# Resistance to oncolytic virotherapy: Multidimensional mechanisms and therapeutic breakthroughs (Review)

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**Abstract.** Oncolytic viruses (OV) are an emerging form of immunotherapy that utilize naturally occurring or engineered viruses to specifically infect and lyse tumor cells. They achieve tumor treatment through direct tumor cell killing or by inducing immunogenic cell death to enhance immune responses. However, the efficacy of OV has been suboptimal in clinical trials. This review comprehensively examines mechanisms of resistance to OV through three interconnected dimensions: The characteristics of tumors and tumor cells, factors related to stromal cells and the extracellular matrix (ECM) and the host immune status. Potential solutions targeting these mechanisms are also proposed. For instance, OV typically achieve tumor selectivity through tumor-specific receptors or specific promoters. However, due to inter- and intratumoral heterogeneity, the lack of such specific receptors or promoters in tumor cells can lead to off-target effects of OV, resulting in treatment resistance. The ECM in the tumor microenvironment, such as hyaluronic acid, may also impede viral transport. Additionally, the clearance of OV by immune cells can contribute to suboptimal therapeutic outcomes of OV treatment. Consequently, investigating predictive biomarkers of OV efficacy, utilizing ECM-degrading enzymes and combining with immune checkpoint inhibitors represents a promising strategy to augment the therapeutic effects of OV. Synthesizing current evidence, it is anticipated that future investigations will optimize the therapeutic effects of OV treatment and bring better immunotherapeutic outcomes for cancer patients.

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*Abbreviations:* Ad, adenovirus; CAFs, cancer-associated fibroblasts; CAR, Coxsackie and Ad receptor; CPA, cyclophosphamide; CTLA-4, cytotoxic T-lymphocyte associated protein 4; CXCR4, C-X-C chemokine receptor type 4; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; EVs, extracellular vesicles; FAP, fibroblast activation protein; FR, folate receptor; GFRα1, GDNF family receptor α1; GSDME, gasdermin E; HA, hyaluronic acid; HER-2, human EGFR2; HMGB1, high-mobility group box 1; HPV, human papillomavirus; HSV, herpes simplex virus; ICB, immune checkpoint blockade; ICD, immunogenic cell death; IFN, interferon; ISGs, IFN-stimulated genes; JAK/STAT, Janus kinase/signal transducer and activator of transcription; JAM-A, junctional adhesion molecule-A; LDLR, low-density lipoprotein receptor; MMPs, matrix metalloproteinases; MSI, microsatellite instability; MV, measles virus; MXRA8, matrix remodeling associated 8; NDV, Newcastle disease virus; NETs, neutrophil extracellular traps; NK cells, natural killer cells; NLR, neutrophil-to-lymphocyte ratio; NSCLC, non-small cell lung cancer; OV, oncolytic virus; PANoptosis, pyroptosis, apoptosis and necroptosis combined cell death; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PDAC, pancreatic ductal adenocarcinoma; PI3K, phosphoinositide 3-kinase; PRRs, pattern recognition receptors; PSMA, prostate-specific membrane antigen; RAS, rat sarcoma; T-VEC, Talimogene laherparepvec; TGF-β, transforming growth factor β; TILs, tumor-infiltrating lymphocytes; TMB, tumor mutational burden; TME, tumor microenvironment; TNF-α, tumor necrosis factor α; TNBC, triple-negative breast cancer; TRAIL, TNF-related apoptosis-inducing ligand; UPAR, urokinase-type plasminogen activator receptor; UPR, unfolded protein response; VEGF, vascular endothelial growth factor; VSV, vesicular stomatitis virus

*Key words:* oncolytic virus, treatment resistance, immune checkpoint, extracellular matrix

## Contents



## 1. Introduction

Cancer remains one of the leading causes of mortality worldwide, driving the relentless pursuit of novel therapeutic strategies (1). Oncolytic virus (OV) therapy is a novel treatment

that utilizes viruses to specifically infect and lyse tumor cells. Unlike traditional radiotherapy and chemotherapy, OVs work through the following mechanisms: Selectively infecting tumor cells, inducing immunogenic cell death and enhancing immune responses. OVs have been explored across a broad spectrum of malignancies, encompassing various solid tumors and some hematological cancers. However, despite decades of research and promising preclinical data, the clinical efficacy of OV therapy, as measured by objective response rates and durable remissions, has often been suboptimal in larger trials. While some patients experience significant and sometimes long-lasting benefits, a substantial proportion derive limited or no clinical benefit (2). This discrepancy between promising mechanisms and variable clinical outcomes highlights a critical challenge: Resistance to OV therapy.

The development of resistance is a complex and multifaceted phenomenon, likely arising from dynamic interactions within the intricate ecosystem of the tumor and its microenvironment (TME). Factors contributing to this resistance span multiple interconnected levels: The heterogeneity of patients and tumors, changes in the components of tumor stromal cells and extracellular matrix (ECM), and the complexity of the tumor immune microenvironment. This review aims to provide an in-depth understanding of the reasons for OV resistance and offer a basis for future research directions and optimization of treatment plans (Fig. 1).

## 2. Tumor cells

The foundation of OV application lies in its ability to specifically infect tumor cells, replicate within them and not harm normal cells. This is typically achieved by exploiting the hallmarks of cancer cells, such as differences in surface protein expression, metabolic characteristics and genetic features. However, due to the significant heterogeneity of tumors, when the strategies targeting the tumor are not applicable to the respective patient, resistance to OV therapy can occur (3).

*Virus entry*. OVs typically require the recognition of specific membrane receptors to enter tumor cells. Taking advantage of the receptor recognition process is an important approach for achieving tumor selectivity for OVs. Certain OVs are designed to target tumor cells via natural receptors, which have differing expression levels between tumor and normal cells (4). For instance, Nectin-like protein 5 (CD155) is the natural receptor for poliovirus and is typically weakly expressed in normal human tissues but widely overexpressed in malignant tumors, and is associated with poor prognosis and tumor progression (5,6). Adenovirus type 5 (Ad5) infects cells via the Coxsackie and Ad receptor (CAR), while Ad3 has a different tropism and enters cells through CD46 (7). Expression of CAR and CD46 was reported to influence therapeutic effectiveness of oncolytic virotherapy, while neither CAR nor CD46 is highly expressed in all tumors (8-10). Although not all viral sensitivities are associated with the expression levels of viral receptors, the presence of Galectin-1, which is involved in the entry of H-1PV, a kind of protoparvovirus, into cells, was found to have a positive correlation with the cell-killing effect induced by H-1PV (11,12). Cell lines of pancreatic ductal adenocarcinoma are known to exhibit reduced expression of the low-density lipoprotein receptor, which is also the receptor for vesicular stomatitis virus (VSV) and pancreatic ductal adenocarcinoma cells thereby had an OV-resistant phenotype (13). Besides natural receptors, another strategy is to genetically modify the virus to target tumor-specific membrane antigens, such as CD20 (14), epidermal growth factor receptor (EGFR) (15) and human EGFR2 (16). Notably, tumor heterogeneity poses a significant clinical challenge. The absence of these receptors on tumor cells compromises OV specificity and infectivity, thereby driving therapeutic resistance (17). The macrophage receptor with collagenous structure (MARCO) exhibits context-dependent duality in viral pathogenesis. As a viral entry facilitator, MARCO directly binds herpes simplex virus type 1 (HSV-1) glycoprotein C to mediate keratinocyte adsorption (18), enhances vaccinia virus infection in skin epithelia (19) and serves as an Ad entry receptor via hexon protein interactions in macrophages (20). Consistently, Ghosh *et al* (21) found that MARCO-deficient mice exhibited significantly reduced morbidity and mortality due to influenza pneumonia compared to wild-type controls. Conversely, in systemic defense roles, $MARCO^+$ lymphatic endothelial cells sequester arthritogenic alphaviruses in draining lymph nodes to limit viremia, while $MARCO^+$ Kupffer cells clear circulating virions in the liver (22). In respiratory immunity, High *et al* (23) identified a loss-of-function single nucleotide polymorphism in the human MARCO promoter that ablates gene expression, amplifies *in vitro* inflammatory responses to respiratory syncytial virus (RSV) infection, and confers an increased risk of severe disease in two independent pediatric cohorts with RSV. Examples of OV achieving tumor selectivity through natural receptors and tumor-specific antigens are listed in Table I (24-33). Additionally, mutations in the PI3K/AKT pathway within tumor cells have been found to be associated with the ability of CF33, a novel chimeric orthopoxvirus, to enter tumors (34). However, AKT mutations occur at low frequencies (~3-5%) and in tumors without mutations in the AKT pathway, this OV may exhibit therapeutic resistance (35). The successful and selective entry of OVs into tumor cells is a crucial step in their therapeutic effectiveness. However, due to the heterogeneity of tumor cells, some may resist the entry of OVs, which directly affects the therapeutic outcome. If this resistance mechanism cannot be overcome, OV therapy will struggle to spread effectively and exert its function, potentially leading to treatment failure. Therefore, understanding and addressing the impact of tumor heterogeneity on OVs is vital for improving the success rate of OV therapy (36).

*Virus replication*. Another feature that makes OVs selectively target tumor cells is their ability to selectively replicate and proliferate within tumor cells. This selectivity relies on the characteristics of high metabolism and rapid division of tumor cells, as well as mutations in tumor genetic features (37). The world's first approved OV, H101, has the E1B 55kD gene deleted (38). Cells infected with the virus lacking the E1B 55kD gene are more susceptible to p53-induced apoptosis, which is typically mutated in tumor cells, leading to selective replication of OV in tumor cells (39). Modification of E1A protein, whose interaction with retinoblastoma protein (Rb) is the initiation for Ad infection, is a common approach to achieve selective replication in tumor cells (40). Mutations

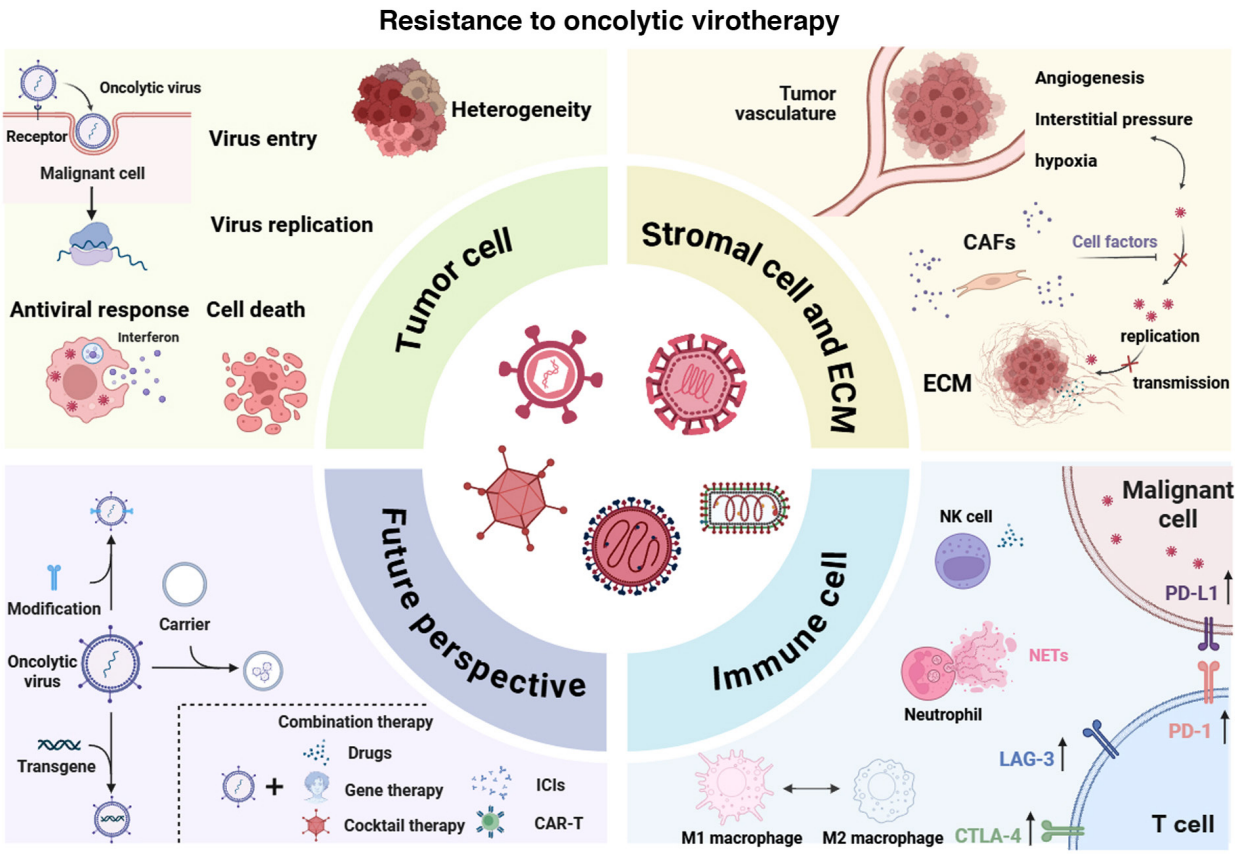


Figure 1. Schematic diagram of the main content of this review. The figure summarizes the multiple layers of resistance that limit the success of OV. In the tumor center, heterogeneous malignant cells with variable receptor expression, hypoxia and antiviral signaling restrict initial virus entry and replication. Peripherally, a dysregulated vasculature-characterized by leaky vessels, high interstitial pressure and angiogenic growth factors-impairs intratumoral OV distribution. CAFs and a dense ECM constitute physical and biochemical barriers that further diminish viral spread. Innate and adaptive immune cells can either promote or suppress OV activity. Therapeutic strategies depicted include combination therapy with ICIs, CAR-T cells, gene-armed OVs and drug cocktails designed to overcome these barriers. CAFs, cancer-associated fibroblasts; CAR-T, chimeric antigen receptor T cell; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; ECM, extracellular matrix; ICIs, immune-checkpoint inhibitors; LAG-3, lymphocyte-activation gene 3; NETs, neutrophil extracellular traps; NK, natural killer; OV, oncolytic virus; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1.

in the E1A gene prevent it from binding to the Rb, which diminishes the virus's replication capability in normal cells. However, due to the typical dysregulation of the Rb gene and over-activated state of E2F in tumor cells, the virus is able to replicate within them (41,42). However, the E2F family is not activated in all tumors. Among the target genes of E2F, some have been identified as negative regulators of growth, which can suppress tumor growth (43-45). High activity of telomerase is a crucial characteristic that allows tumor cells to maintain telomere length and achieve immortality, which is different from the low activity of telomerase in normal cells (46,47). Based on this, controlling the replication of OVs selectively in telomerase-expressing cells can make the OVs tumor-specific (48,49). Using the carcinoembryonic antigen (CEA) promoter, which is highly expressed in numerous tumors, is another approach for targeting tumor cells (50-52). However, telomerase is also expressed in many normal cell types, such as stem cells, germline cells and lymphocytes, which can diminish the targeting specificity of OVs (53). CEA is not universally expressed in all cancers and it has also been detected in mature normal cells, such as goblet cells of the colon and epithelial cells of the prostate (54). In addition, certain OVs can be engineered to possess tissue specificity. For instance, OVs controlled by the cyclooxygenase-2 (COX-2) promoter can specifically target breast cancer, while those with prostate-specific antigen (PSA) selectivity can target prostate cancer (55,56). However, it has been reported that only ~36% of patients with breast cancer express COX-2 and PSA is also expressed in normal prostate tissue and can be elevated in prostatitis (57,58). Notably, the incorporation of a transgene that replaces the E3 region of an HAdV-5 vector enables replication-dependent expression of the transgene in human A549 cells, but not in the murine 4T1 cells (59). The metabolic and genetic alterations in tumors are specific, and when the characteristics of tumor cells change, understanding their overall condition is crucial to minimize resistance to OV therapy.

*Cell death*. After OVs enter tumor cells, sufficient survival time is critical for viral replication and gene expression. Striking a balance is essential: Premature cell death may abort viral replication, while delayed death allows progeny accumulation. This balance varies across cell death modalities impacting therapeutic efficacy (60). Cell death induced by OVs is a form of immunogenic cell death. The oncolytic effect is an important way for OVs to combat tumors (61). OVs can induce tumor cell death through various mechanisms, including apoptosis, necroptosis and pyroptosis.

Table I. Examples of OV achieving tumor selectivity through natural receptors and tumor-specific antigens.

A, OV targets tumor cells through natural receptors

| Name | Viral type | Target | Target characteristics and expression status | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| Ad5/F35 | Ad5 | CAR and CD46 | Widely expressed in the majority of tumor cells | Multiple tumors | (7) |
| MeV-SCD | MV | CD46 | The expression level varies in tumors, which affects the efficacy of OV therapy | Neuroendocrine neoplasms | (9) |
| Ad5/F11p | Ad5 | CD46 | The expression level varies in tumors, which affects the efficacy of oncolytic virus therapy | Bladder cancer | (10) |
| H-1PV | Protoparvovirus | Galectin-1 | Positively correlated with the cytotoxic effect of H-1PV | PDAC | (12) |
| - | VSV | LDLR | Low expression in pancreatic ductal adenocarcinoma cells, leading to resistance to VSV. | PDAC | (13) |
| - | Poliovirus | CD155 | Low expression in normal tissues, but widely overexpressed in malignant tumors and associated with poor prognosis | Multiple solid tumors (such as gliomas) | (30) |
| - | NDV | Sialic acid receptors | Affects the efficacy of OV therapy | - | (26) |
| - | Alphaviru s M1 | MXRA8 | Directly binds to the virus and its expression is positively correlated with the induced tumor lysis | Melanoma | (25) |

B, Targeting tumor overexpressed surface targets with engineered OV

| Name | Viral type | Target | Target characteristics and expression status | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| MVgreenH blindantiCD 20 | MV | CD20 | A marker of B-cell non-Hodgkin lymphoma and also the target molecule for rituximab | B-cell non-Hodgkin's lymphomas | (14) |
| gD:scEGFR Δ2-24 | HSV | EGFR | Lowly expressed in normal tissues but widely overexpressed in various malignant tumors | Glioblastoma, head and neck cancer, lung cancer, breast cancer | (15) |
| R-LM113/ R-LM249 | HSV | HER-2 | Overexpressed in a number of cancers | Malignant epithelial tumor | (16) |
| OV-Cmab-CCL5 | HSV | EGFR | As above | Glioblastoma | (33) |
| AdCMVLac Z 425-S11 | Ad | EGFR | As above | - | (27) |
| KGNEp | HSV | EpCAM | A recognized carcinoma-associated antigen; is associated with poor prognosis | Multiple tumors | (32) |
| Ad5-ffCXCL12 | Ad5 | CXCR4/ CXCR7 | Expression is associated with the occurrence and progression of breast cancer | Breast cancer | (31) |
| KNTc-gD:GDNF | HSV | GFRα1 | Low expression in normal tissues, but highly expressed in breast cancer | Breast cancer | (28) |
| R-593 | HSV | PSMA | Specifically overexpressed in prostate cancer; has been widely used in clinical applications | Prostate cancer | (29) |

Table I. Continued.

| B, Targeting tumor overexpressed surface targets with engineered OV | | | | | |
|---|---|---|---|---|---|
| Name | Viral type | Target | Target characteristics and expression status | Related tumor types | (Refs.) |
| VSVΔG-αFR | VSV | FR | Usually highly expressed in tumor tissues, while its expression is low or absent in normal tissues | Ovarian cancer and myeloid leukemia | (24) |

Ad, adenovirus; MV, measles virus; CAR, Coxsackie and adenovirus receptor; PDAC, pancreatic ductal adenocarcinoma; NDV, Newcastle disease virus; VSV, vesicular stomatitis virus; LDLR, low-density lipoprotein receptor; MXRA8, matrix remodeling associated 8; HSV, herpes simplex virus; HER-2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; CXCR4, C-X-C chemokine receptor type 4; GFRα1, GDNF family receptor alpha 1; PSMA, prostate-specific membrane antigen; FR, folate receptor; OV, oncolytic virus.

Apoptosis modulated by viral infection has been found to be mediated by a death-inducing signaling complex formed by death receptors, such as Fas, TNF-related apoptosis-inducing ligand-receptor and TNF-R. A study has reported that the introduction of the second mitochondria-derived activator of caspase/diablo IAP-binding mitochondrial protein gene can enhance tumor apoptosis and improve OV efficacy, thereby overcoming resistance to OV therapy caused by insufficient oncolytic effects (62). A study by Dobson *et al* (63) also found that the combination of VSV with an antagonist of apoptosis inhibitors can enhance the oncolytic effect, thereby improving therapeutic efficacy. Using Ad armed with secretable trimeric TNF-related apoptosis-inducing ligand promotes apoptosis and enhances the killing and therapeutic efficacy against glioblastoma (49). However, on the contrary, Zhou *et al* (64) discovered that animal cells infected with diarrhea virus promote apoptosis by upregulating the phosphorylation of p53, thereby inhibiting viral replication. Mansour *et al* (65) discovered that the resistance of tumor cells to apoptosis is the basis for the oncolytic selectivity of OVs. They used a non-small cell lung cancer (NSCLC) cell line overexpressing the anti-apoptotic protein Bcl-xL and found that it was resistant to early apoptotic stimuli from Newcastle disease virus (NDV), yet paradoxically exhibited increased apoptosis later on (65). This paradox arises as apoptotic resistance extends viral replication windows, facilitating syncytia formation to form syncytia. Stanziale *et al* (66) also found that cell death induced by HSV-1 OV infection in human gastric cancer cell lines may hinder the generation of viral progeny. The use of the apoptosis inhibitor N-acetylcysteine was shown to enhance the efficacy of the virus (66). These studies highlight the paradoxical and complex role of apoptosis in OV therapy, where both the inhibition and promotion of apoptosis can potentially enhance the efficacy of OVs. Apoptosis may facilitate tumor killing effects and could also prematurely terminate viral replication, reducing virus spread. The net effect may depend on gene mutation status, viral status and tumor type. Therefore, the role of apoptosis in the treatment resistance of specific OVs and particular cancer types requires further in-depth investigation.

Necroptosis, a form of programmed cell death, typically involves the activation of receptor-interacting protein kinase 1 (RIPK1) and RIPK3. RIPK1 and RIPK3 transmit signals by forming a complex known as the 'necrosome', leading to necroptosis (67). Oncolytic HSV intervention can induce early necroptosis after formation of multinucleated giant cells (68). Research by Okamura *et al* (69) found Coxsackievirus A11 could induce various modes of cell death in malignant pleural mesothelioma, including necroptosis. OV M1 can induce necroptosis in triple-negative breast cancer and its combination with doxorubicin further enhances the level of necroptosis (70). In a previous study, a necroptosis-inducing factor, MLKL, was inserted into Vaccinia viruses to further promote necroptosis in tumor cells and the therapeutic efficacy was found to be enhanced (71). In addition to focusing on the role of necroptosis in promoting tumor cell death, attention should also be paid to the potential impact of necroptosis on viral infection and replication. Wen *et al* (72) found that RIPK3-dependent necroptosis is induced and restricts viral replication in human astrocytes infected with Zika virus. Similar conclusions have also been made with pseudorabies virus (73). However, Hu *et al* (74) found that RIPK3-driven necroptosis induced by Coxsackievirus A10 actually enhances viral replication. Furthermore, Cotia poxviruses possess proteins that closely resemble the pseudoactive domain sequence of MLKL. By utilizing their kinase domain to sequester RIPK3, these proteins can impede the engagement and phosphorylation of MLKL, subsequently suppressing the process of necroptosis (75). The M45 protein from cytomegaloviruses (CMV) effectively suppresses necroptosis and the activation of the nuclear factor-κB pathway. This capability to inhibit necroptosis is vital for CMV's infection in certain cell types that are sensitive to this form of cell death, as well as for the virus's spread throughout the host organism (76). These results demonstrate that, from an evolutionary and genetic perspective, necroptosis is a way for host cells to clear viral infections. It is noteworthy that van den Wollenberg *et al* (77) found that reoviruses can induce necroptosis in mouse cells, but this is not common in human cell lines. This indicates differences between animal experimental models and clinical applications and may also account for the resistance to OVs observed in clinical trials.

Pyroptosis, orchestrated by the Gasdermin (GSDM) family, is another type of programmed cell death. It involves distinct

morphological alterations such as cellular swelling, breakdown of the plasma membrane and chromatin condensation. This process culminates in the discharge of pro-inflammatory factors from the cell, subsequently instigating an inflammatory response (78). Lin *et al* (79) discovered that oncolytic Parapoxvirus can promote pyroptosis in tumor cells by reducing the ubiquitination of GSDME, particularly in cells with low expression of GSDME. The recombinant measles virus vaccine, rMV-Hu191, also demonstrates oncolytic activity against esophageal squamous cell carcinoma through the induction of caspase-3/GSDME-dependent pyroptosis (80). Genetically modified OVs can more effectively induce pyroptosis to provide anticancer effects (81,82). Certain novel nanoparticle drugs are also used in combination with OVs to promote pyroptosis in tumor cells and enhance the effectiveness of cancer treatment (83). Furthermore, mouse CMV M84 protein engages with the pyrin domain found in absent in melanoma 2 (AIM2) and Apoptosis associated speck like protein containing a CARD, thereby inhibiting the formation of the inflammasome complex. This interaction can prevent caspase-1-dependent pyroptosis (84). Another protein from human CMV UL83 was also proven to interact with AIM2 and downregulate interleukin (IL)-1β, which is a vital molecule in pyroptosis (85). However, these results do not seem to focus on the impact of pyroptosis on the replication and spread of OVs. In fact, certain studies have found that pyroptosis can promote viral infection, including Coxsackievirus A16, A10, B3 and enterovirus 71 (86,87). By contrast, other studies have found that pyroptosis can enhance antiviral immunity and inhibit viral replication and spread (88,89). Furthermore, the chronic inflammatory tumor microenvironment (TME) induced by pyroptosis may promote tumor growth (90). Therefore, the therapeutic role of pyroptosis-inducing OV therapy within tumors requires further comprehensive exploration (91).

Autophagy involves the encapsulation and transport of cellular waste, including dysfunctional organelles and proteins, by autophagosomes to the lysosomes for destruction and subsequent recycling. It plays a vital role in preserving cellular stability, eliminating damaged elements and coping with stress (92). Lei *et al* (93) found that enhancing cellular autophagy via arming with an autophagic gene, Beclin-1, can boost the antitumor effects of vaccinia virus in leukemia and myeloma. However, autophagy may also impede the production of viral progeny. For instance, Li *et al* (94) discovered that the virus M1 induces autophagy in glioma cells, and inhibiting the inositol-requiring enzyme 1α-mediated autophagy pathway can enhance the oncolytic effect. Xu *et al* (95) also found that modifying and inhibiting cellular autophagy can enhance the effectiveness of OVs in pancreatic cancer.

Collectively, these findings suggest that different forms of cell death seem to play contradictory roles in viral infections. Based on this, it may be hypothesized that the death of tumor cells and the replication of OVs are in a delicate balance (Fig. 2). During the infection of tumor cells by OVs, if an overly strong cell death response is triggered, it can reduce the survival of tumor cells, but it may also inhibit viral replication and spread, thereby diminishing the therapeutic effect of the virus within the tumor. Therefore, in the treatment of different types of cancer, exploring the appropriate administration methods and dosages of OVs is crucial to achieve the best induction of cell death, enhance the therapeutic effect of the virus, and suppress tumor growth and metastasis to the greatest extent. Such targeted strategies can help increase the success rate of OV therapy and play a greater role in personalized cancer treatment to avoid OV resistance. The forms of tumor cell death caused by OVs and the impact of cell death on the efficacy of OVs are summarized in Table II (82,96-111).

*Anti-virus response*. The interferon (IFN) pathway is a primary route of cellular antiviral innate immunity (112). Pattern recognition receptors identify viral infections to activate signaling pathways, leading to the production of IFN. IFN induces the expression of IFN-stimulated genes (ISGs), and the antiviral function is carried out by numerous approaches, including degrading viral genetic material, blocking the synthesis of viral mRNA, preventing the transport of viral nucleoproteins and affecting the release of viruses (113). For instance, a recent study identified an ISG, tripartite motif containing 21, which can affect the ubiquitination of the channel transient receptor potential cation channel subfamily V member 2 through autocrine and paracrine mechanisms, leading to its degradation and thereby modulating the susceptibility of itself and neighboring cells to viral infections (114,115). Within the TME, OVs exhibit significant sensitivity to the antiviral state that is triggered by IFNs. This sensitivity is crucial because the IFN-induced antiviral response can substantially impact the efficacy of OVs (116). In addition, this antiviral effect is thought to be regulated by an intracellular amplifier circuit that depends on the unphosphorylated and phosphorylated forms of IFN-stimulated gene factor 3 (ISGF3) and gamma activation factor complexes, as well as IFN regulatory factor 1 (IRF1) to enhance or fine-tune the antiviral response by modulating the expression of other ISGs or IFNs (117). At the same time, viruses have developed various strategies to resist host elimination and to avoid IFN-dependent innate immunity, such as hiding their genetic material, disrupting interactions with host factors that trigger IFN responses, altering phosphorylation and ubiquitination pathways within host cells, degrading proteins essential for IFN stimulation through cleavage or tagging, controlling the transcription and translation of host genes, manipulating RNA processing and transport, and employing protein-based deception tactics (118). For instance, cells infected with Dengue virus can activate the cyclic guanosine monophosphate-adenosine monophosphate synthase (cGAS) pathway by releasing mitochondrial DNA, which in turn generates an antiviral immune response through the production of cyclic GMP-AMP (119). Additionally, it has been found that the cofactor of the NS2B protease from Dengue virus targets cGAS for degradation in lysosomes, thereby avoiding the detection of mitochondrial DNA during the infection. This process ultimately inhibits the production of type I IFNs in the infected cells (120). This is a game of cellular resistance to viral infection and viral evasion (121).

Cancerous cells that lack a functional IFN induction or response mechanism can gain benefits in terms of survival and proliferation. Consequently, the accumulation of IFN signaling pathway mutations is closely linked to the process of cancer development (122,123). The lack of IFN response in tumor cells is also one of the foundations of OV therapy for cancer. However, research has found that tumor cells still exhibit IFN

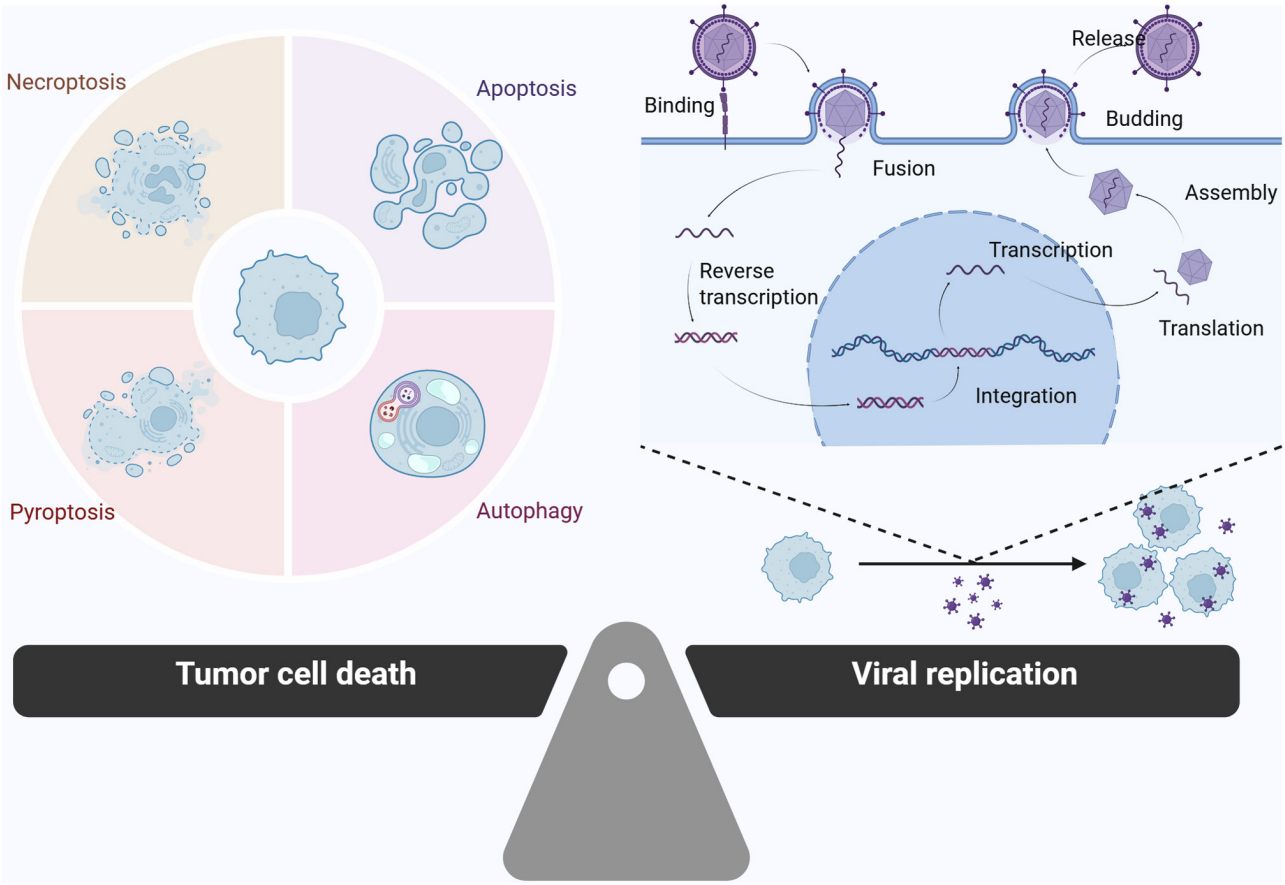


Figure 2. Balance between cell death and viral replication. Oncolytic viruses follow a multistep life cycle that determines whether tumor cell death or viral propagation predominates. The virus first binds to surface receptors, enters the cell and is trafficked to the nucleus or cytoplasm for uncoating. Viral genes are transcribed and translated, followed by genome replication and assembly of progeny virions that bud from the cell surface. Concurrently, infected tumor cells can undergo various death modalities-apoptosis, necroptosis, pyroptosis or autophagy - that limit viral yields but enhance immunogenicity. Conversely, successful viral replication may delay or inhibit these death pathways, perpetuating infection. The balance at any moment dictates net tumor cell killing vs. viral persistence.

responses and activation ISGs. Delaunay *et al* (124) found that in pleural mesothelioma, the homozygous deletion of IFN-I genes is very frequent and increases the sensitivity to oncolytic measles virus. However, they also discovered that even IFN-I$^{-/-}$ cell lines can still produce some IFN-I-independent IFN-I responses and pro-inflammatory responses when exposed to the virus (124). Lipatova *et al* (125) discovered that in patient-derived glioblastoma multiforme, the expression levels of ISGs are similar to those in normal cells, and the silencing of certain IFN pathway-related genes such as IFN-induced protein with tetratricopeptide repeats 3 and phospholipid scramblase 1 can actually reduce the virus's ability to enter cells. If there is activation of the IFN pathway in tumors, it may potentially develop resistance to OV therapy by interfering with viral replication and degrading viral RNA. In addition, the pathways involved in antiviral actions are characterized by both cell and virus specificity (113).

Furthermore, it is important to recognize the differences in viral immunity between animal experimental models and the human body. In patients deficient in ISG15, there is no increased sensitivity to the virus, a finding that stands in marked contrast to the findings with ISG15-deficient mice (126). These differences may also be one of the reasons for the resistance to OV treatment observed in clinical trials.

*Possible solution*. To avoid OV therapy resistance, comprehensive gene testing of the tumor may provide a personalized treatment plan for the patient. Screening for receptors of OVs can help select the type of OV to be used to help OV entry. For instance, the expression level of nectin-1 has also been described as being associated with the sensitivity to OV therapy derived from HSV (127). Matrix remodeling associated 8 has been identified as an entry receptor for the OV M1, and together with the intracellular factor zinc finger antiviral protein, it can serve as a predictive biomarker for OV therapy (128). Furthermore, certain gene variations were reported to be correlated with OV replication and OV sensitivity. For instance, Höti *et al* (129) found that the integrity of p21 in normal cells affects the normal life cycle of OVs and mutations in p21 can enhance Ad replication. If genetic testing can detect mutations in genes related to the IFN pathway, it may, to a certain extent, predict the sensitivity to OV therapy (130). The expression of CD24 can predict weaker antiviral capabilities and increased sensitivity to Zika virus in neuroblastoma cells (131). Mutations in IDH1 also lead to impaired type I IFN antiviral response in glioma cells (132). Other biomarkers can also be anticipated to be further discovered (133,134). For instance, tumor mutational burden (TMB) refers to the total number of mutations within a tumor, indicating the level of genomic instability in

Table II. Potential impact of different forms of cell death on resistance to OV.

A, Apoptosis

| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| H5CmTERT-Ad/TRAIL | Ad | Improve | Ad armed with secretable trimeric TRAIL promotes apoptosis and enhances efficacy | Glioblastoma | (49) |
| VSV-S | VSV | Improve | Introducing the Smac gene to promote apoptosis and overcome OV resistance | Breast cancer | (62) |
| VSVΔ51-GFP | VSV | Improve | Combining apoptosis-inducing agents can enhance the efficacy of OV | Rhabdomyosarcoma | (63) |
| - | NDV | Decrease | The activity and replication of OV are significantly enhanced in tumor cells overexpressing anti-apoptotic proteins | Non-small-cell lung cancer | (65) |
| NV1066 | HSV | Decrease | Combination with an inhibitor of apoptosis increased tumor killing | Gastric cancer | (66) |
| CV-B5/F | Coxsackievirus | Improvement | Oncolytic activity through apoptosis induced by endoplasmic reticulum stress | Non-small-cell lung cancer | (99) |

B, Necroptosis

| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| M1 | Alphavirus | Improvement | Combining with doxorubicin can significantly trigger necroptosis and inhibit tumor growth | Triple-negative breast cancer | (70) |
| MVA/MLKL | Vaccinia virus | Improvement | Insertion of MLKL gene promotes necroptosis and enhances therapeutic efficacy | Multiple tumors | (71) |
| - | Reoviruses | - | Reoviruses have difficulty inducing necroptosis in human cells | Colorectal adenocarcinoma | (77) |
| WR-GS | Vaccinia virus | Improvement | Combining with radiotherapy can enhance necroptosis and anti-tumor efficacy | Lung cancer | (98) |
| - | NDV | Improvement | Blocking necroptosis has blocked the immunogenic death of tumor cells | Glioma | (102) |

C, Pyroptosis

| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| rMV-Hu191 | MV | Improvement | Shows antitumor effect through caspase-3/GSDME-mediated pyroptosis | Esophageal cancer | (80) |

Table II. Continued.

C, Pyroptosis

| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| VSV-S | VSV | Improve | Introducing the Smac gene to promote PANoptosis to produce a more potent antitumor effect | Head and neck squamous cell carcinoma | (97) |
| rM1-mGSDME_FL | Alphavirus | Improvement | Arming gasdermin E can promote pyroptosis and improve the efficacy | Breast cancer | (82) |
| HSV-1Δ34.5/Δ47 | HSV | Improvement | Combining with novel nanoparticle drugs can promote pyroptosis and improve the effectiveness of treatment | Breast cancer and oral cancer | (106) |
| H101 | Ad | Improve | Promotes anti-tumor immunity via inducing pyroptosis | Melanoma | (109) |
| CVB3 | Coxsackie virus | Improve | Promotes gasdermin E-mediated pyroptosis to induce oncolytic activity | Colon cancer | (111) |

D, Autophagy

| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| M1 | Alphavirus | Decrease | Blocking the UPR-autophagy axis significantly enhances the anti-tumor effect | Glioma | (94) |
| $O^{Ad}$.R.shPKM2 | Ad | Decrease | Inhibiting autophagy can exhibit a potent cytotoxic effect | Pancreatic cancer | (95) |
| dl922-947 | Ad | Decrease | Inhibition of autophagy enhances the effects | Glioma | (96) |
| saOAs | Ad | Improve | Promotes anti-tumor effects through sonosensitizer-induced autophagy | Melanoma | (100) |
| - | NDV | Improvement | Inhibiting autophagy can inhibit tumor cell death and fail to enhance the efficacy of OV | Breast cancer | (105) |
| OBP-301/OBP-702 | Ad | Improvement | Ad armed with p53 induces autophagy and promote anti-tumor effect | Colorectal cancer | (108) |
| rMV-Hu191 | MV | Decrease | Inhibiting autophagy with inhibitors can enhance the efficacy of OV | Colorectal cancer | (110) |

E, Ferroptosis

| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
|---|---|---|---|---|---|
| - | NDV | Improvement | NDV kills tumor cells through ferroptosis | Glioma | (101) |
| KD01 | Ad | Improvement | Ferroptosis inducer increased the cytotoxicity of oncolytic virotherapy | Multiple tumors | (103) |

Table II. Continued.

| E, Ferroptosis | | | | | |
|---|---|---|---|---|---|
| Name | Viral type | Impact on the OV efficacy | Related findings | Related tumor types | (Refs.) |
| vvDD-IL15Rα | Vaccinia virus | Improvement | Ferroptosis inducer promotes OV efficacy | Multiple tumors | (104) |
| - | NDV | Improvement | NDV kills tumor cells through ferroptosis | Multiple tumors | (107) |

OV, oncolytic virus; Ad, adenovirus; VSV, vesicular stomatitis virus; HSV, herpes simplex virus; NDV, Newcastle disease virus; GSDME, gasdermin E; UPR, unfolded protein response; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; PANoptosis, pyroptosis, apoptosis and necroptosis combined cell death.

the cancer cells and microsatellite instability (MSI) describes the variation in microsatellite regions within tumor cells due to errors during DNA replication, reflecting a heightened mutation rate in these areas (135-137). They are commonly used as biomarkers to predict the effectiveness of immune checkpoint therapies. However, the relationship between TMB or MSI and the sensitivity and efficacy of OV therapy has remained to be elucidated. However, in addition to the heterogeneity between tumors leading to the failure of OV therapy, the heterogeneity within a tumor can also result in the following scenario: Even if the presence of a certain biomarker is detected, the OV can only have a limited effect on tumor restriction (138). Therefore, in this case, to better cover the mutated characteristics of the tumor, the combination of multiple different types of OVs can broaden the therapeutic spectrum of OV therapy, thereby enhancing the efficacy of OVs.

Rationally combining already clinically used antitumor drugs with OVs can potentially enhance the therapeutic efficacy of cancer treatment. Certain drugs can facilitate the entry of OVs. For instance, the use of histone deacetylase inhibitors can upregulate the expression of virus receptor CAR on the surface of tumor cells, thereby facilitating OV entry (139-141). Jaime-Ramirez *et al* (142) also found that histone deacetylase inhibition could increase the levels of reovirus receptor junctional adhesion molecule 1 in head and neck squamous cell carcinomas and the combination could facilitate both virus infection and tumor lysis. Certain drugs may enhance antitumor efficacy by promoting tumor cell death. Phosphoinositol-3 kinase (PI3K) inhibitors can enhance the antitumor effects of OVs by promoting the expression of death receptor 5 on multiple myeloma cells, thereby facilitating apoptosis (143). PI3K inhibitors have also been shown to enhance the activity against prostate cancer stem cells when used in combination with HSV, while no significant effects were observed when applying a combination of OV with radiotherapy, docetaxel or inhibitors of the Wnt or NOTCH pathways (144). It is worth mentioning the contradictory role of EGFR/RAS signaling in viral replication and tumor growth. The activation of the EGFR/RAS signaling pathway is quite common in tumors and plays a crucial role in their initiation and progression (145). Oncolytic HSV in combination with EGFR inhibitors such as erlotinib or monoclonal antibodies like cetuximab has shown superior therapeutic effects compared to monotherapy in the treatment of malignant peripheral nerve sheath tumors, pancreatic cancer and colorectal cancer (146-148). Jha *et al* (149) also found that the combination of sunitinib can inhibit innate immunity to enhance VSV oncolytic virotherapy in prostate, breast and kidney malignant tumors. Notably, the activation of the RAS signal is also an important factor for the selective infection and replication of certain OVs in tumors. For instance, the infection and the propagation of the virus following the death of cancer cells of reovirus requires the activation of the RAS signaling pathway (150,151). In addition, the presence of RAS mutations boosts the replicative capacity and oncolytic activity of M1 virus within cancer cells and dampening the RAS signaling pathway can hinder M1 infection and the subsequent cytopathic effects (152). Based on current evidence, it may be proposed that whether this combination yields synergistic or antagonistic effects may primarily depend on the specific stage of the viral life cycle involved. Thus, while EGFR inhibitors can suppress tumor growth and exert synergistic effects when combined with OVs, they may also impair the replication of RAS-dependent OVs (153). Therefore, it is conducive that such combinations should not be employed in a random or trial-and-error manner. Instead, they should be used logically, based on an understanding of the underlying mechanisms. Otherwise, it could more readily lead to therapeutic failure.

## 3. Stromal cells and ECM

Stromal cells such as cancer-associated fibroblasts (CAFs), endothelial cells and pericytes are integral components of the TME and exert significant regulatory effects (154). The intricate interplay between cancer cells and stromal cells, in addition to the interactions between immune cells and stromal cells, plays a critical role in the development, advancement, metastasis and resistance to therapy of solid tumors. Dalin *et al* (155) discovered that pancreatic stellate cells can induce drug resistance to gemcitabine treatment by the secretion of deoxycytidine through equilibrative nucleoside transporters. Besides promoting the formation of new blood vessels, factors originating from tumors can also modify the genetic transcription in endothelial cells of

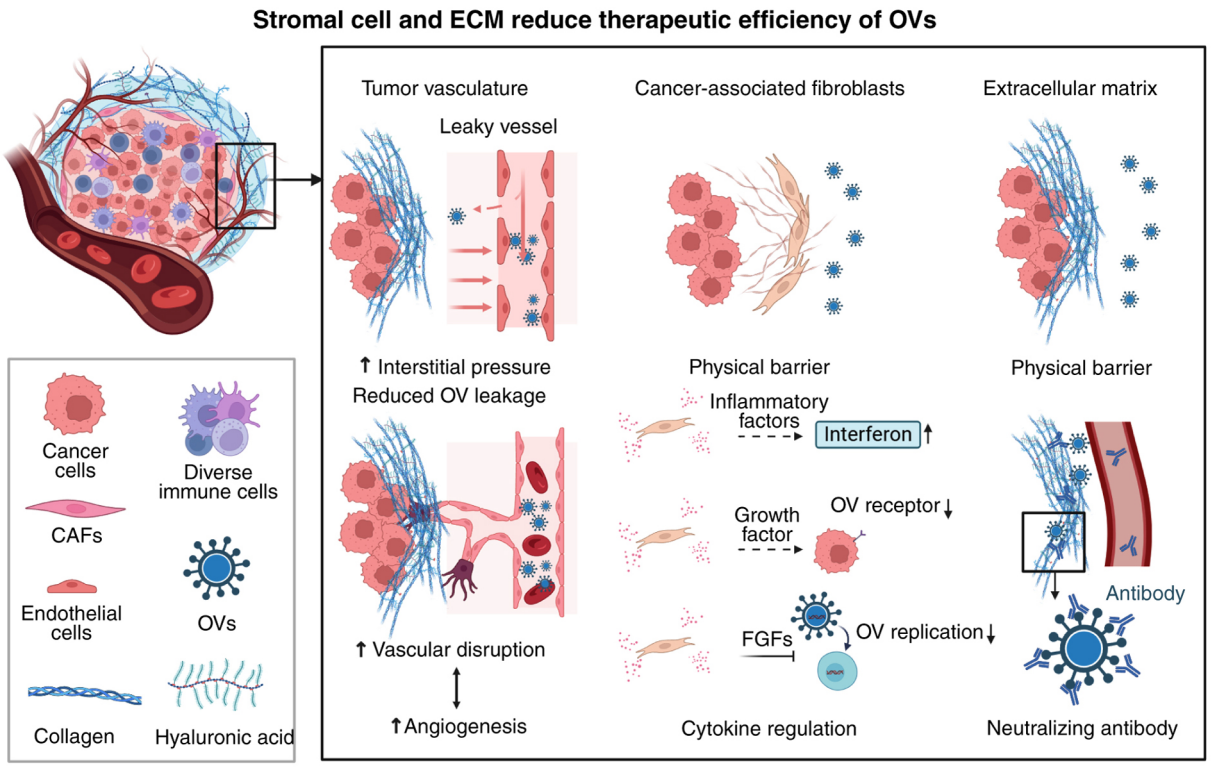


Figure 3. Stromal cells and ECM reduce the therapeutic efficiency of OVs. The tumor microenvironment presents structural and biochemical obstacles to OV delivery and spread. CAFs secrete collagen, hyaluronic acid and other ECM components that create a dense physical barrier, elevating interstitial fluid pressure and compressing blood vessels. This ECM condensation reduces OV extravasation from leaky tumor vessels and retards interstitial diffusion. CAFs also release growth factors such as FGFs that stimulate angiogenesis and vascular normalization, paradoxically decreasing vessel permeability to viruses. Inflammatory cytokines and type-I interferons produced by stromal and immune cells further activate antiviral states in cancer cells, limiting viral replication. Neutralizing antibodies can bind and inactivate OVs before they reach target cells. CAFs, cancer-associated fibroblasts; ECM, extracellular matrix; FGFs, fibroblast growth factors; IFN, interferon; OV, oncolytic virus.

the tumor, resulting in resistance to treatment (156,157). The ECM, which encompasses a variety of structural molecules secreted by cells and encircling them within tissues, is primarily made up of collagen, fibronectin, elastin, laminin and hyaluronic acid (HA) (158). The stiffness of the matrix triggered lipolysis in hepatic stellate cells through the focal adhesion kinase/Yes-associated protein signaling pathway, which contributed to the resistance against anti-angiogenic monoclonal antibody therapy observed in liver metastases from colorectal cancer (159). Cescon *et al* (160) also discovered that collagen VI maintains the stem-like properties and chemotherapy resistance in glioblastoma cells. It is evident that stromal cells and components of the ECM contribute to therapy resistance in tumors. Moving forward, this review will focus on their role in resistance to OV treatment (Fig. 3).

*Tumor vasculature*. Currently, the administration of OVs is mostly through intratumoral injection, which, however, is ineffective for small lesions, multiple metastatic foci and deeply located tumors that are difficult to access anatomically (161). In such cases, intravenous administration could be an alternative. Yet, when administering intravenously, the successful delivery of the virus becomes a critical factor in determining the efficacy of OV therapy. Tumor vasculature differs significantly from healthy blood vessels, being immature and chaotic, often manifesting as spherical and twisted vessels that impede efficient blood flow (162). The discontinuity of basement membranes, incomplete endothelial coverage and poor pericyte wrapping contribute to the irregularity and dysfunction of tumor blood vessels, which are also prone to leakage. While the leaky nature of these vessels permits the retention in the perivascular area and the seepage of viruses into areas beneath the endothelium, the elevated interstitial pressure from leakage also hinders the convective transport of OVs from the tumor's periphery towards its core (163). Miller *et al* (164) identified perfusion pressure as an essential factor influencing both the delivery and effectiveness of OVs within the tumor environment. Their research indicated that increased blood pressure, induced by physical activity, improves the ability of OVs to target tumors effectively (164). Consistent with this, Hong *et al* (165) found that under conditions of cell death or stress, the active or passive release of high-mobility group box 1 (HMGB1) into the extracellular environment leads to a significant increase in endothelial permeability, which results in further leakage and edema in the tumor vasculature, contributing to higher tumor interstitial pressure and blocking HMGB1 can lead to improved outcomes with OV therapy. Nevertheless, it has been posited that OV vectors are large particles to face challenges in extravasating from the tumor vasculature and enhancing tumor vascular permeability through increasing leakage can substantially boost the impact of OVs (166). OV therapy has also been observed to increase vascular permeability and immune cell infiltration in rat glioma models,

yet the relationship between this enhanced tumor vascular permeability and the efficacy of the OV has not been discussed in the study (167).

In addition to affecting OV extravasation from blood vessels, endothelial cells are also involved in the process of angiogenesis. Angiogenesis is considered to be closely associated with the occurrence, progression, metastasis and therapy resistance of tumors and inhibiting angiogenesis is considered an important target in cancer therapy (168). However, the relationship between current OV therapy and tumor angiogenesis has yielded some contradictory conclusions. On the one hand, OVs have been shown in certain studies to disrupt the tumor vasculature and reduce tumor perfusion by leveraging platelet pathways, neutrophil infiltration or downregulating the vascular endothelial growth factor (VEGF) signaling pathway (169-173). Various OVs have been reported to target tumor endothelial cells and it has been found that OV intervention significantly reduces tumor vascular density (174). While these phenomena may appear linked, it is not yet clear whether the infection of endothelial cells is related to the collapse of tumor blood vessels or whether the infection of endothelial cells by OVs leads to some form of cell death (174). In *in vivo* experiments, tumor endothelial cells were able to effectively clear OVs without cell lysis (175). It is indeed thought-provoking to consider whether the reduction in tumor perfusion due to the destruction of tumor vasculature may impede the penetration of OVs into the tumor, particularly for OVs administered intravenously (176). On the other hand, studies have found that OVs exhibit characteristics that promote angiogenesis, but this phenomenon appears to have been only observed in HSVs thus far (176,177). Angiogenesis is a common phenomenon in viral infections and its mechanisms may be associated with tissue damage, immune cell infiltration and cytokine storms (178,179). The evidence presented above shows conflicting angiogenesis roles of OVs. These differing results may be due to variations in viral strains, tumor types and experimental conditions. At the same time, Arulanandam *et al* (180) found that VEGF signaling can increase the sensitivity to OVs and promote OV infection by inhibiting type I IFN. Further research is needed to determine whether OV-induced angiogenesis may further promote the metastasis and drug resistance of tumor cells.

*CAFs*. The influence of CAFs on tumors is complex and involves multiple factors. CAFs can promote tumor growth by regulating inflammatory responses, modifying the ECM to prevent T-cell infiltration and enhancing cell survival. However, certain subtypes of CAFs have been shown to have tumor-inhibiting effects (181). CAFs are also associated with resistance to cancer therapies. For instance, CAFs can suppress ferroptosis and induce gemcitabine resistance in pancreatic cancer cells and can promote castration resistance in prostate cancer after antiandrogen treatment (182,183). Similarly, CAFs can also affect the efficacy of OVs, ultimately leading to treatment resistance. Vähä-Koskela *et al* (184) found that after virus-infected cells undergo death, CAFs can rapidly form connective tissue to protect tumor cells. Arwert *et al* (185) discovered that direct interaction between cancer cells and CAFs stimulates fibroblasts to produce various inflammation-modulating factors. This process activates STING and induces the expression of IFN-β1 and other cytokines through IRF3, which significantly impairs the effectiveness of OVs in both *in vitro* and *in vivo* settings (185). Yasui *et al* (186) found that hepatocyte growth factor secreted by fibroblasts under *in vitro* culture conditions can inhibit Ad infection of pancreatic ductal adenocarcinoma cells by downregulating the expression of virus receptors. In addition, fibroblast growth factors (FGFs), such as FGF9 and FGF16, have been shown to inhibit the replication of viruses such as influenza A, vesicular stomatitis virus and Coxsackievirus (187,188). These factors secreted by fibroblasts are thought to be upregulated during early infection, inducing local IFN responses and inflammatory effects, thereby exerting antiviral activity. However, certain FGFs, including FGF7 and FGF10, have been found to promote the replication of viruses such as HSV I, lymphocytic choriomeningitis virus and Zika virus (189). It is important to note that part of these studies about FGFs were not conducted in tumor models and the TME may be more complex.

Given the crucial role of CAFs in remodeling the TME and contributing to therapy resistance, OVs to directly target CAFs have been designed, achieving success in preclinical research. For instance, i-leader truncation of Ads can target CAFs to enhance antitumor effects (190). Additionally, targeting specific markers of CAFs can be effective, with commonly used markers including fibroblast activation protein (FAP), the urokinase-type plasminogen activator receptor (uPAR) and junctional adhesion molecule-A with Ad and reovirus (191-194). These studies have found that targeting CAFs can effectively disrupt the tumor stromal barrier and reverse the tumor-suppressive immune microenvironment, while also reducing antiviral immune responses, promoting the spread and replication of OVs. Notably, clinical trial outcomes have demonstrated limited efficacy (195). On the one hand, this can be attributed to the limitations of preclinical models, and on the other hand, it may be strongly related to the heterogeneity of CAFs (196). The subtypes of CAFs have been extensively elaborated on in previous reviews (197). Certain subtypes promote tumor growth, suppress immune activation and enhance treatment resistance, while others, such as myofibroblastic CAFs, inhibit tumor growth. It is important to note that the targets of OV modifications aimed at CAFs do not actually distinguish between CAF subtypes and are not exclusively expressed on CAFs. For instance, the FAP protein is not only expressed in CAFs that inhibit tumor growth but also in macrophages (198). Similarly, another target, uPAR, is expressed on macrophages and other bone marrow-derived cells (199). This situation can easily lead to off-target effects of OVs, resulting in suboptimal therapeutic efficacy.

Furthermore, CAFs can regulate the composition of the ECM by secreting matrix metalloproteinase (MMP) proteins, synthesizing ECM components and mechanically reshaping the microenvironment to form physical barriers for virus spread. The relationship between the ECM and OVs will be discussed in the next chapter.

*ECM*. After the lysis of tumor cells by OVs, they are released into the ECM. However, most targeted therapeutics encounter substantial delivery obstacles within the tumor, and OVs, being larger in size than small molecule drugs, face even greater challenges (200). Solid tumors efficiently restrict macromolecule

transport via physical barriers, with high interstitial fluid pressure and the composition of the ECM being the main physical barriers to the effective delivery of OVs (201). Asiatic acid was reported to improve the drug delivery and antitumor efficacy of pegylated liposomal doxorubicin by degrading collagen within the tumor stroma (202). Haseley *et al* (203) found that ECM protein cellular communication network factor 1 (CCN1) restricts the effectiveness of OV in glioma. Therefore, manipulating the ECM may also potentially enhance the spread of OV.

For instance, early studies have shown that pre-treatment with elastase can increase the efficiency of Ad gene transduction, and proteases such as collagenase/dispase and trypsin can also enhance the effectiveness of Ad gene therapy for cancer treatment by facilitating transduction (204-206). The co-injection of bacterial collagenase to modify the ECM results in a marked expansion of the initial viral distribution area within the tumor, increasing it to three times the original size in melanoma xenografts (207). HA, another key component of the ECM primarily composed of glycosaminoglycans, plays a significant role in regulating the tumor immune microenvironment, tumor migration and invasion, and resistance to cancer therapies (208). Ganesh *et al* (209) discovered that combination of hyaluronidase enzyme and Ads can enhance the antitumor activity of the OV and improve the prognosis of mice in animal models. Furthermore, relaxin, a peptide hormone, can effectively prompt the restructuring of collagen by enhancing the expression and activity of specific MMPs, including MMP-1, MMP-2 and MMP-14 (210,211). The intratumoral dissemination, apoptosis induction and therapeutic effectiveness of OV can be enhanced when expressing relaxin (212). Chondroitinase ABC, an enzyme derived from bacteria, facilitates the dissemination of OVs within tumors by degrading chondroitin sulfate glycosaminoglycans, a major component of the ECM (213). Additionally, MMP-1, MMP-8 and MMP-9 improve the distribution and effectiveness of OVs by influencing sulfated glycosaminoglycans in tumors (214,215). The use of epithelial junction opener, derived from *Escherichia coli*, in combination with oncolytic Ads can also enhance therapeutic efficacy (216). It should be noted that hyaluronidase can facilitate the growth of glioblastoma in mouse models via astrocytes, and elevated levels of relaxin may contribute to the metastasis of breast cancer (217,218). Hence, a more cautious consideration is required when disrupting the ECM. Bacterial chondroitinase ABC has been shown to enhance the spread and antitumor efficacy of OVs without significantly promoting the migration and invasion of glioma cells (219). Additionally, neutralizing antibodies present in the stromal components can significantly impact the infection and spread of OVs. Compared to the relatively simple environment in animal models, the complex environment of clinical trial patients may lead to a greater presence of anti-viral antibodies, thereby reducing the efficacy of OVs (220). Evidence indicates that in tumors with high stromal reaction, nanoparticles administered directly into the tumor mass do not disperse widely from the injection point (221). The aforementioned investigations into ECM degradation and reconfiguration, which have been shown to boost the distribution and effectiveness of OVs, further demonstrate the ECM's role as a barrier to viral spread. The failure of viral spread is a significant barrier to tumor resistance against OV therapy.

*Possible solution*. To address the suboptimal therapeutic effects of OVs due to stromal cells and ECM factors, three approaches can be summarized to enhance antitumor efficacy: Optimizing the OV delivery system, genetically modifying the OV and combining it with other treatments.

Using novel delivery systems of OVs can provide advantages such as improvement of the *in vivo* distribution of the virus, offering better biocompatibility, enhanced ability to penetrate high-pressure ECMs and protection against neutralizing antibodies (222). The main approach for 'cloaking' OVs involves encapsulation using a variety of agents, including synthetic and biological materials such as immune cells, copolymers, nanoparticles and biodegradable substances (223). For example, using polyethylene glycol as a carrier to encapsulate OVs has been found to prevent neutralization by antibodies (224). Wang *et al* (225) utilized a dendrimer platform and injectable hydrogel encapsulation system to deliver OVs, which reduced the generation of reactive antibodies. In fact, the exploration and application of using polymers to encapsulate OVs have been widely discussed (226,227). Nanotechnology also represents an emerging avenue for OV carriers. The use of nanoparticles to encapsulate OVs can effectively increase the circulation lifespan and overcome the insufficient penetration caused by high interstitial pressure in tumors and obstruction of dense ECM (228). The application of magnetic nanoparticles can even enable non-invasive therapeutic monitoring (229). However, it is important to note that certain nanoparticles may have inhibitory and cytotoxic effects on OVs (230). Another promising delivery system is extracellular vesicles (EVs). The use of EVs to encapsulate OVs has been explored by certain researchers. It has been found that EVs can bypass the issue of low viral receptor expression and protect the virus from neutralization by antibodies (231). Additionally, EVs offer advantages such as high biocompatibility and ease of modification. Hong *et al* (232) have explored the use of PH20 hyaluronidase to modify EVs for the degradation of the ECM, which holds great promise for overcoming ECM-induced resistance. Furthermore, tumor-derived EVs have a natural tropism for tumors (233). The use of effective delivery systems not only prevents OVs from being neutralized by antibodies but also, to a certain extent, avoids phagocytosis by circulating immune cells, a point that will be discussed later in the text.

Modifying the genome of OVs can enable them to express specific proteins upon infection of tumor cells. To address the physical barrier posed by the ECM, genetic modification of OVs to express ECM-degrading proteins, such as relaxin, MMPs and hyaluronidase, is a common strategy. For instance, Jung *et al* (234) used a relaxin-expressing oncolytic Ad that effectively degraded ECM components and increased the permeability of other drugs. OVs expressing hyaluronidase PH20 or Hyal1 have been shown to effectively induce tumor regression in both immunocompetent and immunodeficient animal models, improving OV infection and spread (201,235,236). A genetically modified vaccinia virus expressing MMP9 has been shown to promote the degradation of collagen IV, facilitating OV spread and demonstrating stronger antitumor effects (237). Regarding endothelial cells, Swanner *et al* (238) found that oncolytic HSV-1 armed with a decoy receptor for advanced glycation end products can block

the signaling of high-mobility group box 1 (HMGB1), thereby preventing the activation of endothelial cells in response to OV infection, thus promoting the efficacy of OVs. Jeong and Yoo (239) constructed an oncolytic vaccinia virus carrying the angiopoietin 1 gene to promote tumor vascular normalization, which further facilitated the activation of antitumor immunity and enhanced antitumor effects. Armed with internalizing Arginine-Glycine-Aspartic tumor-penetrating peptide, by binding to αv-integrins on the tumor vasculature or tumor cells, and through proteolysis exposing a C-terminal motif that binds to neuropilin-1 and facilitates cell internalization, the entry of OVs into tumor cells is enhanced (240,241). OV modifications targeting CAFs have also been explored in preclinical studies. However, due to the limitations of specificity for CAFs and CAF subtypes, more research is needed on OV targeting CAFs (242).

Combining OVs with other treatments can also enhance their therapeutic efficacy. Similar to having OVs express small-molecule proteins, the use of ECM-degrading molecules in combination can effectively improve the replication and spread of OVs, thereby increasing their anti-tumor efficacy. In addition to the aforementioned MMPs, hyaluronidase and relaxin, Lavilla-Alonso *et al* (243) demonstrated that macrophage metalloelastase can enhance the antitumor effects of OVs in subcutaneous colorectal cancer xenografts. Furthermore, epithelial cell junctions within tumors also form an important mechanism of therapeutic resistance. Therefore, OVs combined with epithelial junction openers have been found to have better intratumoral spread and therapeutic effects (216). Targeting tumor vasculature, β-adrenergic receptor inhibitors can enhance the effect of oncolytic herpesvirus in colon cancer through anti-angiogenic actions (244). Interestingly, anti-angiogenesis has also been observed to degrade ECM components, thereby enhancing the efficacy of OVs (245). Other approaches, such as using neutralizing antibodies against HMGB1, can reduce vascular leakage and edema, thereby decreasing interstitial pressure and improving the therapeutic effect on tumors (165). To prevent the neutralization of intravenously injected OVs, certain studies have used the immunosuppressant cyclophosphamide to inhibit both innate and induced neutralizing antibody responses against HSV, thereby promoting the spread of oncolytic HSV *in vivo* (246,247). In addition, certain physical methods can be employed. For instance, Carlisle *et al* (248) and Bazan-Peregrino *et al* (249) found that the use of ultrasound to induce cavitation can also enhance the penetration and infection of OVs. Sun *et al* (250) found that reversible electroporation can induce the PI3K/Akt pathway in pancreatic cancer cells, thereby promoting M1 infection and enhancing its oncolytic effect.

## 4. Immune cells

OV therapy is considered a new generation of immunotherapy, promoting antigen-presenting cells to activate the immune system by inducing immunogenic cell death. However, the activation of the immune system is partly due to tumor antigens released from dying tumor cells and partly due to a series of antiviral responses triggered by viral infection. There is a balance between antitumor and antiviral responses during OV therapy. Moderate antiviral immunity can enhance antitumor immune effects, while excessive antiviral immunity can lead to viral clearance and resistance to OV therapy (251). In addition, Donat *et al* (252) found that the oncolytic vaccinia virus GLV-1H68 has a lymphangiolytic effect when treating human PC-3 prostate cancer xenografts. The authors suggest that this could prevent lymphatic metastasis of the tumor, but it remains to be determined whether this effect would also impact the process of tumor antigen presentation and tumor infiltration. Therefore, the relationship between the tumor, the immune system and the virus becomes even more complex after the introduction of the virus. Below, the potential mechanisms by which immune cells may cause failure of OV therapy are discussed (Fig. 4).

*Immune checkpoint expression*. Immune checkpoints refer to a series of regulatory mechanisms within the immune system that normally maintain the balance of immune responses, preventing excessive reactions that could lead to autoimmune diseases. The upregulation of immune checkpoints typically suppresses antitumor immunity, making immune checkpoint inhibitors a promising approach for cancer treatment (253). Originally characterized as regulators of T-cell function, further research has revealed that various immune cells, including macrophages, natural killer (NK) cells, dendritic cells, regulatory T cells and B cells, are also affected by the expression of immune checkpoints (254). Consequently, the upregulation of immune checkpoints in the tumor immune microenvironment can create a suppressive immune environment, leading to resistance and failure of immunotherapy. In preclinical models, numerous studies have observed evidence of OVs upregulating immune checkpoints. Kwan *et al* (255) also found that the introduction of HSV1716, a modified HSV, can lead to increased expression of programmed death-1 (PD-1) on T cells in a breast cancer model. In melanoma, the use of Talimogene laherparepvec has also been observed to lead to an upregulation of programmed death-ligand 1 (PD-L1) (256). Mostafa *et al* (257) found that oncolytic reovirus treatment in breast cancer also leads to an upregulation of PD-L1 expression in tumor cells. MEDI5395, a recombinant NDV, has similarly been found to upregulate PD-L1 after infecting myeloid cells (258). In treatments with oncolytic vaccinia virus, upregulation of PD-1, PD-L1, cytotoxic T-lymphocyte associated protein 4 (CTLA-4) and lymphocyte activation gene 3 (LAG-3) in the tumor immune microenvironment has also been observed (259). In clinical trials, similar observations of immune checkpoint upregulation following OV intervention have also been made (260). Bernstock *et al* (261) found that treatment with oncolytic HSV-1 G207 in a Phase 1 trial of pediatric patients with glioblastoma induced significant upregulation of immune checkpoint proteins PD-1, PD-L1, and CTLA-4. In bladder cancer, a Phase I clinical trial found an increase in PD-L1 and LAG-3 following treatment with Coxsackievirus A21 (262). In a study on oncolytic HSV-2, an increase in PD-L1 was also observed following intratumoral injection in solid tumors (263). In fact, the upregulation of immune checkpoints following viral introduction is rational. On the one hand, for immune cells, an activated immune system requires the upregulation of immune checkpoints to achieve

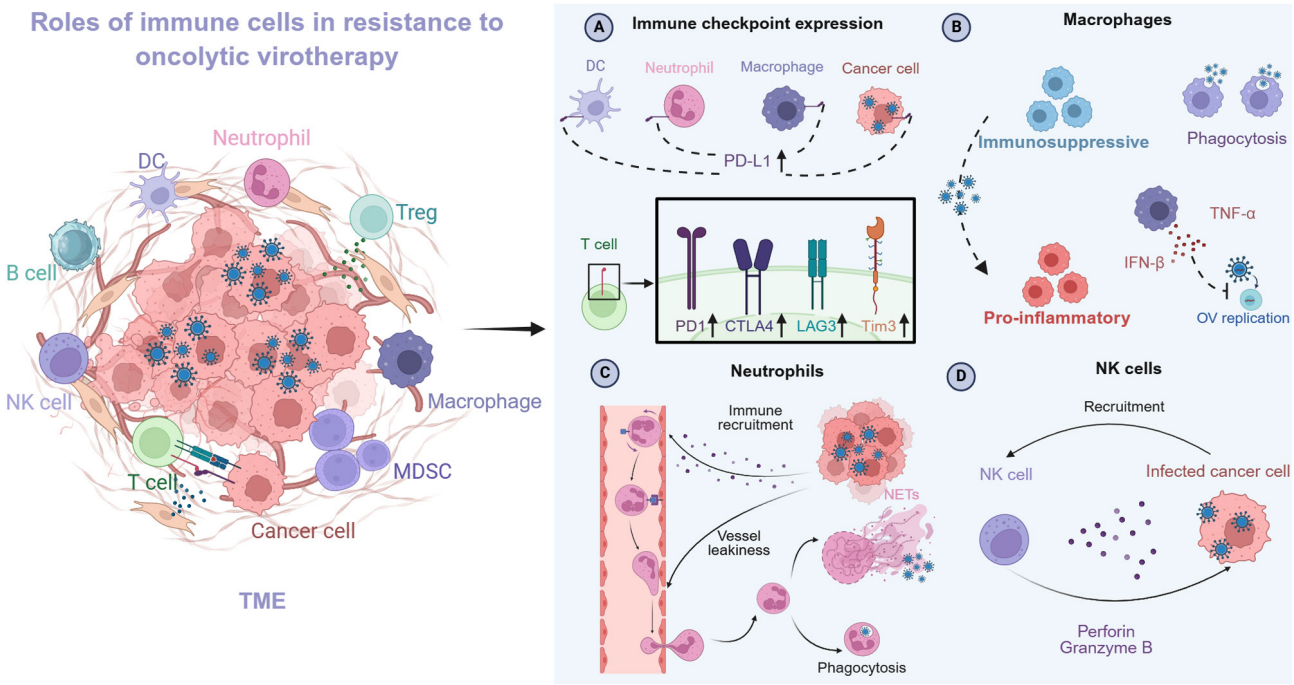


Figure 4. Roles of immune cells in resistance to oncolytic virotherapy. (A) Roles of immune checkpoint expression in resistance to oncolytic virotherapy. T cells express checkpoint receptors including PD-1, LAG-3, TIM-3 and CTLA-4, while myeloid cells and tumor cells upregulate PD-L1. This interaction contributes to T cell exhaustion and diminishes their antitumor response. (B) Roles of macrophages in resistance to oncolytic virotherapy. Macrophages can phagocytose infected cancer cells or produce IFN-β and TNF-α, enhancing antiviral responses. (C) Roles of neutrophils in resistance to oncolytic virotherapy. Neutrophils can phagocytose the virus or release NETs that physically trap the virus. (D) Roles of NK cells in resistance to oncolytic virotherapy. NK cells exert direct cytotoxicity against infected cells via perforin and granzyme B. CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; IFN-β, interferon β; LAG-3, lymphocyte-activation gene 3; MDSC, myeloid-derived suppressor cell; NET, neutrophil extracellular trap; NK, natural killer; OV, oncolytic virus; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; TIM-3, T cell immunoglobulin and mucin domain-containing protein 3; TME, tumor microenvironment; TNF-α, tumor necrosis factor α; Treg, regulatory T cell.

negative feedback regulation and prevent excessive immune effects. On the other hand, for tumor cells, the upregulation of PD-L1 is a self-protective biological response to cope with the stress from viruses and the immune system.

Based on this, combining immune checkpoint inhibitors or genetically modifying OVs are the most feasible solutions. For instance, Saha *et al* (264) used a triple combination of anti-CTLA-4, anti-PD-1 and oncolytic HSV to treat glioblastoma, which can modulate the proportion of CD8-positive T cells in the immune microenvironment and the polarization of macrophages. An Ad has been designed to express a soluble fusion protein of PD-1/CD137L, which can simultaneously block PD-1 signaling and maintain T-cell activation signals (265). Certain studies have also used small molecule inhibitors to intervene in the upregulation of immune checkpoints, such as reducing PD-L1 upregulation in NSCLC following OV treatment through JAK/STAT inhibition (266). It is worth noting that, as previously mentioned, the IFN response of tumor cells is a factor limiting the efficacy of OVs, while mutations in the IFN pathway can lead to resistance to immune checkpoint inhibitor therapy and enhance sensitivity to OV therapy (267). This further provides a rationale for the combined use of OVs and immune checkpoint inhibitors. Therefore, several clinical trials are currently exploring the feasibility and effectiveness of combining immune checkpoint inhibitors with OVs. Current clinical trials of OV in combination with immune checkpoint inhibitors are summarized in Table III.

*Macrophages*. Macrophages are pivotal in the antiviral response, capable of identifying a range of virus-specific antigens such as double-stranded RNA, single-stranded RNA and DNA. Recognition of these antigens leads to membrane reorganization and the creation of new vacuoles that engulf and transport the virus into the cell's cytoplasm. These immune cells can also engulf infected cells, processing and identifying viral elements within phagocytic vesicles (268). Activated macrophages secrete reactive oxygen species and nitric oxide, directly inhibiting viral replication through ribosomal reductase impairment (269). It has been found after extensive research that simply classifying macrophages into M1 and M2 types is somewhat outdated. In order to avoid using outdated terminology, the descriptions of 'pro-inflammatory' (historically M1) and 'immunosuppressive' (historically M2) phenotypes are now used for clarity (270). Here, the pro-inflammatory type represents the classically activated antitumor phenotype, while the immunosuppressive type is generally considered the protumor phenotype. Current research typically finds that after OV intervention, the proportion of pro-inflammatory macrophages in the immune microenvironment increases. The intervention can promote the polarization of pro-inflammatory macrophages and can even revert the immunosuppressive phenotype back to the pro-inflammatory phenotype (271,272). The pro-inflammatory macrophage phenotype, on one hand, promotes the generation of antitumor immunity, and on the other hand, also generates anti-OV immunity. Applying microglia with the pro-inflammatory

Table III. Clinical trials of oncolytic viruses in combination with immune checkpoint therapy.

| Viral name | Viral type | Checkpoint inhibitor | Checkpoint | Indication | Clinical phase | Identifier |
|---|---|---|---|---|---|---|
| RT-01 | Ad | Nivolumab | PD-1 | Advanced solid tumors | Phase I | NCT05228119 |
| H101 | Ad | Camrelizumab | PD-1 | Pancreatic cancer | Phase II | NCT06196671 |
| RT-01 | Ad | Nivolumab | PD-1 | Advanced solid tumors | Phase I | NCT05122572 |
| Revottack | Vesicular stomatitis | PD-1 Inhibitor | PD-1 | Advanced solid tumors | Phase I | NCT05644509 |
| - | Ad | Anti-PD1 | PD-1 | Pancreatic cancer | Phase I | NCT06346808 |
| MG1-E6E7 | Maraba virus | Atezolizumab | PD-L1 | HPV-associated cancers | Phase I/1b | NCT03618953 |
| Pexa-Vec | Vaccinia virus | Ipilimumab | CTLA-4 | Metastatic/advanced solid tumors | Phase I | NCT02977156 |
| ADV/HSV-tk | HSV | Pembrolizumab | PD-1 | MetastaticTNBC/NSCLC | Phase II | NCT03004183 |
| DNX-2401 | Ad | Pembrolizumab | PD-1 | Glioblastoma/gliosarcoma | Phase II | NCT02798406 |
| Pexa-Vec | Vaccinia virus | Durvalumab | PD-L1 | Colorectal cancer | Phase I/II | NCT03206073 |
| Pelareorep | Reovirus | Nivolumab | PD-1 | Multiple myeloma | Phase I | NCT03605719 |
| RP1 | HSV | Nivolumab | PD-1 | Cutaneous squamous cell carcinoma | Phase II | NCT04050436 |
| MEM-288 | Ad | Nivolumab | PD-1 | Solid tumors/NSCLC | Phase I | NCT05076760 |
| CF33-hNIS | Vaccinia virus | Pembrolizumab | PD-1 | Advanced solid tumors | Phase I | NCT05346484 |
| TILT-123 | Ad | Avelumab | PD-L1 | Ovarian cancer | Phase I | NCT05271318 |
| VSV-IFNβ-NIS | Vesicular stomatitis | Pembrolizumab | PD-1 | Solid tumors/NSCLC | Phase I/II | NCT03647163 |
| Olvi-Vec | Vaccinia virus | Pembrolizumab | PD-1 | NSCLC | Phase II | NCT06463665 |
| H101 | Ad | Camrelizumab | PD-1 | Malignant pleural mesothelioma | Phase I | NCT06031636 |
| rHSV2hGM-CSF | HSV | HX-008 | PD-1 | Melanoma stage IV | Phase I | NCT05070221 |
| rHSV2hGM-CSF | HSV | HX-008 | PD-1 | Melanoma stage IV | Phase I | NCT05068453 |
| BioTTT001 | Ad | Toripalimab | PD-1 | Gastric cancer | Phase II | NCT06283121 |
| M1-c6v1 | M1 | SHR-1210 | PD-1 | Hepatocellular carcinoma | Phase I | NCT04665362 |
| TILT-123 | Ad | Avelumab | PD-L1 | Solid tumors | Phase I | NCT05222932 |
| T3011 | HSV | Toripalimab | PD-1 | Colorectal cancer | Phase I | NCT06283303 |
| OH2 | HSV | HX-008 | PD-1 | Melanoma | Phase I/II | NCT04616443 |
| BioTTT001 | Ad | Toripalimab | PD-1 | Colorectal cancer | Phase I | NCT06283134 |
| TBio-6517 | Vesicular stomatitis | Pembrolizumab | PD-1 | Solid tumors | Phase I/II | NCT04301011 |
| LOAd703 | Ad | Atezolizumab | PD-L1 | Malignant melanoma | Phase I/II | NCT04123470 |
| ONCR-177 | HSV | Pembrolizumab | PD-1 | Solid tumors | Phase I | NCT04348916 |
| RP3 | HSV | Nivolumab | PD-1 | Head and neck squamous cell carcinoma | Phase II | NCT05743270 |
| HF10 | HSV | Ipilimumab | CTLA-4 | Melanoma | Phase II | NCT02272855 |
| VG161 | HSV | Nivolumab | PD-1 | Gastric cancer | Phase I/II | NCT06008925 |
| CVA21 | Coxsackievirus | Ipilimumab | CTLA-4 | Uveal melanoma | Phase I | NCT03408587 |
| OBP-301 | Ad | Pembrolizumab | PD-1 | Head and neck squamous cell carcinoma | Phase II | NCT04685499 |
| VSV-IFNβ-NIS | Vesicular stomatitis | Pembrolizumab | PD-1 | Solid tumors | Phase I/II | NCT03647163 |
| H101 | Ad | Camrelizumab | PD-1 | Cervical cancer | Phase II | NCT05234905 |
| H101 | Ad | Tislelizumab | PD-1 | Pancreatic cancer | Phase I | NCT05303090 |
| Oncorine | Ad | Tislelizumab | PD-1 | Hepatocellular carcinoma | Phase I | NCT05675462 |

Ad, adenovirus; VSV, vesicular stomatitis virus; HSV, herpes simplex virus; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; CTLA-4, cytotoxic T-lymphocyte associated protein 4; NSCLC, non-small cell lung cancer; TNBC, triple-negative breast cancer; HPV, human papillomavirus.

phenotype to organotypic slice cultures implanted with glioma results in reduced infection of tumor cells (273). Liu *et al* (274) found that during OV therapy for breast cancer, low concentrations of IFN-β derived from macrophages mediated a robust antiviral immune state. Additionally, TNF-α is also considered a key effector molecule in the antiviral immune response of macrophages (275). In addition to activated inflammatory macrophages exerting antiviral effects locally within tumors, macrophages also play a role in clearing OVs when administered intravenously. This necessitates a larger dose of OV to achieve an appropriate and effective titer of OV in the TME (276). It is worth noting that OVs can also infect macrophages, turning them into carriers for dissemination and establishing chronic infections to achieve long-term therapeutic effects. Although these infections are found non-productive, whether they could lead to off-target effects and a decrease in viral therapeutic efficacy requires further consideration and discussion (277).

Based on the above evidence, strategies targeting macrophages focus on two aspects: Preventing the phagocytosis of OVs and inhibiting the function of immunosuppressive-type macrophages. Administering the PI3Kδ selective inhibitor IC87114 or the clinically approved drug Idelalisib (CAL-101) to immunocompetent mice before intravenous injection of tumor-tropic vaccinia virus can prevent viral attachment to macrophages and achieve better anti-tumor effects (278). Meisen *et al* (271) discovered that the therapeutic efficacy of oncolytic HSVs in treating glioblastoma was significantly improved through the use of TNF-α blocking antibodies or macrophages derived from TNF-α knockout mice. Blocking integrin β1 can also inhibited macrophage migration and enhanced viral replication and oncolytic effects (279). Thorne *et al* (280) found that the activation of CCN1 promotes macrophage activity and increases viral clearance. Therefore, neutralizing integrin αMβ2 can reverse this activation and the macrophage-mediated viral clearance (280). A novel oncolytic Ad engineered to express IL-13 has been shown to decrease the proportion of immunosuppressive macrophages within the TME, resulting in improved antitumor activity in oral cancer (281).

*Neutrophils*. Neutrophils are the most abundant subgroup of white blood cells in the peripheral blood. In recent years, the role of neutrophils in tumor occurrence, development and treatment has gradually gained attention (282). Typically, the infiltration of neutrophils in tumors is associated with poor clinical outcomes (283). Neutrophils can promote angiogenesis, tumor spread and metastasis in tumors. However, there is also a type of neutrophil, known as N1, which has been identified in humans and mice and is capable of exerting antitumor effects (284). Neutrophils were previously thought to play a crucial role primarily in combating bacterial and fungal infections, but have recently been found to also have a key role in antiviral responses. Neutrophils can act similarly to macrophages by phagocytosing viruses, thereby activating antiviral responses (285). Another powerful function of neutrophils is the release of neutrophil extracellular traps (NETs) to capture and inactivate viruses. The production of NETs is also commonly associated with poor clinical outcomes such as low immune infiltration in tumors, treatment resistance and metastasis (286,287). The above discussions have explored the relationships between neutrophils and tumors, as well as between neutrophils and viral infections. However, current research directly investigating the role of neutrophils in OV treatment resistance is not yet sufficient (288). Dai *et al* (289) noted that the use of oncolytic HSV in treating gliomas triggers insulin-like growth factor (IGF) 2 mRNA binding protein 3-induced NETosis. This is a specific form of inflammatory cell death in neutrophils, marked by the creation of NETs, which contributes to resistance against therapy. Price *et al* (290) found that vaccinia virus Ankara can rapidly recruit the infiltration of white blood cells, particularly neutrophils. The direct phagocytosis of vaccinia virus by neutrophils has also been observed in radiophagocytosis assays (291). Breitbach *et al* (292) also found that vesicular stomatitis virus intervention increased neutrophil infiltration in tumors, and pre-clearing neutrophils would allow the virus to replicate and spread more widely within the tumor. Taipale *et al* (293) found in clinical trials that patients with a lower neutrophil-to-lymphocyte ratio (NLR) prior to Ad oncolytic immunotherapy had significantly longer overall survival, and He and Lin (294) also found that in the treatment of hepatocellular carcinoma with Ad5 H101, the NLR was associated with a higher hazard ratio, indirectly suggesting that the level of neutrophils may negatively regulate the efficacy of OV therapy. Furthermore, inhibiting neutrophil function can significantly enhance the antitumor effects of intravenously administered oncolytic vaccinia viruses (295). Of note, certain studies have also found that neutrophils play a key role in tumor regression during OV therapy (296,297). Given the heterogeneity of neutrophils, further precise molecular subtyping of neutrophils may be necessary to more comprehensively explore the dual roles of neutrophils in different cancer types and with different OVs.

There are certain strategies that can inhibit the function of neutrophils to enhance the therapeutic effect of OV. For instance, pharmacological targeting of PI3Kγ can mitigate immune suppression mediated by tumor-associated myeloid cells, thereby improving the therapeutic efficacy of OV M1 in ovarian cancer (298). IFNs secreted by myeloid cells can enhance the resistance of malignant neural progenitor cells to Zika virus (299). However, inhibiting the JAK1/2 signaling pathway can restore their susceptibility. It should be noted that the term myeloid cells used here is a general reference and does not specifically pinpoint a particular subpopulation. Noh *et al* (300) found that in patients with recurrent glioblastoma after treatment with oncolytic HSV-1, IGF2 was upregulated. By expressing a decoy receptor for IGF2 via OV, the infiltration of neutrophils was reduced, thereby decreasing the production of immunosuppressive molecules and enhancing the efficacy of OV in animal models (300). This target is consistent with the findings of Dai *et al* (289) reported earlier.

*NK cells*. NK cells are an important component of the innate immune system, playing a crucial role in both antitumor and antiviral immunity. NK cells can directly kill tumor cells or virus-infected cells by releasing cytotoxic granules or through antibody-dependent cellular cytotoxicity. They can also enhance antitumor immunity and viral clearance by activating adaptive immunity (301). Alvarez-Breckenridge *et al* (302) observed that during the treatment of glioblastoma with

oncolytic HSV, NK cells were recruited to the infected area within a few hours after infection and preferentially lysed infected cells through recognition by NK cell natural cytotoxicity receptors. Meanwhile, Reale *et al* (303) found that after infecting glioblastoma with HSV expressing green fluorescent protein, it took a period of time measured in days to observe significant viral spread and replication. It is required to consider whether the rapid response of NK cells to viral infection is too quick for the virus to survive and spread within the tumor. Of note, depleting endogenous NK cells two days before OV treatment and replenishing exogenous NK cells three days after OV intervention to control the number of NK cells can effectively extend survival (304). It is worth mentioning that OV treatment potentiates exogenous NK-cell cytotoxicity (305). Specifically, Wang *et al* (306) found that oncolytic HSV can upregulate checkpoint molecule NK group 2 member A on NK cells, indicating a more complex interaction between oncolytic virotherapy and tumor immune microenvironment. Expressing IL-15 via OVs can stimulate the activation of NK cells and more effectively promote tumor regression (307). Of course, this cytokine-mediated antitumor effect may not be generated through NK-cell activation, as no strict causal relationship has been identified.

Overall, most studies have found that NK cells do not seem to be a helpful ally in OV therapy. Therefore, most strategies targeting NK cells are aimed at suppressing their numbers and functions. In an orthotopic glioblastoma mouse model, pretreatment with valproic acid can reduce the recruitment of NK cells and macrophages, and valproic acid can also inhibit the function of NK cells (308). The recombinant virus rVSV-UL141 has been proposed, which expresses a protein that downregulates the expression of the NK-cell activation ligand CD155, reducing NK-cell recruitment and activation and enhancing viral replication, leading to increased tumor necrosis and extended survival (309). Administering a single dose of TGF-β before OV treatment for glioblastoma can temporarily inhibit innate immune cells, primarily macrophages and NK cells, that restrict therapeutic efficacy, thus improving the effectiveness of OV therapy (310).

## 5. Future perspective

The potential mechanisms of OV treatment resistance identified in the above studies are mostly based on biological foundations. Therefore, addressing OV treatment resistance will likely require further in-depth exploration of the effects of different virus types on different tumors and their underlying biological mechanisms in order to better intervene in this process. In addition to the exploration of biomarkers, pathways and immune cells mentioned earlier, metabolic changes within tumors may represent a new avenue for investigation. For instance, a chemical derivative of itaconate, a Krebs cycle-derived metabolite, has been found to enhance the efficacy of OV in various tumor models (311). The homeostasis of calcium ions in the TME also has a significant impact on the antitumor effects of OV (312).

Additionally, emerging biological research techniques could be highly significant for OV studies. For instance, considering the spatial dynamics within tumors, 3D cell culture techniques may more accurately reflect real tumor treatment scenarios. These techniques can systematically investigate how viral characteristics (such as viral spread rate), tumor features (such as the emergence rate of drug-resistant cells and the cost of resistance), the resistance of healthy stromal cells to the virus and the timing of treatment collectively influence therapeutic outcomes (313,314). Brachtlova *et al* (315) proposed a method that can detect virus-associated RNA through liquid biopsy in preclinical *in vivo* models, which can provide a minimally invasive detection method for *in vivo* replication of OV. Going further, to address animal model limitations in tumor immune microenvironment studies, humanized mouse models offer a translational platform for assessing the impact of OVs on the tumor immune microenvironment (316).

In addition to biological techniques, the intersection of biology with other disciplines is more conducive to the study and development of OV. For instance, according to mathematical models, monotherapy alone is insufficient to completely eradicate tumors and needs to be supplemented with other therapies (317). By employing a finite volume method coupled with a fractional step method and utilizing the nonlinear solver nksol for algebraic systems, Kim *et al* (304) determined the optimal number of endogenous immune cells to deplete and exogenous immune cells to reinfuse in order to best enhance the effects of OV therapy. Assuming the support of more powerful computing capabilities and more refined models, which could provide more accurate predictions of real-world scenarios would significantly advance the study of OV resistance. For instance, agent-based models simulating spatial OV spread in 3D tumor architectures and ordinary differential equation models optimizing immune cell depletion kinetics could predict resistance evolution (318,319).

## 6. Literature search strategy

The literature review for this article was conducted using major databases such as PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (https://www.webofscience.com/) and Scopus (https://www.elsevier.com/en-in/products/scopus/search). Key words included 'oncolytic virus therapy', 'drug resistance', 'tumor microenvironment' and 'immune response'. Studies were selected based on their relevance to the topic and quality of evidence, with a focus on recent advancements and key findings in the field.

## 7. Conclusion

In this review, the challenges of drug resistance in OV therapy for cancer treatment were thoroughly explored. OVs demonstrate significant antitumor potential by selectively infecting and lysing tumor cells and inducing immunogenic cell death. However, the clinical application of OV therapy is still limited due to factors such as the high heterogeneity of tumor cells, the complexity of the TME and the diversity of host antiviral immune responses. The drug resistance mechanisms of tumor cells were analyzed in detail, including the obstruction of viral entry, the imbalance of cell death mechanisms, the activation of antiviral responses, the physical barrier effect of tumor stromal cells and the ECM, and the factors related to immune cells. Meanwhile, this paper proposes several possible solutions, such as personalized genetic testing (e.g. to test

mutations in E1A or E2F, or the expression status of OV receptors to prevent treatment resistance caused by improper virus selection), combination therapy strategies (e.g. to combine with immune checkpoint inhibitors to address treatment resistance caused by immunosuppressive microenvironments), optimization of viral delivery systems (e.g. to address treatment resistance caused by antibody neutralization and insufficient tissue penetration) and genetic modification of viruses (e.g. to express ECM-degrading enzymes to overcome resistance due to physical barriers for viral spread), in order to improve the clinical efficacy of OV therapy. Future studies should prioritize investigating the interaction mechanisms between OVs and tumor cells and immune cells, develop more effective biomarkers and therapeutic targets to achieve the goal of precision medicine and facilitate broader clinical translation of OV therapy in cancer treatment.

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JX performed the literature search and manuscript writing. ZX contributed to the literature search. SW was involved in manuscript review & editing. QX was responsible for study design and supervision. All authors have read and approved the final version of the manuscript. Data authentication is not applicable.

**Ethics approval and consent to participate**

Not applicable.

**Patient consent for publication**

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**Competing interests**

The authors declare that they have no competing interests.

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