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Research progress and development potential of oncolytic vaccinia virus

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Abstract

Oncolytic virotherapy is a promising therapeutic approach treating tumors, where oncolytic viruses (OVs) can selectively infect and lyse tumor cells through replication, while also triggering long-lasting anti-tumor immune responses. Vaccinia virus (VV) has emerged as a leading candidate for use as an OV due to its broad cytophilicity and robust capacity to express exogenous genes. Consequently, oncolytic vaccinia virus (OVV) has entered clinical trials. This review provides an overview of the key strategies used in the development of OVV, summarizes the findings from clinical trials, and addresses the challenges that must be overcome in the advancement of OVV-based therapies. Furthermore, it explores potential future strategies for enhancing the development and clinical application of OVV, intending to improve tumor treatment outcomes. The review aims to facilitate the further development and clinical adoption of OVV, thereby advancing tumor therapies.

Keywords: Oncolytic virotherapy; Vaccinia virus; Tumor therapies Immune response; Clinical

Introduction

Discovery of OV

OV therapy is a type of cancer treatment in which viruses specifically replicate within tumor cells, exerting viral cytotoxicity while minimizing damage to normal cells. Both natural and genetically modified OVs are capable of spreading throughout the tumor, offering a level of tumor selectivity that is exceptional compared to other cancer therapies.[1] The concept of oncolytic virotherapy (OVT) dates back to the 19th century, when septic dressings containing viruses were occasionally applied to ulcerated tumors for cancer treatment. In more recent history, viral microorganisms have garnered attention as potential therapeutic agents. For instance, at the end of the 19th century, a 42-year-old woman suffering from leukemia experienced a spontaneous remission of her tumor after a suspected influenza virus infection;[2] similarly, in 1912, an Italian physician reported a case of significant tumor regression in a patient with advanced cervical cancer following rabies vaccination.[3] Common features in these cases included viral infection and relatively young patients with well-functioning immune systems. Building on these observations, in 1949, 22 patients with Hodgkin's disease were treated with serum or tissue extracts containing hepatitis viruses,[4] and numerous clinical trials were conducted between 1950 and 1980. In 1950, early attempts to treat various cancers with wild-type or naturally attenuated viruses—including hepatitis virus, West Nile virus, yellow fever virus, dengue virus, and adenovirus (Ad)—were initiated, with around 150 patients involved in these studies.[5] Subsequent research on Ad as an oncolytic agent for cervical cancer in 1956 demonstrated its ability to induce extensive tumor necrosis, although it failed to prevent tumor progression.[6] In the 1970s, the oncolytic activity of several other viruses was tested in patients with various solid tumors,[7] and a clinical trial involving mumps virus as an OV showed tumor regression in about 40% of patients, although metastasis was not controlled, ultimately leading to patient death.[8] At that time, there was limited knowledge about controlling viral virulence and maintaining viral replication within cancer cells, as well as a poor understanding of the tumor immune response. The concurrent development of radiotherapy, which provided an alternative method for cancer treatment, led to a decline

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in the enthusiasm for oncolytic virotherapy. With the rapid advancements in genetic engineering and immunotherapy, there has been a renewed interest in the use of OVs for tumor treatment. A particularly notable case contributing to this resurgence involved a 50-year-old female virologist with locally recurrent, muscle-invasive breast cancer. After receiving multiple intratumoral injections of an experimental viral agent, she was able to undergo a simple, non-invasive tumor resection.[9] This case further underscores the clinical potential of OVs as a therapeutic modality.

With a deeper understanding of immunology, researchers have recognized that the antiviral defense mechanisms in most tumor cells are impaired, such as the disruption of the interferon (IFN)-γ and IFN-β production pathways,[10] and that the metabolic rate of tumor cells is significantly higher than that of normal cells. This accelerated metabolism facilitates much faster viral replication within tumor cells. As a result, it is theoretically possible for OVs to replicate specifically in tumor cells while avoiding replication in normal cells, though achieving this selectivity remains a significant challenge. It was not until 1991 that Martuza *et al*[11] pioneered the concept of cancer cell-specific replication by modifying the viral genome. They engineered herpes simplex virus type I (HSV-1) by mutating the thymidine kinase (*TK*) gene, creating a genetically modified HSV-1 capable of selectively replicating in cancer cells. This engineered virus was used to treat gliomas, marking the beginning of a rapidly growing field of OV development. Subsequently, an increasing number of viruses—both DNA viruses (e.g., adenovirus, HSV, vaccinia virus [VV]) and RNA viruses (e.g., coxsackievirus, measles virus, Newcastle disease virus, poliovirus)—have entered clinical trials with similar genetic modifications.[1,12] The approval of genetically modified HSV-1-based OVs by the Food and Drug Administration (FDA), followed by European Medicines Agency (EMA) approval in 2015 for the treatment of advanced melanoma, marked the peak of oncolytic virotherapy research.[13]

Mechanisms of action of OV

The primary mechanism by which OVs eliminate tumors is through the lysis of tumor cells following extensive viral replication, which directly induces tumor cell destruction. This selective replication and spread within tumor cells allows for a more targeted and safer method of tumor eradication compared to conventional chemotherapy. However, it is crucial to limit the replication capacity of OVs, particularly when using pathogenic viruses, to ensure they remain confined to tumor cells. Once tumor cells are lysed by the OV, tumor antigens are released, which can activate the immune system and trigger a systemic anti-tumor response. The advantage of these antigens is that they do not require prior knowledge of specific tumor targets nor the screening of particular reagents to ensure their efficacy.[14] Building upon this, the immune response against tumor cells can be further enhanced by genetically modifying the OV to include sequences encoding immune-stimulatory cytokines, such as granulocyte-macrophage colony-stimulating factor, interleukin (IL)-2, or IL-12, or by incorporating sequences that encode immune checkpoint inhibitors, such as anti-programmed cell death protein 1 (PD-1) or anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4), which block immunosuppressive pathways and enhance anti-tumor immunity.[15] In addition, OVs can target tumor-associated stromal cells. For example, OV targeting of tumor vascular endothelial cells can disrupt tumor vasculature, leading to nutrient deprivation and subsequent tumor cell death,[16,17] as well as induce neutrophil infiltration, resulting in blood clot formation and vascular collapse.[18]

Tumor cell-selective replication and oncolysis

Tumor cells replicate at a faster rate than normal cells, which necessitates a more robust metabolism, thus providing OVs with an opportunity to exploit the tumor-specific metabolic mechanisms for replication. Tumor-selective replication of OVs is achieved by modifying the virus, such as deleting non-essential virulence genes and inserting tumor-specific promoters or combinations of microRNAs.[19,20] At the same time, the dysfunctional immune response in tumor cells allows for the evasion of the antiviral clearance mechanisms that normally operate in healthy cells. In contrast to normal cells, which produce antiviral factors such as signal transducer and activator of transcription or janus kinase (STAT) or JAK proteins to limit viral replication upon infection,[21,22] tumor cells typically lack this response. However, it should be noted that some patient-derived tumor cells with mutations in the interferon pathway may still exhibit OV clearance.[23] Another strategy to enhance cancer selectivity involves modifying the OV surface to carry ligand proteins that specifically bind to cell surface molecules expressed by cancer cells.[24] This approach can alter the tropism of the OV by replacing viral membrane surface proteins or adding soluble bispecific junction proteins, such as bispecific antibodies.[25] This strategy relies on the expression of specific cell surface molecules on all, and only, the targeted cancer cells. Similar to other therapeutic approaches that target cell surface molecules (e.g., chimeric antigen receptor T-cell therapy), this opens up the possibility of developing alternative delivery methods for OVs beyond traditional injection-based routes.

Anti-tumor immune response

OVs replicate and lyse tumor cells, a process known as immunogenic cell death, which plays a critical role in inducing anti-tumor immunity. This form of cell death, which can be apoptotic, necrotic, or autophagic, results in the release of danger-associated molecular patterns (DAMPs) and tumor-associated antigens (TAAs).[26,27] In addition, OV-infected tumor cells release cytokines, such as IFNs and IL-12, which promote the maturation of antigen-presenting cells (APCs) [Figure 1]. Furthermore, OV infection enhances the expression of major histocompatibility complex class I (MHC I) molecules on tumor cells, which aids in the activation and recruitment of $CD8^+$ T cells to the tumor site. These T cells subsequently differentiate into cytotoxic effector T cells, mediating systemic anti-tumor immunity [Figure 1]. Innate immunity induced by OV infection is also crucial for the anti-tumor immune response. The release of DAMPs and

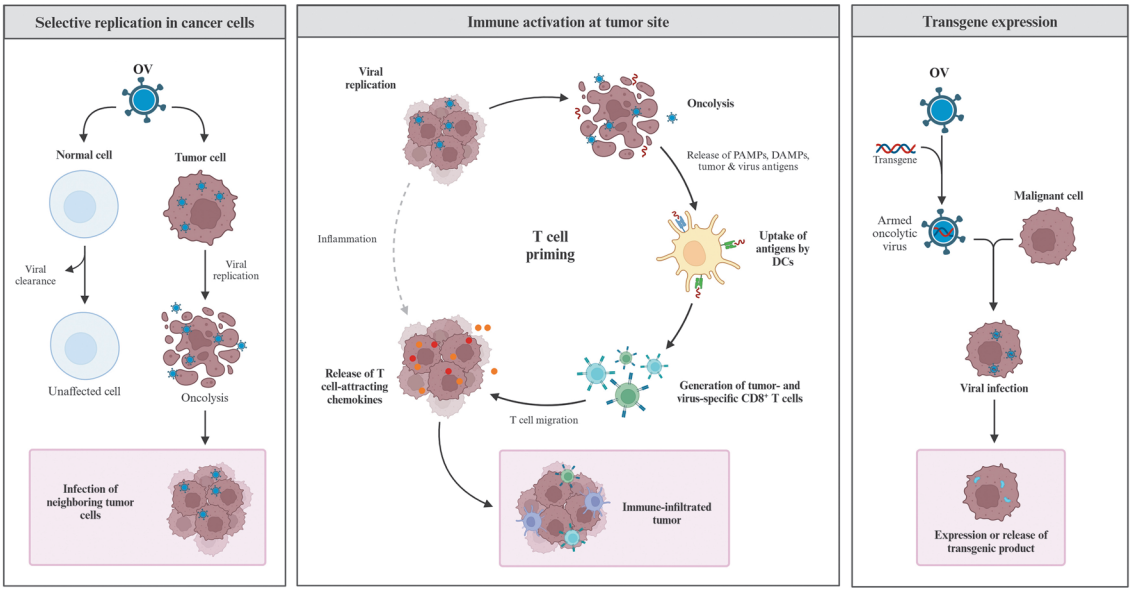

Figure 1: Schematic diagram of the mechanism of OV action. OV replicates in tumor cells without affecting normal cells; OV is released after replicative lysis of tumor cells and subsequently infects adjacent tumor cells (left); OV releases tumor antigens and pro-inflammatory cytokines after lysis of tumor cells, recruits DCs, and activates CD8+ T cells to achieve the activation and enhancement of anti-tumor immunity (middle); OV can insert exogenous genes to encode proteins that favor anti-tumor responses, such as cytokines (right). DAMP: Damage-associated molecular patterns; DC: Dendritic cells; OV: Oncolytic virus; PAMP: Pathogen associated molecular pattern.

type I IFNs activate natural killer (NK) cells,[28] which, in turn, kill OV-infected tumor cells through FAS-FASL signaling. This interaction also triggers the release of cytokines such as IFN-γ and TNF-α, which promote the conversion of tumor-supportive M2 macrophages into pro-inflammatory M1 macrophages.[29,30] In addition, these cytokines recruit more immune cells to the tumor microenvironment (TME), amplifying the anti-tumor immune response. Although OV infection typically stimulates a strong immune response through the presentation of viral antigens, it also induces immune responses against TAAs. The immune system's response to viral antigens can be redirected by TAAs, potentially enhancing the convergence of antiviral immunity and anti-tumor immunity.[27,31] Although the precise dynamics remain unclear, maintaining a delicate balance between anti-OV and anti-tumor immunity is essential for the success of oncolytic virotherapy.[32] The activation of the immune system plays a dual role in the efficacy of OVs, serving both as an aid and an obstacle. On one hand, OV activation of the immune system is beneficial for inducing anti-tumor immunity, enhancing tumor cell destruction. However, on the other hand, it can also limit the persistence of the OV, making it more susceptible to immune-mediated clearance. Various immune cells, such as NK cells and cytotoxic T lymphocytes (CTLs), are recruited to the site of infection, where they contribute to the direct lysis of tumor cells by the OV. However, the killing of OV-infected tumor cells by viral antigen-specific CTLs can impede the spread of the OV, and the presence of anti-OV antibodies further complicates the situation. These antibodies hinder the effective intravenous delivery of the OV, often necessitating the use of a carrier to facilitate its administration. Therefore, it is crucial to design OVs that can replicate and spread rapidly within tumors, maximizing anti-tumor effects before the immune system clears the virus.

The immune processes induced by OVs lead to the enhanced recognition and elimination of tumor cells infected by the virus, compared to uninfected tumor cells. This can result in the transformation of "cold" tumors–characterized by low immune cell infiltration, few pro-inflammatory cytokines, and poor responsiveness to immunotherapy–into "hot" tumors, which are more infiltrated with immune cells and pro-inflammatory cytokines.[33] Hot tumors are more susceptible to immune-mediated attack and eradication,[34] and they are also more responsive to immune checkpoint inhibitors (ICIs), such as anti-PD1 and CTLA4 antibodies.[35,36] Consequently, the conversion of cold tumors into hot tumors represents a critical strategy in tumor therapy. In addition, OVs can reduce the population of immunosuppressive cells within the TME, thereby restoring the anti-tumor function of immune cells and overcoming the intrinsic immunosuppression that characterizes the TME.

Targeting tumor vasculature

When pro-angiogenic cytokines are secreted at the tumor site, they promote the degradation of the tumor extracellular matrix and the formation of capillary-like structures from existing blood vessels. During this process, quiescent endothelial cells are activated to migrate, while bone marrow-derived endothelial progenitor cells are recruited to the tumor site, where they differentiate into endothelial cells to form new blood vessels. These tumor-associated blood vessels are morphologically and functionally distinct from normal blood vessels.[37] In studies of OVs, it has been observed that, in addition to directly targeting and killing tumor cells, OVs can inhibit tumor angiogenesis, thereby controlling tumor progression by cutting off the supply of nutrients to the tumor. Some OVs have the ability to infect developing or

established tumor vasculature without causing damage to normal blood vessels [Figure 2], potentially similar to the effects of vascular endothelial growth factor (VEGF) in endothelial cells or the Ras-activated "proviral" state in cancer cells.[38] By specifically modifying OVs to target VEGF, it may be possible to disrupt the tumor vasculature, thereby depriving tumor cells of their nutrient supply.

Vaccinia as an OV

Characterization of VV

VV is a member of the genus *Orthopoxvirus* in the subfamily Chordopoxvirinae. Its genome is a double-stranded DNA molecule larger than 190 kb [Figure 3], encoding approximately 250 proteins. The entire viral life cycle occurs in the cytoplasm of mammalian cells, not in the nucleus,[39] which makes VV a safer OV compared to other viral vectors. The VV life cycle involves three forms of the virus: intracellular mature virus particles (IMVs), cell-associated enveloped virus particles (CEVs), and extracellular enveloped virus particles (EEVs) [Figure 3]. IMVs enter the host cell by fusion with the plasma membrane, whereas EEVs enter via endocytosis,[40,41] providing a broad range of tumor tropism. In normal cells, VV is typically cleared by the cellular antiviral mechanisms, such as the IFN pathway. However, in tumor cells, this clearance is often impaired, allowing the virus to replicate rapidly despite the hypoxic conditions commonly found in tumors.[42] Viral progeny are produced in approximately 6 hours. The large genome of VV is capable of incorporating around 50 kb of exogenous DNA,[43] and the replication cycle is dependent on a high-fidelity DNA polymerase,[44] which ensures the integrity of the VV genome and facilitates the efficient expression of exogenous proteins without compromising its replication capacity.[45] The four commonly used strains of VV in research are Tiantan, Lister, Western Reserve, and Wyeth. VV has been studied extensively for many years and played a critical role in the development of the smallpox vaccine, effectively eliminating one of the deadliest viruses in human history. The long history of VV research and its widespread use in humans offer strong evidence of its safety as an OV, as VV does not cause significant disease in the general population, and any adverse effects can be controlled with antiviral drugs.[46] However, it is worth noting that approximately 50% of the proteins encoded by VV remain functionally uncharacterized, which, while contributing to the unpredictability of VV, also presents significant potential for further development.

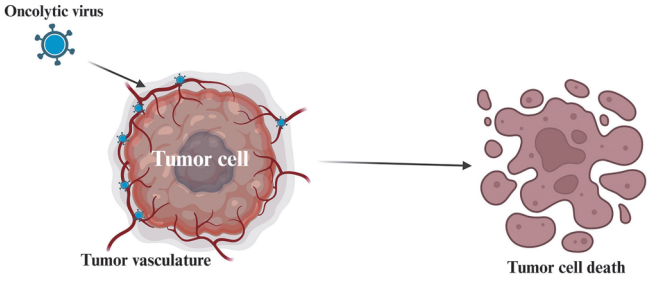

Figure 2: Oncolytic virus attacks tumor vascular cells to cut off the supply of tumor cells, thus causing tumor cell death.

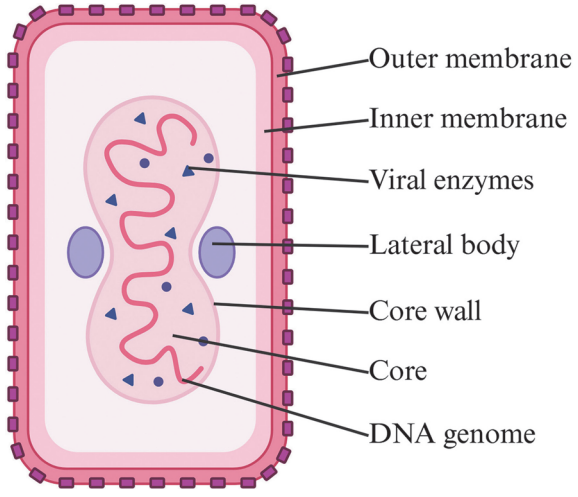

Figure 3: The poxviridae are the most structurally complex group of viruses known to date. Poxvirus particles have an elliptical or brick-like structure, with a length of approximately 200–400 nm and an axial ratio of 1.2–1.7. The membrane is a typical 50–55 nm lipid-protein bilayer encapsidated around the core, and the outer surface is covered with randomly arranged STEs (averaging 7 nm in width and 100 nm in length). Viral particles, consisting of a nucleoprotein core and associated lateral bodies surrounded by a membrane, are sufficiently infectious; however, for certain strains of viruses and for infection of certain cells, viral particles acquire an additional lipid bilayer with its own unique chemical composition called the envelope. This envelope contains approximately twice as much phospholipid as an unenveloped virus particle. Numerous studies have shown that the viral antigens present in the envelope elicit immunity and protect the host against poxvirus attack. The poxvirus envelope contains at least seven different glycoproteins, as well as a major non-glycosylated acylated polypeptide. STEs: Small tubulin elements.

Modification strategies for VV

To enhance the utility of VV as an OV, several key modification strategies have been developed. These modifications are designed to increase the virus's immunogenicity and optimize its anti-tumor efficacy, based on the characteristics of tumor cells. For example, genes encoding immune-enhancing cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-12, or genes ICIs like anti-PD-1 and anti-CTLA4, are incorporated into VV vectors. These modifications promote T-cell activation and enhance the tumor-killing ability of the virus. In addition, efforts are made to minimize the virulence of VV by removing or modifying virulence-related proteins, thereby reducing pathogenicity and ensuring the safety of the virus for therapeutic use.

Deletion of specific genes to enhance viral replication and reduce virulence

The natural antiviral immune signaling pathways of host cells typically interact with proteins in VV, which can alter its immunogenicity.[47] Therefore, modifications to VV are designed to improve several key properties, including enhanced tumor cell selectivity, stronger expression of therapeutic genes, increased tumor immunogenicity, reduced toxicity, and lower immunogenicity of the vector itself. One of the most common genetic modifications involves the deletion of the *TK* gene. *TK* is essential for VV DNA synthesis, and the deletion of the *J2R* gene,

which encodes TK, significantly reduces VV replication in normal cells. However, tumor cells generally exhibit high levels of TK expression, allowing for continued replication of VV within tumors despite the *J2R* gene deletion. This modification not only reduces the virulence of VV but also enhances its safety profile.[48] All current clinical oncolytic vaccinia virus (OVV) designs including JX-594 and GL-ONC1 carry a *J2R* gene deletion. To further enhance the efficacy of VV as an OV, researchers, armed with a deeper understanding of VV, have explored double, triple, or even quadruple deletions in the viral genome. Common deletion sites include the ribonucleotide reductase (RR) large subunit and small subunit genes (*I4L* and *F4L*). VV strains that combine mutations in both the *J2R* and *I4L* or *F4L* genes demonstrate selective replication in tumor cells, as compared to viruses with a single *TK* deletion. These modified VV strains not only exhibit tumor-selective replication but also enhance tumor cell killing while significantly reducing virulence.[49,50] The deletion of the anti-apoptotic viral gene *F1L* reduces the likelihood of OVV being cleared, as infected cells are less prone to undergoing apoptosis. In contrast, the deletion of the type I IFN inhibitor gene *B18R* enhances the tumor selectivity of OVV, as many tumor cells are deficient in type I IFN production compared to normal cells. Deleting *B18R* also facilitates the clearance of OVV from normal cells. When both *F1L* and *B18R* are deleted in combination with the *J2R* gene, the oncolytic potential of OVV is significantly enhanced, leading to increased tumor cell death and improved efficacy, particularly with systemic intravenous administration.[51,52] Triple or quadruple deletions, including the *J2R* gene, have also been explored.[53,54] Collectively, these findings indicate that VV with such modifications holds significant promise as an ideal candidate for OVV.

In addition to *TK*-related deletions, mutations in other genes encoding viral proteins can also enhance tumor selectivity and reduce virulence. The B5R protein, located on the membrane of the EEV form of VV, is recognized and neutralized by the complement system.[55,56] Partial deletion of a conserved repeat sequence within the *B5R* gene allows the virus to escape neutralization, thereby enhancing its oncolytic effect and enabling it to evade clearance by certain immune responses. Furthermore, pentameric deletions, including genes such as *C7L-K2L*, *E3L*, *A35R*, *B13R*, and *A66R* in the VV Tiantan strain, have been shown to produce more potent OVVs for cancer therapy.[57] Another promising strategy to enhance the efficacy of OVV involves genetic modifications aimed at boosting viral replication. Liu *et al*[58] conducted a small interfering RNA screen to explore the role of necroptotic kinase receptor-interacting protein kinase 3 (RIPK3) and its viral inducer of RIPK3 degradation (vIRD). Their findings suggest that vIRD may facilitate the ubiquitination and proteasomal degradation of RIPK3, thereby promoting viral replication.

Enhancement of OVV-induced anti-tumor immune response

Initially, researchers focused on the direct tumor-lysing effect of OVs as the primary mechanism of their anti-tumor action. However, with the advancement of tumor immunotherapy, the role of OV-induced anti-tumor immune responses has gained increasing attention. OVV, like other OVs, triggers an anti-tumor immune response upon lysis of tumor cells, which releases tumor antigens. This immune response can be enhanced by cytokines, chemokines, and immune checkpoint inhibitors. In addition, the disruption of the tumor microenvironment through OV-induced lysis can facilitate the infiltration of anti-tumor immune cells into the tumor. These processes can convert a "cold" tumor, with minimal immune cell infiltration, into a "hot" tumor, characterized by increased immune cell presence.

Arming VV with genes encoding cytokines that enhance immunogenicity

With the growing understanding of tumor immune mechanisms, several immunostimulatory genes have been incorporated into the genome of VV to strengthen the anti-tumor immune response triggered or amplified following OVV-induced tumor lysis. These genes include cytokines, chemokines, and co-stimulatory factors. Inflammatory cytokines, which are soluble proteins secreted by cells, play a crucial role in regulating both the innate immune response and the anti-tumor immune response after OVV expression. Pro-inflammatory cytokines are primarily used to recruit immune cells, enhance the effector functions of immune cells, and, to some extent, inhibit the activity of immunosuppressive cells.

GM-CSF is one of the most commonly used cytokines in OVV therapy, as it promotes the recruitment of dendritic cells (DCs) and NK cells. GM-CSF enhances the antigen-presenting capacity of DCs, which in turn activates $CD8^+$ T cells and strengthens anti-tumor immunity.[59] JX-594, a recombinant VV engineered with a *TK* gene deletion and *GM-CSF* gene insertion [Figure 4], has shown promising results in clinical trials targeting various solid tumors. Building upon JX-594, BT001 introduces further modifications by deleting the *I4L* gene and inserting a gene encoding anti-CTLA-4.[60] The incorporation of

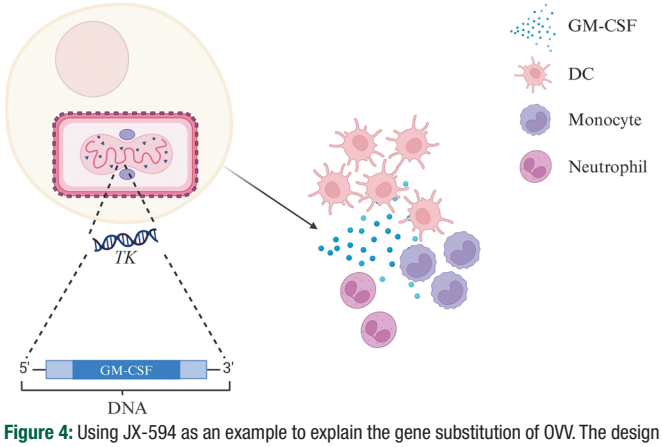

Figure 4: Using JX-594 as an example to explain the gene substitution of OVV. The design strategy of JX-594 is to knock out the *TK* gene in VV and replace it with a gene encoding GM-CSF. JX-594 infects cells and expresses and releases GM-CSF, recruiting immune cells such as DCs, monocytes, and neutrophils. DCs: Dendritic cells; GM-CSF: Granulocyte-macrophage colony-stimulating factor; OVV: Oncolytic vaccinia virus; *TK*: Thymidine kinase; VV: Vaccinia virus.

additional pro-inflammatory cytokines, such as IL-2 and IL-12, into OVVs has demonstrated enhanced anti-tumor effects by boosting NK cell activity, T-cell activation, and IFN-γ production.[61,62] For example, Transgene (France) replaced the *GM-CSF* gene in BT001 with the *IL-12* gene to create TG6050, a novel OVV currently under clinical evaluation.[63] Similarly, IL-21 enhances OVV-mediated anti-tumor responses by expanding effector $CD8^+$ T-cell populations.[64] On the other hand, inhibiting anti-inflammatory cytokines, such as IL-10, TGF-β, and IL-35–which are commonly produced by regulatory T cells (Tregs)–can further potentiate anti-tumor immunity. Among these cytokines, IL-10 production plays a negative role in the synthesis of IL-12 by DCs. This reduction in IL-12 production weakens the support that T cells receive from IL-12, thereby impairing their ability to secrete IFN-γ.[65] In addition, IL-10 suppresses the immediate tumor immune response triggered by OVV infection.[66] Inhibiting TGF-β, a well-known immunosuppressive cytokine that promotes the differentiation of Tregs, can further enhance the effectiveness of OVV therapy. Notably, Delgoffe *et al*[67] demonstrated that arming OVV with a TGF-β inhibitor enhanced IFN-γ-driven immune responses and counteracted the suppressive TME. In addition to immunomodulatory approaches, novel strategies are emerging. Hirvinen *et al*[68] developed a lysogenic VV that expresses the DNA-dependent activator of IFN regulatory factors (DAI), an intracellular pattern-recognition receptor. This approach showed promise in melanoma by boosting both innate and adaptive immune responses. In a related study, Rivadeneira *et al*[69] metabolically reprogrammed T cells with leptin, enabling them to exert immunological control over leptin-expressing melanoma cells in mice. These findings highlight the potential of arming OVVs with components that can trigger robust anti-tumor immune responses, offering a versatile platform for advancing cancer immunotherapy.

Arming VV with ICI

For the use of OVV in tumor treatment, the mainstream delivery method of ICIs is synergistic with OVV therapy. However, some researchers have explored the direct insertion of genes encoding ICIs into the VV genome, achieving promising results. Direct expression of ICIs by VV provides an alternative approach to enhancing anti-tumor immunity [Figure 5]. Zuo *et al*[70] engineered an oncolytic cowpox virus encoding a single-stranded variable fragment targeting T cell immunoreceptor with Ig and ITIM domains (TIGIT), which induced potent anti-tumor immunity and synergized with PD-1 or LAG-3 blockade to remodel the tumor immune environment, effectively converting a cold tumor into a hot one. Wang *et al*[71] used GM-CSF in combination with anti-PD-L1 to co-arm VV-generated OVVs, overcoming PD-1/PD-L1-mediated immunosuppression. Semmrich *et al*[72] applied anti-CTLA-4 in conjunction with GM-CSF as part of an OVV strategy, successfully suppressing Tregs and increasing $CD8^+$ T cell activity, thereby enhancing the strength and durability of the anti-tumor immune response. In addition to BT001 and TG6050, which also used anti-CTLA-4 armed with VV in combination with pro-inflammatory cytokines such as GM-CSF or IL-12, further reinforcing anti-tumor immunity.[60,63]

Destroying the tumor microenvironment

In cold tumors, the effectiveness of ICI therapy is limited because ICI therapy requires immune cell infiltration, which is often hindered by the TME as tumors progress. The TME can alter local stromal cells and immune cell populations, creating a resistant barrier that prevents immune infiltration, recognition, and function. These alterations include the formation of physical barriers to infiltration, changes in the local metabolic environment,

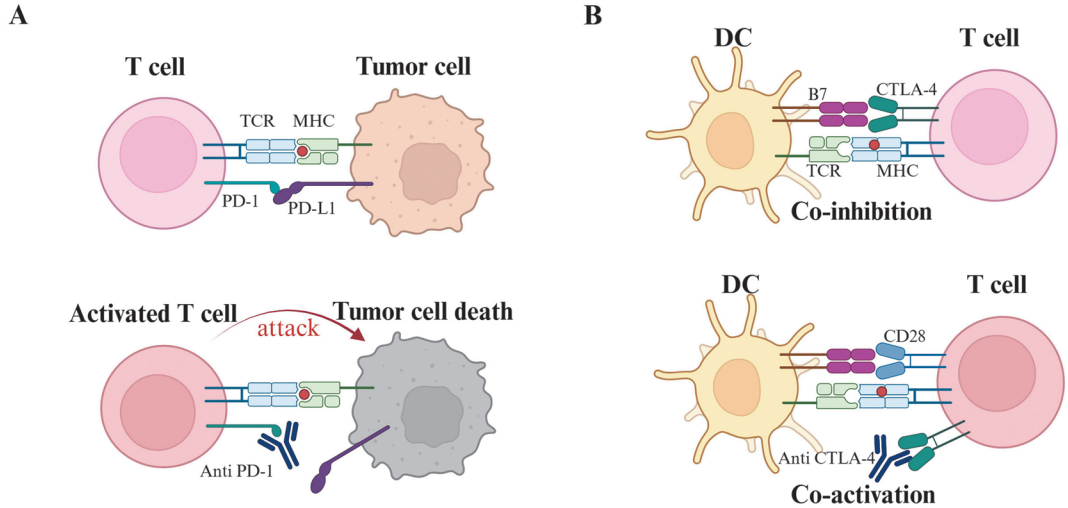

Figure 5: Mechanism of action of immune checkpoint inhibitors. (A) The tumor microenvironment induces infiltrating T cells to overexpress PD-1 molecules, resulting in the continued activation of the PD-1 pathway in the tumor microenvironment and the suppression of T-cell function, which prevents the killing of tumor cells. Inhibitors of PD-1 can block this pathway, partially restoring the function of T-cells so that these cells can continue to kill tumor cells. (B) The *CTLA-4* gene encodes a transmembrane protein with a high degree of homology to the co-stimulatory molecule receptor (CD28) on the surface of T cells. CTLA-4 and CD28 are both members of the immunoglobulin superfamily, and both bind to the same ligands, CD86 (B7-2) and CD80 (B7-1), but with opposing functions (CTLA-4 inhibits T-cell activation, and CD28 activates T-cell activation). Inhibition of CTLA-4 results in increased T-cell activation. CTL: Cytotoxic T lymphocytes; CTLA4: Cytotoxic T-lymphocyte associated protein 4; DC: Dendritic cell; GM-CSF: Granulocyte-macrophage colony-stimulating factor; MHC: Major histocompatibility complex; PD: Progressive disease; PD-1: Programmed cell death protein 1; TCR: T cell receptor; TK: Thymidine kinase.

recruitment of immunosuppressive cell types, and the elevation of soluble factors that may suppress immune responses. As previously mentioned, arming OVs with TGF-β inhibitors aims to block the effects of these immunosuppressive cells.[67] An important strategy is to disrupt the TME matrix to allow for sufficient immune cell infiltration [Figure 6]. GL-ONC1, which is armed with β-galactosidase and bacterial glucuronidase to lyse TME stroma, is currently progressing to clinical phase III trials.[73] In addition, Wang *et al*[74] engineered a recombinant OVV encoding a soluble hyaluronidase gene to remodel the TME, demonstrating promising anti-tumor efficacy.

Increased OVV positioning capability

Although VV naturally exhibits a tumor enrichment effect, OVV is primarily administered locally, and its accumulation is generally confined to the site of injection, resulting in limited systemic effects. In addition, certain tumor sites are challenging to target with direct in situ injection of OVV. If intravenous administration were possible, it could potentially overcome these limitations, improving the broader applicability of OVV. This approach is feasible because neutralizing antibodies against VV are not pre-existing in humans, making intravenous delivery a viable option. VV exhibits broad cytophilicity, with a notable preferential enrichment in the ovary, a characteristic that may be leveraged for targeted therapeutic purposes. This selective affinity was recently investigated by Erica *et al*[75] and the potential application of OVV in ovarian tumors is discussed further in subsequent sections. The TK deletion discussed earlier enables VV to passively target tumor cells, and will not be reiterated here. In addition, genetic modifications to VV can facilitate targeted delivery, with the tumor cell surface marker MUC1 serving as an alternative target site for such modifications.[76] Other methods of targeting tumor cells used by different OVs have not yet been applied to OVVs and will be addressed in later sections. Another approach to enhancing OVV delivery involves the use of polymer coatings, such as Chol-PEG(10K)-NHS, which can help prevent neutralizing antibodies from binding to VV,[77] thus enabling intravenous administration of VV for second or subsequent treatments. This strategy represents a potential method for improving OVV delivery.

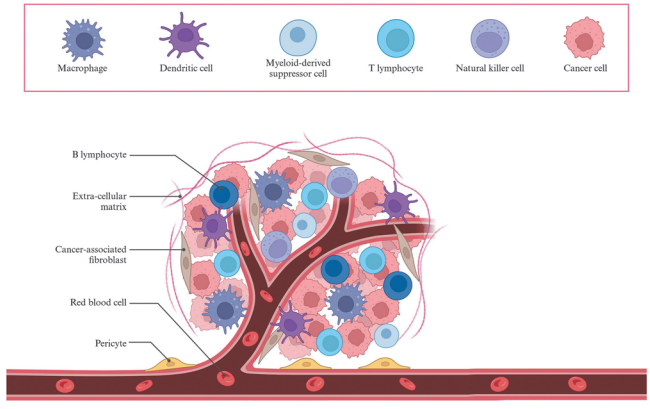

Figure 6: Disruption of the tumor extracellular matrix as a means to allow full infiltration of immune cells.

Administration of OVV

The effectiveness of OVVs is often limited by individual variations in the immune system, immune clearance, and tumor resistance mechanisms. To enhance the efficacy of OVVs, they are typically used in combination with other anticancer therapies. This combined approach helps overcome tumor resistance to single therapies and strengthens the antitumor immune response triggered by OVVs. This article outlines the common co-administration strategies for OVVs, focusing on the most widely used approaches.

OVV and chemotherapy

Chemotherapy is a widely used treatment modality for tumors, primarily exerting its effects by inhibiting DNA synthesis or disrupting microtubule structures, ultimately leading to tumor cell death. Combining OVV therapy with chemotherapy has shown promising potential and has been the focus of numerous studies, yielding improved outcomes. For instance, the combination of JX-593 with sorafenib has demonstrated significant antitumor effects.[78,79] Similarly, the co-administration of GLV-1h68 (Lister strain, ΔF14.5L, ΔA56R, ΔTK) with cyclophosphamide (CPA) produced synergistic antitumor effects in a mouse lung cancer model.[80] In addition, chemotherapy-resistant tumor patients exhibited enhanced antitumor responses when GLV-1h68 was combined with chemotherapeutic agents,[81] suggesting that OVVs not only act in synergy with chemotherapy but also enhance the effectiveness of chemotherapeutic agents. Although other OVVs combined with chemotherapeutic drugs have been explored, they are not detailed here. In summary, the combination of OVVs with chemotherapy improves the overall antitumor response and enhances patient survival compared to OVV monotherapy.

OVV and radiotherapy

Radiotherapy for tumor treatment works by inducing DNA damage and promoting apoptosis, typically administered locally either before or after surgery. The combination of OVV therapy with radiotherapy has also emerged as a promising therapeutic approach. Sunil *et al*[82] demonstrated that radiotherapy-induced tumor cell irradiation enhanced the replication of GLV-1h68 in the treatment of U87 tumors. Building on this finding, Michelle *et al*[83] showed that the combination of GLV-1h68 and radiotherapy exhibited synergistic antitumor effects by inducing apoptosis. In a rat model, this combined therapy significantly prolonged survival and inhibited tumor growth compared to GLV-1h68 alone. Furthermore, in a clinical trial involving GLV-1h68 for head and neck cancer, the combination of GLV-1h68 with cisplatin and radiotherapy resulted in an overall patient survival rate of 74.4%.[84] However, JX-594 has not yet been studied in conjunction with radiotherapy.

OVV and ICI

The strategy of arming OVVs with ICIs to block anti-tumor immunosuppression has been discussed previously.

Similarly, combining ICIs with OVV therapy can enhance tumor sensitivity to ICIs by modulating the TME and the immune system, thus facilitating the complementary action between OVV and ICI therapies. Just as OVVs armed with ICIs can augment anti-tumor immunity, OVVs that express pro-inflammatory cytokines (such as IL-12, GM-CSF, etc.) or stromelytic enzymes targeting the TME can also remodel the TME, enhancing the efficacy of ICIs. Kowalsky *et al*[85] demonstrated that OVs expressing a fusion protein of IL-15 and IL-15Rα, which promote the survival, proliferation, and activation of NK, NKT, and $CD8^+$ T cells, induced strong anti-tumor immunity. In addition, combining this treatment with PD-1 blockade led to significant tumor regression and prolonged survival in mice with colon or ovarian cancer.[85,86] Furthermore, Lou *et al*[87] reported that OVV-MnSOD (manganese superoxide dismutase) enhanced intratumoral inflammation, improved systemic anti-tumor efficacy, and increased lymphoma sensitivity to PD-L1 blockade, offering a promising strategy to overcome ICI resistance.

OVV and molecularly targeted therapies

Unlike chemotherapy, molecularly targeted therapy is designed to selectively kill tumor cells by targeting specific molecules that are overexpressed in these cells but not in normal cells, using specific inhibitors. Due to its relatively low toxicity, molecularly targeted therapy has emerged as a promising strategy for cancer treatment. As noted previously, OVV-induced antiviral immunity can result in the rapid clearance of the virus. Molecularly targeted therapy can enhance the antitumor efficacy of lysogenic VV by using several mechanisms, such as evading antiviral immune responses and boosting antitumor immunity. For instance, inhibition of the MEK-ERK pathway promotes the accumulation of lysosomal VV in doxorubicin-resistant ovarian cancer by disrupting cytoplasmic DNA sensing and viral defense mechanisms.[88] The FDA-approved drug ruxolitinib, a selective inhibitor of JAK-1/2, enhances the therapeutic activity of various OVs by counteracting antiviral JAK/STAT signaling, with no observed toxicity in numerous preclinical studies.[89] The combination of molecularly targeted therapy and tumor-targeting oncolytic VV represents a promising new approach to cancer therapy. Further development of tumor-specific molecules and corresponding therapeutic agents is necessary to enhance the efficacy of oncolytic virotherapy.

OVV and gene therapy

Inhibitors of intracellular proteins, as described above, can effectively silence the functions of their target proteins; however, not all intracellular proteins are amenable to inhibitor development. Gene therapy offers an alternative approach by selectively silencing specific intracellular genes to achieve targeted functional blockade, or by introducing specific genes for expression. With advances in gene therapy, the combination of OVV and gene therapy has shown considerable promise. Recently, microRNAs (miRNAs)–single-stranded, mature RNA molecules approximately 22 nucleotides in length–have been explored as therapeutic agents, particularly for cancer treatment. miRNAs can function as oncomiRs by targeting tumor suppressor genes or act as tumor suppressors by targeting oncogenes. These molecules exert their effects by binding to complementary sequences in the 3′-untranslated regions (3′-UTRs) of mRNAs, thereby inhibiting post-transcriptional gene expression. Consequently, the integration of miRNA therapy with OVV has the potential to enhance the safety and efficacy of oncolytic virotherapy by removing disease-promoting genes from the virus.

OVV and metabolic reprogramming

Metabolic alterations play a significant role in tumorigenesis and cancer progression. The energy metabolism of cancer cells differs markedly from that of normal cells, with tumor cells often exhibiting a phenomenon known as the "Warburg effect".[90] In this process, cancer cells preferentially produce lactic acid through glycolysis, even in the presence of sufficient oxygen, which contributes to the synthesis of various biomolecules.[91,92] In addition to the Warburg effect, several other forms of metabolic reprogramming occur, enabling the tumor microenvironment to be modified in ways that promote tumorigenesis and progression.[93] Numerous studies have explored how metabolic reprogramming can influence the susceptibility of tumor cells to OVs. For example, Arthur Dyer *et al*[94] demonstrated that antagonizing glycolysis and promoting the reductive carboxylation of glutamine enhanced the activity of oncolytic adenoviruses in cancer cells. Similarly, Barry E. Kennedy *et al*[95] showed that inhibiting pyruvate dehydrogenase kinase increased the antitumor efficacy of oncolytic reovirus.

OVV and other therapies

Gene therapy represents a promising approach for tumor treatment, with RNA-based gene therapies being explored in combination with OVV therapy.[96,97] However, akin to hormone therapy[98] and dietary interventions,[99] the potential of exogenous substances to enhance the antitumor effects of OVV–rather than directly inducing tumor cell death in the traditional sense–limits the focus of this discussion. Consequently, this aspect will not be further elaborated upon here.

Clinical Progress in OVV

As research on OVV advances, several transgenic recombinant OVVs have entered clinical trials. One of the most effective strategies to enhance the tumor-specific targeting of OVV is the deletion of the *TK* gene, which, when combined with the insertion of additional therapeutic genes, can improve antitumor efficacy. A Phase III randomized, double-blind clinical trial of OVV for the treatment of stage II melanoma was conducted as early as 1995. Unfortunately, this trial showed no significant differences in disease-free intervals or overall survival between the treatment and placebo groups.[100] Despite these disappointing results, research into OVV continues, and ongoing advancements in our understanding of VV

and tumor treatment have led to several optimizations aimed at improving clinical outcomes. Currently, only two OVVs have entered Phase III clinical trials worldwide, one of which is JX-594. The backbone of JX-594 is the Wyeth strain, initially developed as a tumor vaccine expressing GM-CSF under the name Pexa-Vec.[101] After about a decade, Pexa-Vec was found to have oncolytic potential, prompting its renaming to JX-594. This strain was subsequently acquired by Jennerex Biotherapeutics after SilliJen Biotherapeutics filed the first patent for it.[102] JX-594 has been tested in multiple Phase I/II clinical trials involving patients with hepatocellular carcinoma, showing that it can effectively secrete GM-CSF and elicit comparable antitumor immune responses.[45,103] In 2013, a randomized Phase II trial demonstrated that JX-594 treatment resulted in significant improvements in patient survival, with a dose-dependent effect observed: median survival was 14.1 months for the high-dose group and 7 months for the low-dose group (hazard ratio = 0.39; $P = 0.020$). In addition, another related study showed that JX-594 could induce antibody-mediated complement-dependent tumor cell killing.[104] However, a Phase III trial of JX-594 combined with the TKI sorafenib for the treatment of hepatocellular carcinoma (NCT02562755) did not yield optimal results, as it failed to extend overall survival in patients. As noted by Jeong *et al*[78], JX-594 exhibits differential replication patterns in mouse models compared to humans, meaning that the results obtained from preclinical animal studies may not be directly applicable to large human populations. In this context, it is crucial to investigate the mechanisms underlying the synergistic effects of JX-594 when combined with sorafenib. Specifically, if JX-594 is capable of sensitizing cells that are otherwise resistant to sorafenib, it is important to understand how this sensitization occurs. However, this mechanism has not yet been thoroughly explored.[78] Expanding the research in this area could potentially enhance the success rates of such therapeutic combinations.

Another OVV that made it to phase III is GL-ONC1 (name of GLV-1h68 after entering clinical trials) with a Lister strain backbone. GL-ONC1 is a replication-competent lysogenic VACV that is endowed with the ability to replicate selectively in tumor cells by deletion of the J2R, F14.5L, and hemagglutinin genes, and is replication-blocked in normal cells,[105] followed by insertion of the genes encoding β-glucuronidase, β-galactosidase, and the Ruc-GFP marker.[106] A 67-year-old ovarian cancer patient with CA-125 of 4112 U/mL recurred after multiple chemotherapies, and when she received GL-ONC1 lysovirus therapy followed by chemotherapy, her CA-125 was reduced to 99 U/mL in the fourth cycle,[81] demonstrating the promising use of GL-ONC1. GL-ONC1 is safe in patients with locally advanced head and neck cancer (NCT01584284)[84] or peritoneal cancer (NCT01443260)[107] receiving standard radiotherapy, and has been tested in a phase II clinical trial in patients with ovarian cancer (NCT02759588). The results can be satisfactory in phase I and phase II clinical trials, and the phase III clinic has not yet been concluded. GL-ONC1 has the ability to transform immunologically "cold" tumors into "hot" tumors by modulating the tumor microenvironment and overcoming platinum drug resistance.[106] However, as previously mentioned, the exact mechanism by which GL-ONC1 eliminates platinum drug resistance remains unclear.[106] From the perspective of these clinically advanced OVVs, the role of OVVs in reversing chemoresistance in tumor cells appears to be similar. A deeper understanding of the mechanisms underlying this phenomenon would be valuable for the future design of targeted OVVs. Furthermore, as GL-ONC1 is an OVV capable of disrupting the TME matrix, it could potentially be combined with immune-enhancing strategies, such as arming the OVV with GM-CSF. This combination could theoretically amplify the anti-tumor immune response.

In addition to the two OVVs discussed earlier, several other OVVs have reached the clinical stage. A summary of key OVV-related clinical trials is provided in the following tables. The major OVV clinical trials that have been completed or terminated, as reported by Li *et al*[108] in September 2023, are cited as complete and have not been updated. However, significant new OVVs that have since entered clinical trials have been included in the updated tables [Supplementary Tables 1, 2, and 3, http://links.lww.com/CM9/C407].

Issues to be Addressed

As an OVT, the body's immune response to foreign antigens is an expected outcome. Such reactions may diminish the effectiveness of OVV treatment, for instance, through the neutralization of the virus by preexisting or newly generated antibodies. In addition, these immune responses could potentially lead to side effects beyond the intended tumor cell lysis, such as off-target effects that result in damage to normal cells. Efforts are ongoing to address and mitigate these challenges.

Protecting OVV safety through immunity interception

As with other OVs, intratumoral injection of OVV has proven effective; however, this approach is limited by its applicability to tumors and metastatic cells that are difficult to inject directly. Consequently, preventing OVV clearance by the immune system has become a focal point of research.[109] Similar to other viruses, the entry of VV into cells stimulates the secretion of IFNs, triggering an inflammatory response. This is followed by an influx of innate immune cells, such as NK cells, to clear virus-infected cells. In addition, VV presentation to T cells by APCs activates an adaptive immune response. Given that smallpox has been eradicated and most individuals have not been vaccinated against it, there is a lack of preexisting antibody responses to VV, suggesting that OVVs could be delivered systemically via intravenous injections. However, this is only effective for a single injection; a second injection still faces the immune response induced by the first. One promising strategy to evade immune clearance is encapsulation, where biofilms, liposomes, or polymers are used to shield OVVs from immune detection. Narayanasamy *et al*[110] demonstrated the use of poly(lactic acid)-hydroxyacetic acid copolymers (PLGAs) to encapsulate OVVs, which showed significant antitumor efficacy against a CT26 cell line, highlighting the

feasibility of this approach. Another promising modality involves the use of cellular vectors for tumor cell delivery. Dobrin *et al*[111] showed that adipose-derived stem cells (ADSCs) could effectively eradicate drug-resistant tumor cells through enhanced viral amplification and sensitization of tumor cells to viral infection. This approach is particularly valuable in cases where traditional encapsulated vectors are not suitable, such as in the challenge of penetrating the blood–brain barrier. However, challenges remain for these methods, including the significantly increased cost of viral encapsulation and the difficulty of scaling up production, which present obstacles to commercial viability.[112] Furthermore, the impact of the encapsulation layer on the therapeutic efficacy of OVVs requires further investigation. It is evident that balancing antiviral and antitumor immunity is crucial, and efforts to reduce the immunogenicity of OVVs are currently a major focus of research.

Ensuring the safety of OVV subjects

OVs are designed to selectively target and destroy tumor cells; however, this effect is not flawless, and there is a risk of inadvertent infection of normal cells,[113] which can be adversely affected.[114] A significant side effect of OVV therapy is that viral entry into cells triggers the release of IFNs from infected cells, which can cause cytotoxicity in neighboring non-infected cells, leading to their death.[115] In addition, the activation of the immune system by the virus results in prolonged release of pro-inflammatory cytokines, and excessive or prolonged inflammation can exacerbate the pathology. Although this issue has not yet been fully addressed in studies involving VV, the virus's greater compatibility with exogenous genes may offer potential solutions, such as loading genes that specifically target proteins on the tumor surface.

OVVs retain the self-replicating ability of VV, which introduces the potential risk of adverse reactions in patients who have been vaccinated with OVVs.[116] Despite rigorous monitoring of the vaccination status of patients before OVV administration, there remains a risk of harm, particularly in oncology patients, many of whom have compromised immune systems, especially after chemotherapy.[117] To minimize these risks, OVV developers typically use two strategies. One approach involves direct modification of the VV genome to remove virulence factors (such as thymidine kinase, or *TK*, as previously discussed) or the insertion of genes encoding cytokines.[118–120] The other approach is to use pharmacological control, although no specific drug has been approved for treating VV infections. In an experiment to develop an HIV-1 vaccine using replicative VV as a vector, Zhang *et al*[49] introduced the HSV-TK/GCV suicide system into the replicative VV vector for the first time. In this system, HSV-TK was incorporated into VV, and exogenous administration of ganciclovir (GCV) eliminated VV-infected cells, thereby controlling the replicative VV without posing a risk to immunocompromised individuals. Although the introduction of HSV-TK into OVVs may not be directly applicable (as HSV-TK might interfere with the selective replication of tumors in the absence of VV-TK), this approach provides valuable insights. For example, OVs like T-VEC, based on HSV-1, can use similar strategies to ensure safety. Other suicide gene systems have also been successfully integrated into OVV constructs.[121] These findings offer new avenues for enhancing OVV safety, and alternative suicide gene systems could be explored in OVV development to further ensure patient safety.

Antitumor effects of OVV when administered alone

The effects of OVV when administered alone are generally suboptimal, often due to inadequate retention of the virus in the body or insufficient intensity of its action. As with other OVVs,[122,123] maintaining OVV in the body long enough to achieve the desired therapeutic effect is a significant challenge. Achieving this through multiple high-dose injections is difficult and raises safety concerns due to the virus being a foreign antigen. Consequently, OVV treatment is typically combined with small molecule antitumor drugs, which further raises biosafety concerns, as well as increases both the cost and discomfort for patients. A more comprehensive understanding of viral biology and immune responses is necessary to identify more suitable modifications to OVVs that could enhance their antitumor efficacy while minimizing these challenges.

Determination of tumors suitable for OVV treatment

Different types of tumors exhibit varying sensitivities to specific OVVs as they progress. For example, T-VEC, an OV based on HSV-1, has shown promising results in the clinical treatment of gliomas but has been less effective in the treatment of melanomas.[124] OVVs, like other therapeutic approaches, carry inherent risks. Furthermore, most patients in clinical trials have undergone other treatments, such as chemotherapy, which alters their immune systems, making them different from healthy individuals. In addition, tumor cells may have undergone changes that complicate the prediction of OVV efficacy in these patients.

Summary and Discussion

Since the discovery of tumor regression following natural viral infections,[2,3] the use of viruses for cancer treatment has become a promising avenue in cancer immunotherapy, with VV emerging as a prominent subject of research in this field. OVV, like other OVs, infects tumor cells by replicating within them, inducing immunogenic cell death and triggering a systemic anti-tumor immune response, accompanied by the release of tumor antigens. However, OVVs possess broad cytotropism, and the inadvertent infection of normal cells is a significant concern.

With the advent of gene editing technologies, researchers have been able to modify VV, and through further investigation of tumor cells, they discovered that the deletion of VV's *TK* gene does not hinder its replication in tumor cells, while inhibiting replication in normal cells. This modification has become a foundational strategy for developing OVV, with other modifications typically built upon it. Currently, OVV modifications generally focus

on two main areas: enhancing its replication and spread within tumor tissue, and boosting the anti-tumor immune response triggered by tumor cell lysis.

However, the limitations posed by the immune system's ability to eliminate exogenous substances and the variability between individuals present challenges in maintaining OVV activity. Therefore, combining OVV therapy with other anti-tumor treatments to boost efficacy and applicability is crucial. The failure of the Phase III clinical trial of JX-594 underscores the importance of selecting the appropriate combination therapy, as well as carefully considering the timing and sequence of treatment administration.[125] The success observed in mouse models with the administration of JX-594 in combination with sorafenib was not replicated in the Phase III clinical trials. The mechanism by which JX-594 reverses sorafenib resistance in tumor cells, as observed in animals, requires further investigation to clarify its potential for success in human applications. In addition, IL-2, a cytokine secreted by Th1 cells that plays a key role in counteracting Th1 and CTL responses, has attracted significant attention for its role in promoting anti-tumor immunity. Although IL-2 is critical for cellular immunity against tumors, it has been found to induce Treg activity, complicating its therapeutic use due to its dual effects. This has shifted research focus toward IL-12, a cytokine believed to more purely drive the differentiation of Th0 cells into Th1 cells, followed by the later recruitment of DCs through GM-CSF. Although both IL-12 and GM-CSF have shown efficacy, they may not offer a perfect solution. It is well established that in the design of infectious disease vaccines, adjuvants play a more significant role in enhancing the immune response than cytokines. Adjuvants generally fall into two categories: those that act as toll-like receptor (TLR) agonists and those that alter the form of the antigen (e.g., transforming the antigen into particles that are more likely to stimulate an immune response or slowing its release). However, adjuvants like aluminum or oil-in-water, which alter the antigenic structure, are unsuitable for tumor immunity. A prevailing theory is that tumor cells lysed by OVVs release antigens that activate TLRs, which, if validated, could enhance anti-tumor immunity. Moreover, metal ions such as Mn^{2+} and Zn^{2+} have been shown to stimulate immune pathways associated with anti-tumor responses (e.g., cGAS-STING), while also mitigating the nonspecific toxicity of cytokines.[126,127] This "metal immunotherapy" approach could represent a novel adjuvant for enhancing OVV therapy.

Another challenge in the development of OVV therapy is the targeting mechanism, which is currently limited to strategies that involve arming OVVs with cytokines to suppress the immune response and ensure viral survival. However, there has been little research on the active targeting of OVVs, and the full potential of this approach remains unexplored. The high failure rate of Phase III clinical trials involving OVVs raises important considerations for their design. Traditionally, OV design has focused on the expression of cytokines or ICIs to enhance immune responses. However, the numerous failures in clinical trials prompt questions about whether cytokines or ICIs are the most effective immune adjuvants for OV therapy. This has led scientists to consider whether enhancing the replicative ability of OVVs to improve their inherent tumor-lytic effects may be more beneficial. Nonetheless, the potential side effects of excessive viral replication must be carefully considered, which is one of the reasons why OVVs cannot currently be administered intravenously. Nanoparticles (NPs) enable the encapsulation of OVs through physical interactions. With particle sizes ranging from 1 to 100 nm, NPs can passively accumulate in tumor tissues due to the enhanced permeability and retention effect[128]. Recently, researchers have increasingly used biomimetic nanomaterials, such as vesicles derived from cells, as viral vectors to reduce the immunogenicity of the vector material. The combined function of NPs and OVs is to protect the OVs from rapid immune clearance through physical shielding, while enhancing their tumor tropism via chemical modifications.[129] Cell-based drug delivery systems offer several advantages over NP-based systems, including prolonged circulation time, improved efficacy, controlled drug release, and reduced immunogenicity and cytotoxicity.[130] However, the effects induced by OVV replication within these systems are more difficult to characterize. These strategies, however, can also be applied to enhance OVV delivery.

The suicide system, such as HSV-TK in combination with GCV, has been used in the context of replicative VV products to control side effects. However, for OVVs requiring TK deficiency, introducing external sources of TK is not ideal. Alternative approaches should be explored. One example is the 5-CFU system, a chemotherapeutic agent that ensures OVV safety, while its potent tumor cytotoxicity contributes to anti-tumor effects. The unique metabolic characteristics of tumor cells can also be targeted in OVV design, as tumor cells exhibit distinct metabolic patterns compared to normal cells.[131] Designing OVVs based on the inherent characteristics of VV itself offers a novel direction for OVV development.[132] Barry *et al*[133] conducted a systematic review on the metabolic reprogramming of tumors in the context of OVVs. Glycolysis, the classic metabolic pathway in tumor cells, is an area of concern for OV design because disrupting glycolysis may hinder OV replication. Balancing these challenges could open new avenues for OV design. In addition, RNA-based gene editing tools, such as those used by Yi *et al*[134] to trace the function of the tumor suppressor gene *TP53*, could be used to restore normal cellular phenotypes in OV-infected tumor cells. This approach might allow for the selective removal of cells without compromising VV replication. Combining this with gene silencing strategies to target specific tumor genes could enhance OV replication and efficacy. Unfortunately, OV designs targeting the metabolic features of tumor cells are less common than those focused on immune modulation. However, combining both approaches could improve OV efficacy, capitalizing on VV's strong compatibility with exogenous genes. These concepts may provide an alternative strategy for enhancing OV therapy.

The field of OV is evolving rapidly with promising results, but the success of clinical trials must be carefully evaluated to optimize therapeutic outcomes. Despite the challenges, the future of OV therapy remains bright. VV's compatibility with exogenous genes offers a unique advantage, and

improving the active targeting of OVVs via gene insertion could enable the treatment of systemic metastatic tumors, making OV therapy a promising option for cancer patients. Continued innovation and research are essential to realizing the full potential of OVVs and establishing them as a cornerstone of cancer treatment.

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Conflicts of interest

None.

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