthermore, the administration of T-VEC is limited to intralesional injections for melanoma treatment due to high prevalence of anti-HSV1 antibodies in humans.[102] Even in the absence of pre-existing antibodies, the immune system will eventually mount a response and severely reduce the period of virus efficacy to between a few days and couple of weeks, requiring multiple/increased doses of the virus.[97,103,104] Even more troublesome is the induction of primary antibody response or augmentation of a low pre-existing antiviral response upon initial administration of low seroprevalence viruses.[105,106] Such evidence arises from neutralization of low human seroprevalence viruses such as vesicular stomatitis virus (VSV) in non-immune human serum as early as one hour after exposure.[107,108]

On the other hand, a major advantage of the stimulating effect raised by OVs on immune system against viruses is that it could enhance epitope spread to tumor antigens. One pre-clinical study on recombinant measles virus VLPs expressing the tumor-specific antigen claudin-6 triggered claudin-6-specific immune responses in melanoma mouse models, ultimately inhibiting tumor metastasis.[109] While generally pre-existing immunity for oncolytic viruses reduces their efficacy, a contrary finding was reported in

the case of Newcastle disease viruses (NDV) where an augmented therapeutic effect was observed in melanoma mouse models in the presence of NDV-specific antibodies through potentiation of systemic anti-tumor immunity.[110]

Solutions to Virus Neutralization

The presence of pre-existing anti–viral immunity or the development of neutralizing antibodies upon systemic administration of oncolytic viruses highlights the importance of developing novel strategies to prolong their availability to access tumors. At proof-of-concept level, novel OV delivery methods have been proposed and can be categorized into four distinct groups (Figure 3 and Table 2).

Cell Carriers

Encapsulation of virus particles in a carrier is a logical approach as a strategy to conceal the antigenicity of native virions. Several molecular and cellular carriers have been investigated. Murine colon carcinoma cells infected with vesicular stomatitis virus (VSV) homed to cancer cells but not to normal cells, when delivered intravenously in a mouse lung tumor model.[111] Reovirus incorporated into dendritic cells and T cells can efficiently deliver the virus into cancer cells in the presence of neutralizing antibodies in vitro[112] and in vivo.[113] Furthermore, antibody-neutralized reovirus complex can be introduced into human monocytes, where internalized complexes were processed to release infectious particles, ultimately targeting cancer cells.[114] Mesenchymal stem cells (MSCs) have been used as a delivery system for chimeric human adenovirus5/3 (HAdV5/3) with the primary purpose of masking the virus from immune attack.[115] The cellular receptor for HAdV5 entry, coxsackievirus and adenovirus receptor (CAR) is poorly expressed in MSCs.[116] This has been circumvented by swapping the receptor binding fiber knob domain of HAdV5 with that of HAdV3, allowing a CAR-independent cell binding.[115]

Liposomes

Liposomes are large hydrophilic spherical vesicles, which have been widely used for encapsulation and delivery of diverse range of drugs since they act as a shield from cellular and humoral responses.[117,118] In a pre-clinical study, liposomes were used to encapsulate oncolytic alphavirus strain

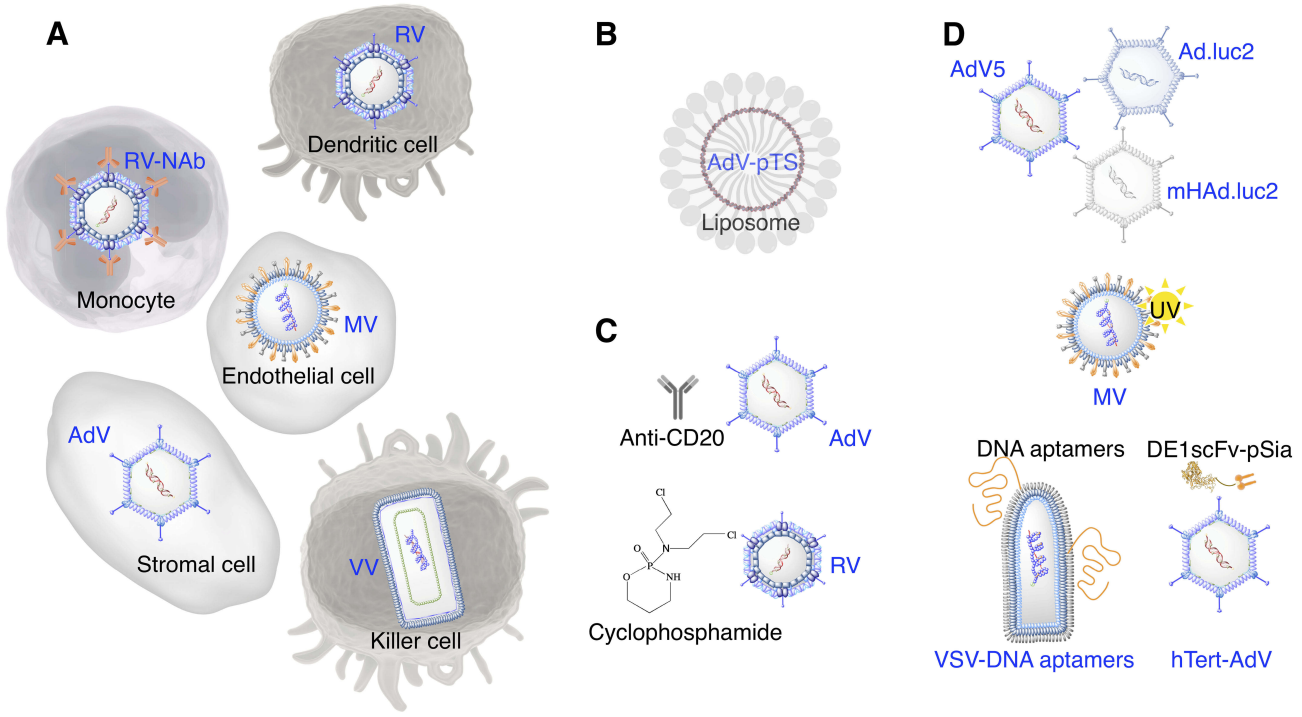

Figure 3 Strategies to avoid virus neutralization. (**A**) Cell carriers such as monocytes (Reovirus-neutralizing antibody complex), dendritic cells (reovirus), endothelial cells (measles virus), stromal cells (adenovirus) and killer cells (vaccinia virus) remain as most extensively researched solutions to bypass the recognition by neutralizing antibodies. (**B**) Liposomes have been used to incorporate plasmids of oncolytic viruses such as Telomerase-specific oncolytic adenovirus (pTS). (**C**) Anti-CD20 and cyclophosphamide (immunomodulators) aid in suppressing the antiviral immune response associated with adenovirus and reovirus treatment. (**D**) Other solutions to virus neutralization include the use of different serotypes of adenovirus strains, sequestration of pre-existing antibodies using UV–inactivated measles virus (decoy virus), and shielding of vesicular stomatitis virus and adenovirus with coadministration of DNA aptamers and bifunctional protein DE1scFv-pSia, respectively.

Table 2 Strategies to Avoid Oncolytic Virus Neutralization

Strategy to Avoid Virus Neutralization	Oncolytic Virus and Modifications	Development Status and Treated Cancer(s)
Cell carriers	• Vesicular stomatitis virus-infected murine colon carcinoma cells • Reovirus incorporated in T cells and dendritic cells • Reovirus-antibody complex loaded into human monocytes • Chimeric adenovirus type 5/3 capsid (OAd)-infected mesenchymal stromal cells • Measles virus-infected endothelial, monocytic or stimulated peripheral blood cells • Vaccinia virus introduced into cytokine-induced killer cells	• In vivo study against lung cancer[111] • In vivo study against metastatic melanoma[112,113] • In vivo study against melanoma[114] • In vitro study against pancreatic cancer[115] • In vivo study against ovarian cancer[131] • In vivo study against ovarian cancer[91]
Liposomes	• Alphavirus strain M1 encapsulated into liposomes (M-LPO) • Modified adenovirus, ONYX-015 plasmid encapsulated into liposomes • Telomerase-specific oncolytic adenovirus plasmid DNA encapsulated into liposomes (Lipo-pTS)	• In vitro study against human colon carcinoma and epidermoid-carcinoma[119] • In vivo study against non-small cell lung cancer[120] • In vivo study against colon cancer[132]
Immunomodulators	• Reovirus therapy in combination with cyclophosphamide • Adenovirus administration after anti-CD20 treatment	• In vivo study against melanoma[121] • Gene transfer study unrelated to cancer therapy[123]
Other strategies	• Vesicular stomatitis virus (VSV) shielded by dual-function DNA aptamers • Oncolytic adenovirus, hTert-Ad fused with bifunctional protein DE1scFv-pSia • UV–inactivated Measles virus as a decoy virus • Different serotypes of adenovirus • Immune-evasive particle forms (extracellular enveloped particles or EEV) of vaccinia virus	• Efficacy study to evaluate the infectivity of VSV in the presence of neutralizing antibodies[124] • In vivo study against murine colon adenocarcinoma[125] • In vitro study against T cell leukemia[126] • In vivo study against breast cancer bone metastasis[127] • Efficacy study to show the resistance of EEV to vaccina virus-specific antibodies[128]

M1 (M-LPO) with anti-tumor efficacy in vitro and a reduced immunogenicity in mice when administered intravenously.[119] A similar approach was used to encapsulate a replication-competent, ONYX-015-based plasmid in liposomes.[120] Liposomes harboring the plasmids were resistant to antibodies neutralizing the parent strain, while the plasmids could only transfect tumor cells which are p53 deficient.

Immunomodulators

With the aid of conventional immunosuppressants used in the treatment of autoimmune disorders, the host immune response can be partially reduced to favor the targeted delivery of oncolytic viruses to tumors. Combination therapy of cyclophosphamide and reovirus has been shown to rescue reovirus when administered intravenously in mice.[121] Currently, there are seven clinical trials that are either completed, active or in recruiting stage for combination or neoadjuvant therapy of metronomic cyclophosphamide with oncolytic viruses such as ONCOS-102 (adenovirus), rQNestin34.5v.2 and T-VEC (HSV-1), MV-NIS (measles virus), and JX-594 (vaccinia virus).[122] In a non-cancer related study, T cells and B cells activated by repeated administration of adenovirus have been inhibited by anti-CD 20 en route to assist a successful immunosuppressive regime of liver gene transfer.[123]

Other Strategies

The use of DNA aptamers to shield viruses from neutralizing antibodies has been shown as a proof-of-concept in vitro study with vesicular stomatitis virus (VSV).[124] In this study, aptamers were developed to bind virus surface as well as the antigen-binding fragment (Fab) of anti-VSV antibodies, providing a dual protection mechanism when used concurrently with VSV. Another example for use of bifunctional adapters arises from a recent study on oncolytic adenovirus hTert-Ad treatment in combination with DE1scFv-pSia protein containing a DE1 domain of adenovirus hexon and a polysialic acid-specific single-chain variable fragment (scFv) to capture neutralizing antibodies and for tumor cell recognition, respectively.[125]

In a different approach, prior treatment of cancer cells with UV–inactivated measles virus prevented the neutralization of the active virus, suggesting the possibility of using a "decoy virus" to sequester pre-existing antibodies.[126]

Another strategy to counteract host anti-viral immunity is to use different serotypes of the virus (native or modified) or immune-evasive particle forms of the same virus. The feasibility of using different serotypes of adenovirus has been shown in a pre-clinical study, where intravenous administration inhibited the formation of bone metastases.[127] Vaccinia virus produces extracellular enveloped particles (EEV) that possess a cell-derived envelope capable of evading neutralizing antibodies.[128] Therefore, high EVV-producing strains of vaccinia virus can be engineered to improve the spread of the virus upon systemic delivery.[129] In the case of measles virus, N-linked glycosylation of hemagglutinin resulted in strain resistance to a mixture of monoclonal antibodies.[130]

Conclusion

Oncolytic virotherapy is a promising field of cancer treatment with selective targeting of tumors. However, the antiviral immune response is still a limiting factor hindering the outcome of the treatment. While many OVs have a rapid replication in tumors and direct oncolysis, it is often the antitumor immunity induced by oncolytic activity that contributes to preventing the disease progression and recurrence. When OVs are originally pathogenic to humans, specific targeting of tumors has been achieved either through the manipulation of viral genome to exploit de-regulated signaling pathways in tumors or by modifying viral coat proteins to bind receptors overexpressed in cancer cells. However, the expression levels of attachment/entry receptors specific to OVs differ depending on the type of cancer and/or patient, highlighting the importance of understanding of OV-receptor interactions to modify capsid architecture and re-target cancers. The systemic administration still remains a less effective mean of OV delivery due to the existence of pre-existing neutralizing antibodies or rapid anti-viral immune response after initial treatments. To address this issue, novel treatment strategies have been developed and showed promise in various proof-of-concept and pre-clinical studies: encapsulation of OV in carriers, modification of capsid or envelope proteins, the use of decoy viruses to sequester pre-existing antibodies, multiple administration of different viral serotypes and adjuvant therapy with immunomodulators.

Disclosure

Dr John T. Poirier reports personal fees from Perceiver Pharmaceuticals LLC, outside the submitted work; in addition, Dr John T. Poirier has a patent WO2017096201A1 licensed to Perceiver Pharmaceuticals, LLC. The authors report no other conflicts of interest in this work.

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