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Review Article

Advances in targeted delivery and combination immunotherapy of oncolytic viruses for glioma

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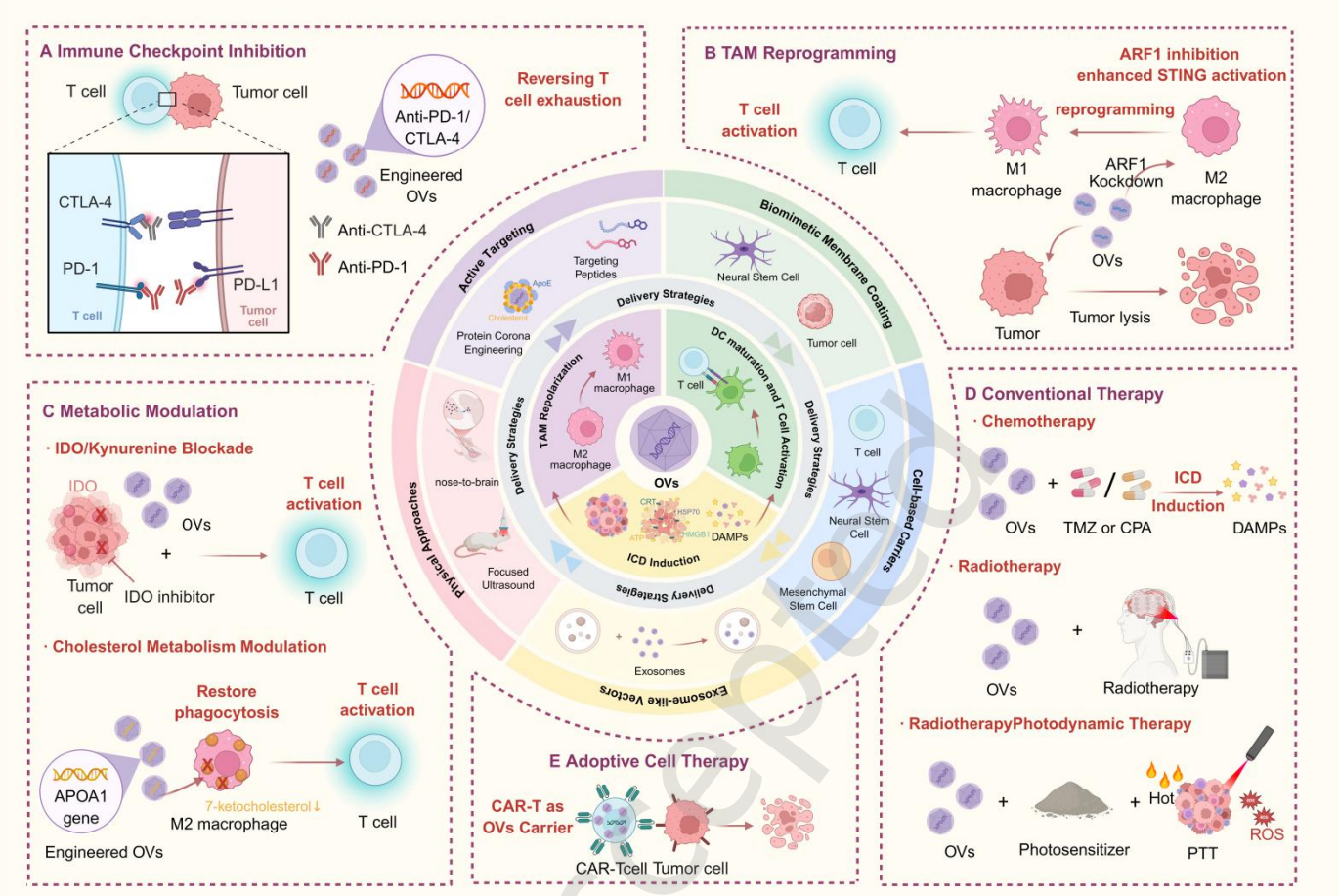
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This review provides a schematic overview of oncolytic virus OVs-mediated immunotherapy for glioma, highlighting delivery strategies and combination therapies that enhance immune activation and antitumor efficacy.

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Advances in targeted delivery and combination immunotherapy of oncolytic viruses for glioma

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ABSTRACT: Glioblastoma (GBM), as the most lethal malignancy of the central nervous system, faces the dual constraints of the physical barrier of the blood-brain barrier (BBB) and a highly immunosuppressive microenvironment, rendering conventional immunotherapies largely ineffective. Oncolytic viruses (OVs), which possess a dual capacity for selective tumor cell lysis and antitumor immunity induction, hold considerable promise for glioma treatment. Nevertheless, the clinical application of OVs remains severely constrained. The constraints include poor intracranial delivery efficiency, swift sequestration by the reticuloendothelial system, and the dampening of therapeutic outcomes. These outcomes are elicited by counter‐regulatory immunosuppressive signals that exist in the microenvironment. This review systematically examines diversified delivery strategies to address delivery challenges. The review also discusses the advantages and limitations of each strategy. Recognizing the suboptimal outcomes associated with single-agent regimens, we further delineate the synergistic interactions across multiple dimensions within combinatorial therapeutic approaches, thus establishing a robust theoretical foundation and proposing practical strategies for clinical translation of OVs-mediated immunotherapy for gliomas. Finally, this review envisions future directions, including personalized combination strategies, safety management, biomarker development, and manufacturing considerations, to inform precision immunotherapy for glioma.

KEYWORDS: oncolytic viruses, glioma, immune microenvironment, delivery strategies, combination therapy, blood-brain barrier

1 Introduction

Glioma ranks as the most common primary malignant tumour in the central nervous system. GBM represents the most malignant subtype of glioma. It shows strong invasive behavior, fast cell growth, and intrinsic resistance to both radiotherapy and chemotherapy[1]. Surgical resection combined with temozolomide chemotherapy and radiotherapy has been the mainstream clinical treatment for twenty years. Patients have a median overall survival of less than 15 months. The 5-year survival rate stays below 10%[2]. The difficulties encountered in glioma treatment are to a great extent ascribed to the distinctive immunosuppressive nature of its tumor immune microenvironment (TIME)[3]. The TIME contains large numbers of immunosuppressive cell populations. These cells consist of M2-type tumour-associated macrophages (TAMs), regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). The microenvironment also accumulates soluble inhibitory molecules including IL-10, TGF-β and PD-L1. In

concert with the physical obstruction imposed by the BBB, these factors collectively preclude standard immunotherapeutic modalities from exerting meaningful efficacy[4-6].

Over the past several years, OVs have become a major focus of research for novel immunotherapeutic approaches. OVs can preferentially replicate in neoplastic cells and induce their lytic destruction. Concurrently, they trigger immunogenic cell death (ICD), with the consequent release of damage-associated molecular patterns (DAMPs) and tumor-associated antigens, culminating in the elicitation of innate and adaptive antitumor immunity. Notably, OVs can reshape the immune landscape of gliomas, effecting a transition from an immunologically "cold" to a "hot" phenotype, which in turn augments the susceptibility of tumors to immune checkpoint blockade and other immunotherapeutic interventions. Multiple OVs have advanced into glioma-related clinical trials. Typical candidates include the oncolytic adenovirus DNX-2401, the oncolytic herpes simplex viruses G47Δ and G207, and the oncolytic poliovirus PVSRIPO. Current evidence confirms reliable safety profiles and preliminary therapeutic effects of these OV candidates[3, 7].

Efficient delivery of OVs to intracranial tumours remains a major unmet clinical challenge. The BBB prevents passive diffusion of OVs into the brain parenchyma[8, 9]. Intravenous administration faces neutralization by antibodies and rapid clearance by the mononuclear phagocyte system, resulting in very low viral doses accumulating at tumour sites[10, 11]. Even with direct intratumoral injection, neutralizing antibodies penetrate the tumour microenvironment and inactivate OVs particles[12]. Infectivity of OVs depends heavily on viral entry receptor expression on neoplastic cells. Low surface abundance of these receptors causes primary resistance to OVs-based treatment[13]. Novel technical approaches have been engineered to address the BBB delivery limitation of OVs. Common approaches include active targeting modification, biomimetic cell-membrane camouflage, living cell-based delivery vehicles, physical BBB opening, and nose-to-brain administration routes[14-16]. Progress in delivery technology provides multiple practical ways to overcome the limitations of systemic OVs administration.

Although advances in delivery technologies have cleared some obstacles for OVs application, OVs therapy for glioma still faces other non-negligible limitations. Antiviral neutralizing antibodies and virus-specific T cells generated by the host can rapidly clear the virus[12]. Feedback upregulation of immune checkpoint molecules such as PD-L1 in the microenvironment leads to rapid exhaustion of infiltrating T cells[17]. Moreover, glioma stem cells (GSCs) are inherently resistant to OVs, serving as a source of recurrence[18]. To overcome the above bottlenecks, combination therapeutic strategies have emerged. The strategic integration of OVs with immune checkpoint blockade, TAM-targeting agents, metabolic modulators, cytotoxic chemotherapy, ionizing radiation, or adoptive cellular therapies can engender synergistic amplification of antitumor immunity, reversion of the immunosuppressive milieu, and additional mitigation of delivery-related impediments[19-21]. This review commences by delineating the immunosuppressive attributes of the glioma TIME, proceeds to systematically expound the molecular underpinnings of OVs-mediated microenvironmental remodeling, critically examines the shortcomings of single-agent approaches, and places particular emphasis on surveying combination strategies predicated upon microenvironment modulation as well as emerging delivery technologies designed to traverse the BBB. Collectively, this synthesis aspires to furnish a theoretical framework and translational roadmap for OVs-based immunotherapeutic interventions in glioma.

2Immunosuppressive Microenvironment in Glioma

2.1 Immunosuppressive Cell Populations

TAMs represent the largest immune cell population in the glioma microenvironment. These cells make up nearly 50% of all cells residing in tumour tissues[22]. TAMs are classified into two major groups based on their tissue origin. Resident microglia originate locally within the central nervous system. Monocyte-derived macrophages (MDMs) originate from peripheral circulating monocytes. Tumour-secreted chemoattractants including CCL2 and CSF-1 guide MDMs to cross the BBB and infiltrate glioma lesions[23-25]. TAMs in glioma tissues exhibit an M2-like polarization profile. This phenotype differs from the pro-inflammatory M1 polarization state. These cells show high surface expression of CD163 and CD206. They promote tumour progression and immune evasion through multiple biological pathways[23, 26, 27]. TAMs produce high levels of IL-10 and TGF-β via the LILRB4/STAT3/IL-10 signalling axis. These cytokines inhibit the activation and cytotoxic function of $CD8^+$T cells[28, 29]. TAMs exhibit high surface levels of PD-L1 and other immune checkpoint ligands. These ligands bind to programmed cell death receptor 1 (PD-1) on tumour-infiltrating lymphocytes. This interaction delivers inhibitory intracellular signals. The signals lead to functional impairment and exhaustion of T cells[30]. A reciprocal regulatory loop exists between TAMs and GSCs. GSCs release periostin (POSTN) in the tumour microenvironment. This protein acts on the integrin αvβ3 signalling axis. The axis recruits circulating TAMs and drives their differentiation into the M2-like phenotype. Reciprocally, M2-polarized

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TAMs secrete IL-6 and TGF-β, which activate STAT3- and NF-κB-dependent transcriptional programs in GSCs, thereby potentiating neoplastic proliferation, sustaining stem-like properties, and amplifying chemokine output. This bidirectional crosstalk thus consolidates a self-perpetuating immunosuppressive network[31, 32].

Tregs constitute another critical immunosuppressive cell population in the glioma microenvironment, with their proportions significantly elevated in both peripheral blood and tumor tissues of glioma patients, accounting for approximately 20%–30% of $CD4^+$T cells[33]. Tregs typically express high levels of CD4, CD25, and the transcription factor FoxP3, with their functional specialization and immunosuppressive activity governed by multiple signals within the tumor microenvironment[34]. CCL2, produced by both macrophages and microglia within the glioma ecosystem, potently chemoattracts CCR4- expressing Tregs, promoting their accumulation within neoplastic lesions[33]. The elevated expression of indoleamine 2,3- dioxygenase (IDO) in glioma depletes tryptophan and generates kynurenine, which not only directly suppresses effector T- cell proliferation but also induces naïve $CD4^+$T cells to differentiate into $FoxP3^+$Tregs. High IDO expression is significantly correlated with poor patient prognosis[35, 36]. Mechanistically, Tregs exert their functions primarily through three pathways. First, Treg- derived TGF- β acts in a paracrine manner to selectively restrain helper T- cell polarization toward antigen- presenting cells and to disrupt their secretory architecture, thereby precluding productive amplification of the immune response[37]. Through their high- affinity IL- 2 receptor (CD25), Tregs scavenge IL- 2 from the local milieu, constraining the viability and clonal expansion of effector and memory T- cell populations[38]. Tregs curtail interferon- γ (IFN- γ) production by $CD8^+$T lymphocytes, which in turn blocks IFN- γ- driven repolarization of TAMs and indirectly perpetuates their protumoral, immunosuppressive state, collectively establishing a synergistic immunosuppressive circuitry[39]. Of note, tumor- infiltrating Tregs upregulate lipid anabolic pathways dependent on sterol regulatory element- binding protein (SREBP), a metabolic adaptation that enables Tregs to sustain their proliferation and suppressive functions within the hypoxic and nutrient- depleted tumor microenvironment[40].

MDSCs constitute a remarkably heterogeneous ensemble of immature myeloid elements that extensively infiltrate the glioma milieu and execute robust immunosuppressive activities[41]. According to their phenotypic distinctions, MDSCs are broadly categorized into monocytic (M- MDSC) and polymorphonuclear (PMN- MDSC) subpopulations. Within gliomas, the MDSC infiltrate is dominated by the PMN- MDSC subset, which comprises roughly 10%–20% of $CD45^+$ leukocytes[41, 42]. MDSCs suppress immune responses through multiple mechanisms. MDSCs highly express arginase 1 (Arg- 1) and inducible nitric oxide synthase (iNOS), which deplete L- arginine in the microenvironment and directly block the expression of the T- cell receptor- associated CD3ζ chain, thereby inhibiting T- cell proliferation and effector functions[43, 44]. MDSCs produce metabolites such as reactive oxygen species (ROS) that can induce T- cell apoptosis, further exacerbating immunosuppression[45]. MDSCs produce chemokines including CCL22 that attract Tregs into the tumor ecosystem, where they act in concert to dampen effector T- cell activity[46]. More importantly, a bidirectional regulatory crosstalk exists between MDSCs and GSCs. Macrophage migration inhibitory factor (MIF) secreted by GSCs stimulates MDSCs to upregulate Arg- 1 and iNOS expression, enhancing their immunosuppressive activity. Conversely, activated MDSCs sustain GSC stemness and proliferative capacity through secretion of IL- 6 and TGF- β, establishing a positive feedback loop that promotes tumor progression[47]. Exosomes shed by glioma cells are capable of shuttling non- coding RNAs, including miR- 1246 to monocytes, thereby skewing their differentiation toward the M- MDSC phenotype and potentiating their immunosuppressive functions[48]. Therefore, through metabolic competition, ROS- mediated damage, immune cell recruitment, and crosstalk with GSCs, MDSCs constitute a critical component of the glioma immunosuppressive network.

Effector T cells, particularly $CD8^+$ cytotoxic T lymphocytes (CTLs), are the core executors of antitumor immunity. However, within the glioma microenvironment, these cells often exist in a dysfunctional and atypical state, rendering them incapable of effectively eliminating tumor cells[49]. Tumor- infiltrating $CD8^+$T cells within gliomas exhibit elevated surface expression of an array of immune checkpoint receptors, such as PD- 1, TIM- 3, and LAG- 3[50]. Sustained chronic antigen exposure imposes metabolic reprogramming upon T cells. Meanwhile, glioma cells avidly consume glucose via aerobic glycolysis (the Warburg effect)[51], resulting in mitochondrial dysfunction and diminished glucose uptake and utilization, which in turn leads to reduced secretion of effector molecules such as IFN- γ, granzyme B, and perforin, impaired proliferative capacity, and eventual entry into a state of functional exhaustion[52]. The glioma microenvironment is deficient in effective co- stimulatory signals, while MDSCs and Tregs further impede T cell activation and expansion through secretion of inhibitory cytokines and competitive depletion of IL- 2[53]. Abnormal accumulation of cholesterol in the glioma

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microenvironment can directly induce T- cell exhaustion by triggering endoplasmic reticulum stress, which upregulates the expression of inhibitory receptors including PD- 1, 2B4, and LAG- 3 on $CD8^+$T cells[54]. Hypoxia impairs mitochondrial integrity in T cells and elevates ROS generation, which collectively promotes their polarization toward an irreversible, terminally exhausted phenotype[55]. Under the combined influence of the aforementioned multiple mechanisms, effector T cells, even if they successfully infiltrate the tumor site, are incapable of mounting effective antitumor functions.

Collectively, the glioma milieu contains an intricate circuitry of diverse immunosuppressive cellular subsets and their associated soluble mediators, which in concert establish a hierarchical array of barriers against effective antitumor immunity. TAMs, being the most prevalent immune constituents, produce immunosuppressive cytokines including IL- 10 and TGF- β via their M2- polarized state, and concurrently overexpress PD- L1, thereby exerting direct restraint upon T- cell effector functions[29, 31, 56, 57]. Tregs potentiate the immunosuppressive landscape via secretion of TGF- β, scavenging of IL- 2, and engagement of IDO- mediated metabolic circuits[35, 37, 58]. MDSCs, for their part, deplete T cell- essential nutrients via overexpression of Arg- 1 and iNOS and generation of ROS. Additionally, they coordinate with GSCs and Tregs through exosomal crosstalk and chemokine- directed signaling cascades[43, 44, 46-48, 59]. Under the concurrent influence of these three immunosuppressive subsets, effector T cells, even those that successfully navigate to neoplastic lesions, inevitably lapse into a state of functional exhaustion, precipitated by persistent checkpoint receptor engagement, deficiency of co- stimulatory signals, and profound metabolic perturbations within the microenvironment. Consequently, their tumor- clearing potential is abrogated[54, 60, 61]. These cells, interwoven with metabolic products such as cholesterol and lactate as well as inhibitory cytokines within the microenvironment, form a self- reinforcing and highly stable immunosuppressive network that endows gliomas with a typical "cold" tumor phenotype.

2.2 Immunosuppressive Molecular Characteristics

The inhibitory cytokine network in the glioma microenvironment is collectively secreted by multiple cell types, including tumor cells, TAMs, and Tregs, forming a complex regulatory circuitry in which autocrine and paracrine signals are intricately interwoven. IL- 10 and TGF- β stand out as the pivotal immunosuppressive moieties operative in this network.Within the glioma microenvironment, IL- 10 is predominantly produced by M2- like TAMs and Tregs. GSCs, in turn, secrete MIC- 1, TGF- β1, and sCSF- 1, which potently augment TAM- derived IL- 10 and TGF- β output, collectively erecting a robust immunosuppressive bulwark[29, 32]. Mechanistically, IL- 10 primarily engages the IL- 10 receptor on target cells to activate the JAK2/STAT3 signaling pathway, which on one hand downregulates surface expression of MHC class II molecules and the co- stimulatory molecule CD40 on TAMs and dendritic cells, thereby restraining initial T- cell activation. On the other hand, which promotes further M2 polarization of TAMs, amplifying the immunosuppressive effects[28, 62]. TGF- β, mainly derived from neoplastic cells, TAMs, and Tregs, operates via the classical Smad cascade as well as non- classical MAPK and PI3K axes to fulfill a broad spectrum of immunosuppressive roles. It curbs $CD8^+$T cell expansion and effector molecule production, orchestrates the commitment of naive $CD4^+$T cells toward $FoxP3^+$Tregs, dampens NK cell- mediated cytotoxicity, reinforces M2 polarization of TAMs, and impairs dendritic cell antigen presentation[63, 64]. Additionally, upon exposure to IL- 6 and IL- 10, TAMs engage STAT3 signaling to upregulate their own secretion of IL- 6, IL- 10, and TGF- β, thereby engendering a self- amplifying positive feedback cycle that perpetuates immunosuppression[28]. These inhibitory cytokines are interwoven with one another, collectively sustaining the immunosuppressive state of the glioma microenvironment.

Immune checkpoint molecules are a class of regulatory molecules expressed on the surface of immune cells or tumor cells that finely modulate T- cell activation thresholds by transmitting inhibitory or stimulatory signals. In the glioma microenvironment, neoplastic cells and tumor- infiltrating leukocytes aberrantly overexpress an array of co- inhibitory checkpoint receptors, collectively establishing a "molecular braking" circuitry that intercepts T- cell immunity at successive stages, encompassing initial priming, effector function sustenance, and memory differentiation[65]. The PD- 1/PD- L1 axis stands as the most canonical immune checkpoint cascade operative in gliomas. PD- 1 is predominantly displayed on activated T lymphocytes, Tregs, and myeloid lineage cells, its cognate ligand PD- L1, in turn, is widely overexpressed on glioma cells, TAMs, and MDSCs[66]. Upon engagement of PD- 1 with PD- L1, the recruitment of SHP- 2 phosphatase inhibits T- cell receptor (TCR)- downstream PI3K/AKT and RAS/MAPK signaling pathways, leading to impaired T- cell proliferation, reduced secretion of effector molecules such as IFN- γ and granzyme B, and ultimately driving T cells toward an exhausted state[67]. Notably, an adaptive immune resistance mechanism operates dynamically within the glioma niche, wherein IFN- γ produced by activated T cells upregulates PD- L1 expression on neoplastic

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cells, thereby establishing a self-amplifying negative feedback circuit[67]. The CTLA-4/CD80-86 axis constitutes another critical checkpoint pathway. CTLA-4 is induced early during T-cell activation and exhibits a markedly higher binding affinity for CD80/CD86 than the co-stimulatory molecule CD28, thereby competitively blocking the second signal required for T cell activation. It primarily acts in secondary lymphoid organs such as lymph nodes to regulate the initial priming phase of T cells, functionally complementing the role of PD-1 in peripheral tissues[68]. Additional emerging checkpoint receptors likewise exert significant immunomodulatory functions.LAG-3 is overexpressed on both exhausted T lymphocytes and Tregs. Its co-expression with PD-1 synergistically drives severe T-cell dysfunction and exhaustion[69]. TIM-3 is upregulated on T cells infiltrating gliomas. Upon ligation with its ligand Galectin-9, which triggers T-cell apoptosis and fosters Treg proliferation[70]. TIGIT, expressed on both T and NK cells, inhibits effector functions directly upon engagement with its ligand CD155, and is commonly co-expressed with PD-1 on dysfunctional lymphocytes[71]. VISTA is predominantly expressed on T cells, TAMs, and MDSCs. Notably, its expression levels in glioma are tightly associated with unfavorable clinical outcomes[72]. These immune checkpoint receptors do not operate as independent entities. Instead, through synergistic and non-redundant mechanisms, they impose sustained co-inhibitory signaling upon T cells at successive checkpoints, ranging from priming within lymph nodes to effector phase execution within neoplastic lesions, thereby jointly constructing a tiered molecular fortress that obstructs antitumor immune responses.

2.3 Metabolic Byproducts and Microenvironmental Stress

The glioma microenvironment harbors multiple metabolic aberrations, including lactate accumulation, activation of the tryptophan-kynurenine pathway, adenosine accumulation, and cholesterol metabolic reprogramming. These metabolites not only directly impair immune cell functions but also promote immunosuppression through modulation of signaling pathways[73]. Lactate accumulation represents one of the most prominent features of the glioma metabolic microenvironment. Glioma cells produce large quantities of lactate through the Warburg effect, leading to reduced extracellular pH[74]. Upon entering T cells via monocarboxylate transporters, lactate suppresses T cell proliferation and effector functions, promotes M2 polarization of TAMs, and enhances the immunosuppressive activity of MDSCs[75]. The tryptophan-kynurenine metabolic pathway constitutes another important mechanism of immunosuppression in glioma. IDO and TDO are overexpressed in neoplastic cells and TAMs, catalyzing the conversion of tryptophan to kynurenine. Depletion of tryptophan imposes cell-cycle blockade upon T lymphocytes. Concomitantly, kynurenine engages the aryl hydrocarbon receptor (AhR) to promote Treg differentiation while restraining Th17 cell activity[35, 36]. The adenosine pathway also plays a significant role in glioma immunosuppression. Under hypoxic conditions, tumor cells and myeloid cells highly express CD39 and CD73, which sequentially hydrolyze ATP into adenosine.Adenosine, by binding to the A2A receptor on T-cell surfaces, activates the cAMP-PKA signaling pathway, which suppresses T-cell proliferation, activation, and effector molecule secretion[76]. Hypoxia and nutrient scarcity represent additional defining attributes of the glioma niche. Hypoxia-inducible factor HIF-1α transcriptionally regulates the expression of immunosuppressive molecules including PD-L1, CD39, and VEGF, and drives M2 polarization of TAMs[77]. Profound depletion of glucose and glutamine within the milieu disadvantages tumor-infiltrating T cells in metabolic competition, precluding mTOR pathway engagement and ultimately propelling them into functional exhaustion[51]. Cholesterol metabolic reprogramming represents an emerging immunosuppressive mechanism in the glioma microenvironment. TAMs within the glioma milieu are capable of scavenging cholesterol-laden myelin fragments and transferring the liberated cholesterol to neoplastic cells, thereby meeting their elevated metabolic requirements[73]. Aberrant cholesterol accumulation directly impairs antitumor immunity. On one hand, oxidized metabolites such as 7-ketocholesterol suppress mitochondrial translation in TAMs, attenuating their phagocytic function and skewing them toward an M2-like phenotype. On the other hand, excessive cholesterol upregulates the expression of inhibitory receptors including PD-1 on $CD8^{+}$T cells through endoplasmic reticulum stress, inducing T cell functional exhaustion[78].

In sum, the intricate interplay among immunosuppressive cytokines, metabolic byproducts, and immune checkpoint molecules consolidates a hierarchical, self-amplifying immunosuppressive circuitry that confers upon gliomas the prototypical "cold" tumor phenotype(**Fig.1**). This architecture also establishes the microenvironmental foundation upon which OV-mediated disruption of the inhibitory barrier may be examined. To achieve effective immunotherapy for glioma, it is imperative to fundamentally remodel this immunosuppressive microenvironment at its source. The distinctive therapeutic merit of OVs as an emerging immunomodulatory modality resides in their capacity to breach the above-described immunosuppressive barricades via diverse mechanisms, encompassing ICD elicitation, TAM repolarization, and

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recruitment/activation of cytotoxic T cell effectors, thus transforming immunologically "cold" lesions into "hot" ones and setting the stage for combinatorial immunotherapeutic approaches. The subsequent chapters will systematically delineate the molecular underpinnings of OVs- mediated remodeling of the glioma immune microenvironment.

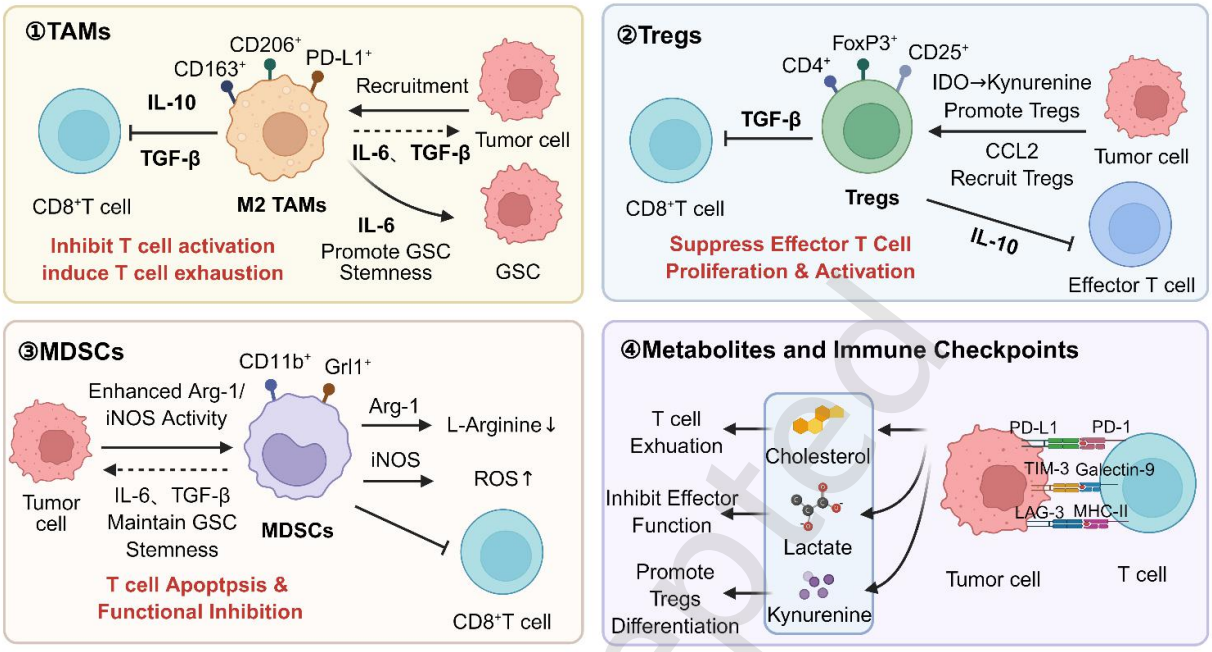


Figure 1 Core components and functional network of the immunosuppressive microenvironment in glioma.

3 Mechanisms of OVs- Mediated Remodeling of the Immune Microenvironment

OVs are a class of viruses capable of selectively replicating within and lysing tumor cells, and have garnered considerable attention in recent years due to their unique immunomodulatory functions. Beyond their conventional direct cytolytic activity, the paramount therapeutic significance of OVs resides in their function as an "in situ vaccine", viral infection precipitates ICD of neoplastic cells, with the consequent release of danger signals that recruit and prime antigen- presenting cells. In parallel, OV- mediated infection restructures the immune cellular landscape of the tumor microenvironment, effecting a transition from an immunologically "cold" to a "hot" phenotype(**Fig.2**)[79, 80]. This section will delineate the molecular underpinnings of OV- mediated remodeling of the glioma immune microenvironment through three interconnected axes: elicitation of ICD to prime antitumor immunity, repolarization of TAM phenotypes, and recruitment/activation of CTLs.

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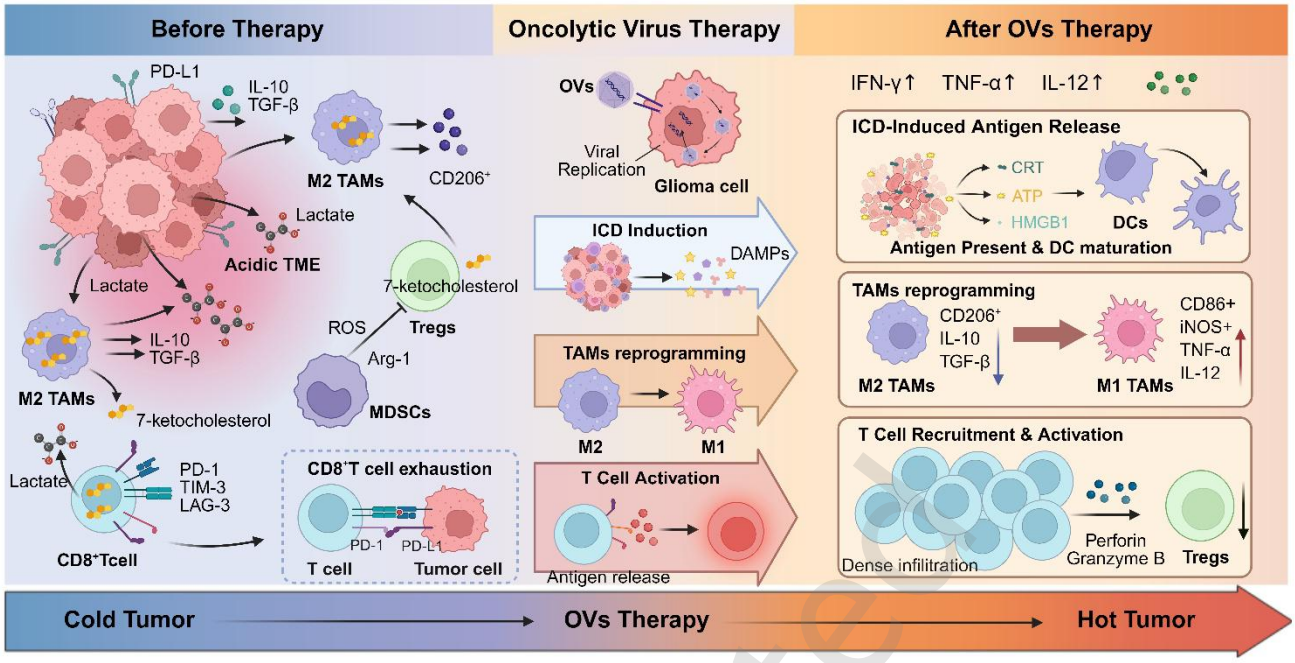


Figure 2 OVs remodel the glioma TIME.The left panel depicts the immunosuppressive microenvironment (cold tumor) before treatment, characterized by abundant infiltration of M2-like tumor-associated macrophages, regulatory T cells and myeloid-derived suppressor cells, along with functionally exhausted $CD8^+$T cells.The right panel illustrates the immunologically activated microenvironment (hot tumor) after OVs therapy, achieved through induction of immunogenic cell death with release of damage-associated molecular patterns, promotion of dendritic cell maturation reprogramming of tumor-associated macrophages, and recruitment and activation of $CD8^+$T cells, collectively driving the conversion from a "cold" to a "hot" tumor.

3.1 Induction of ICD

Following infection of glioma cells, OVs do not simply lyse the cells. Rather, they induce ICD, prompting dying cells to release damage- associated molecular patterns (DAMPs), including calreticulin (CRT), adenosine triphosphate (ATP), high- mobility group box 1 (HMGB1), and heat shock proteins, along with tumor- associated antigens[81]. These molecules act in concert to facilitate the uptake and cross- presentation of tumor antigens by antigen- presenting cells (APCs) such as dendritic cells, thereby initiating tumor- specific adaptive immune responses[82]. Notably, ICD converts the inherently immunosuppressive state of the tumor microenvironment into an immunogenic event capable of activating T cells[81].

The immunogenic potency of ICD is contingent upon the spatiotemporally ordered release or surface exposure of DAMPs throughout the cell death process. CRT undergoes swift translocation from the endoplasmic reticulum to the neoplastic cell surface during the early phase of viral infection. This event is governed by PERK- dependent eIF2α phosphorylation, represents a pivotal checkpoint in the initiation of ICD[83]. CRT displayed on the outer leaflet of the plasma membrane engages CD91 on dendritic cells, thereby promoting the engulfment of apoptotic neoplastic cells and the acquisition of tumor antigens[84, 85]. ATP is massively discharged into the extracellular milieu via autophagy and exocytosis triggered by viral infection. It subsequently recruits and activates dendritic cells and macrophages through ligation of the purinergic receptors P2Y2 and P2X7[86]. Elevated ATP levels engage P2X7 to trigger NLRP3 inflammasome assembly, culminating in the release of IL- 1β and IL- 18, which potently amplify the ensuing immune cascade[87]. HMGB1, liberated from the nucleus into the extracellular compartment upon viral replication- induced compromise of nuclear envelope integrity, activates dendritic cells via engagement of Toll- like receptor 4 (TLR4) and other pattern- recognition receptors, thereby driving their maturation, mobilizing their migration, and potentiating their cross- presenting capability[88, 89]. Furthermore, heat shock proteins HSP70 and HSP90 can also be released extracellularly, where they act as DAMPs and bind to TLR2 and TLR4 on dendritic cells, promoting their maturation and antigen- presenting capacity and further reinforcing adaptive immune responses[90, 91]. The synergistic action of these DAMPs converts viral infection- induced cell death from an immunologically silent to an immunostimulatory event, laying a solid foundation for the subsequent initiation of antitumor immune responses.

A range of OVs have been validated as ICD inducers across glioma and other neoplastic models, although the magnitude of ICD induction is contingent upon both the specific viral backbone and the intrinsic properties of the target tumor cells. Newcastle disease virus (NDV)

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ranks among the most thoroughly investigated viral agents capable of eliciting ICD. Koks and colleagues reported that NDV (Hitchner B1 strain) infection of glioma cells triggers CRT membrane translocation and HMGB1 liberation, while concurrently fostering dendritic cell maturation and T‐cell cross‐priming. In orthotopic murine glioma models, NDV therapy conferred extended survival and, importantly, engendered durable tumor‐specific immune memory[92]. Shao and coworkers subsequently demonstrated in melanoma cells that the NDV/FMW strain likewise elicits CRT surface exposure, liberation of HMGB1 and HSP70/90, and ATP release in melanoma cells[93].

Vaccinia virus similarly qualifies as an ICD inducer. Huang et al.found that vaccinia virus infection induces HMGB1 release from tumor cells in a virus‐mediated cell death‐dependent manner, and that the released HMGB1 enhances the sensitivity of tumor cells to chemotherapy[94]. Chen et al.further demonstrated that stereotactic body radiotherapy combined with vaccinia virus therapy significantly increases the expression of CRT, HMGB1, HSP70, and Annexin A1 in tumor cells, synergistically enhancing immunogenicity[95].

Modified adenovirus (Ad) strains have also achieved significant progress in ICD induction. The CD40L‐armed adenovirus Ad5/3‐hTERT‐E1A‐hCD40L, developed by Diaconu et al., significantly enhances CRT exposure and HMGB1/ATP release upon infection of tumor cells, and exerts antitumor effects through induction of Th1‐polarized immune responses and $CD8^+$T cell activation[96]. Wang and coworkers developed the oncolytic adenoviral variants ADV^{-NE} and ADV^{-PPE}, which encode neutrophil elastase (NE) or porcine pancreatic elastase (PPE), respectively. These agents trigger pyroptotic cell death in neoplastic cells, with concomitant HMGB1 liberation. The released HMGB1 then engages TLR4 on macrophages, activating the MyD88‐NF‐κB‐NLRP3 signaling cascade to drive M1 macrophage polarization, which in turn augments the infiltration of effector memory and effector $CD8^+$T cells, culminating in amplified antitumor activity[97]. Ma et al.compared the ICD‐inducing capacities of wild‐type adenovirus, Semliki Forest virus (SFV4), and vaccinia virus, finding that all three viruses could induce CRT exposure and the release of HMGB1, HSP90, and ATP. However, only SFV4 could significantly trigger dendritic cell secretion of Th1‐type cytokines and induce antigen‐specific T‐cell activation[90].

Parvovirus H‐1 (H‐1PV) has demonstrated immunostimulatory potential in clinical trials for glioma. In a Phase I/IIa clinical trial, Geletneky et al.observed increased $CD8^+$ T cell infiltration in patient tumor tissues following H‐1PV treatment, along with upregulated expression of cytokines such as IFN‐γ and IL‐2, suggesting that the virus had induced antitumor immune responses[98].

3.2 TAMs Repolarization

OVs can influence the polarization state of TAMs through direct or indirect means. It has been demonstrated that conditioned media harvested from breast cancer cells following infection with measles virus (MeV) or mumps virus (MuV) drive MDMs toward an antitumor phenotypic switch, characterized by elevated CD80 expression and enhanced production of M1‐skewed cytokines, among them IL‐1β, TNF‐α, CXCL9, CXCL10, and IL‐6[99].This reprogramming effect, however, does not depend on direct viral infection of macrophages, as MeV and MuV cannot replicate efficiently in macrophages[99]. Instead, soluble mediators shed from virally infected neoplastic cells, including DAMPs and various cytokines, likely constitute the principal impetus for TAM repolarization.

Similarly, in colorectal cancer models, following infection with the oncolytic adenoviruses ADV^{-NE} and ADV^{-PPE}, HMGB1 released from tumor cells can engage TLR4 on macrophages, activating the MyD88–NF‐κB–NLRP3 signaling pathway and inducing macrophage polarization toward the M1 phenotype[97]. Concomitant with this polarization shift are elevated expression of M1‐linked markers CD86 and iNOS, as well as pro‐inflammatory mediators TNF‐α, IL‐6, and IL‐1β, together with reduced levels of M2‐associated molecules Arg‐1 and CD206 and suppressive cytokines IL‐10 and TGF‐β. CD40L‐armed oncolytic adenoviruses, on the other hand, modulate TAMs through a distinct pathway.Diaconu et al. found that following infection with CD40L‐expressing adenovirus, macrophage infiltration in tumor tissues significantly increased, accompanied by the production of Th1‐type cytokines such as IL‐12[96]. Engagement of CD40L with CD40 receptor on macrophages directly activates M1 polarization, and this macrophage reprogramming further promotes the recruitment and activation of $CD8^+$ T cells.

The impact of OVs on TAM polarization exhibits variability contingent upon both the specific viral strain and the tumor model under investigation. In glioma models, an oncolytic adenovirus engineered with ARF1 deficiency potentiates cGAS/STING pathway activation, thereby skewing TAMs toward a pro‐inflammatory phenotype[100]. Conversely, across a range of solid tumor models, the antitumor efficacy of OVsM1 correlates inversely with TAM abundance, and this resistance is predominantly orchestrated by M1‐like TAMs[101]. Thus, TAMs exert a dichotomous role in OV‐based therapy. Activated TAMs may curtail viral propagation through phagocytic clearance of virions and elaboration of antiviral cytokines[102]. Conversely, TAMs are

amenable to repolarization toward antitumor effector phenotypes.

M1 polarization of TAMs exerts important immunological functions. Anti‐CD47 antibody‐armed oncolytic adenoviruses potently augment macrophage‐mediated engulfment of neoplastic cells. However, this phagocytic activity is abrogated under acidic microenvironmental conditions[21]. M1 macrophages orchestrate the recruitment of $CD8^+$ T lymphocytes into neoplastic lesions via the elaboration of chemotactic cues, including CXCL10 and CXCL11[97]. Critically, M1 macrophages are competent to serve as professional antigen‐presenting cells, capable of processing and cross‐presenting tumor‐derived epitopes to prime tumor‐specific T cell immunity. In glioma models, selective ablation of ARF1 in TAMs drives their repolarization toward a pro‐inflammatory phenotype, augments $CD8^+$ T cell infiltration, and potentiates tumor antigen‐specific immunity[100]. Thus, through modulation of TAM polarization, OVs mitigate local immunosuppression and establish a permissive milieu that enables the restoration of $CD8^+$ T cell effector function.

3.3 T Cell Recruitment and Activation

Effector T cells, particularly CTLs, are the core executors of antitumor immunity. In gliomas, however, T‐cell infiltration is sparse and the few T cells that do infiltrate are functionally exhausted. OVs, on one hand, reshape the chemokine network through infection to recruit T cells into the tumor microenvironment. On the other hand, they activate T cells to acquire cytotoxic functions through induction of ICD and antigen cross‐presentation[90, 103]. Moreover, OVs can also weaken the immunosuppressive barrier imposed by Tregs, further unleashing the potential of effector T cells and thereby reversing the immunosuppressive microenvironment[104, 105].

Upon OVs infection, neoplastic and stromal cells elaborate an array of chemokines that collectively establish a chemotactic gradient to guide T cell trafficking. Liu et al. reported that OVsM1 treatment significantly upregulated the mRNA levels of chemokines including CCL3, CCL5, CXCL9, and CXCL10 in tumor tissues, promoting $CD8^+$ T cell infiltration into the tumor site. M1 treatment increased the number of tumor‐infiltrating $CD8^+$ T cells by nearly 2‐fold, and these T cells highly expressed the activation markers CD69 and CD44. Critically, ICD elicited by OVs liberates tumor‐associated antigens that are subsequently internalized by dendritic cells and cross‐presented to $CD8^+$ T lymphocytes, thus priming tumor‐specific adaptive immunity. Supernatants harvested from M1‐infected tumor cells, upon exposure to immature dendritic cells, drove robust upregulation of MHC I and CD86, while concurrently stimulating dendritic cells to produce IL‐12, TNF‐α, and IL‐1β. These licensed dendritic cells subsequently orchestrated the differentiation of naive T lymphocytes into effector CTLs characterized by IFN‐γ, granzyme B, and perforin positivity[103]. Depletion of $CD8^+$ T cells completely abrogated the therapeutic efficacy and survival benefit of OVsM1, further underscoring the critical importance of T cells. Similarly, Webb et al. found in hepatocellular carcinoma models that OV VSV‐IFNβ (vesicular stomatitis virus expressing IFN‐β) treatment induced a robust antiviral $CD8^+$ T cell response. However, when tumor antigens were encoded into the virus, a fraction of the antiviral response could be redirected toward antitumor responses, significantly enhancing $CD8^+$ T cell‐mediated tumor clearance. This suggests that incorporating tumor antigens into viral vectors is an effective strategy to potentiate tumor‐specific T cell activation[106].

$CD4^+$ helper T cells (Th1) play a critical auxiliary role in antitumor immunity. In particular, the Th1 subset promotes the survival, proliferation, and functional maintenance of $CD8^+$ T cells through secretion of cytokines such as IFN‐γ and IL‐2. OVs can activate $CD4^+$ T‐cell responses through multiple pathways. Following infection with CD40L‐armed oncolytic adenoviruses, CD40L is expressed on the surface of tumor cells, binds to CD40 receptor on antigen‐presenting cells, and induces IL‐12 production, which in turn drives $CD4^+$ T cell polarization toward the Th1 phenotype. In vivo experiments demonstrated that splenocytes from mice treated with Ad5/3‐CMV‐mCD40L exhibited significantly elevated levels of Th1‐type cytokines, including IL‐12, IFN‐γ, RANTES, and TNF‐α[96]. In a parallel finding, Delaunay and colleagues reported that following infection and lysis of NY‐ESO‐1‐expressing tumor cells by diverse OVs, including measles virus, vaccinia virus, VSV, and HSV, the liberated tumor antigens were internalized by neighboring HLA‐DP4‐positive tumor cells and presented in the context of MHC‐II to the NY‐ESO‐1‐specific $CD4^+$ T cell clone NY67. This engagement activated the clone to secrete IFN‐γ and execute cytolytic functions[107].

Tregs are a critical suppressive cell population that maintains immune tolerance and accumulate abundantly in the glioma microenvironment, where they inhibit effector T cell functions. An ideal immunotherapy should simultaneously activate effector T cells and attenuate the suppressive barrier imposed by Tregs. Wang et al.reported that following treatment with the armed vaccinia virus VV‐iPDL1/GM (expressing a PD‐L1 inhibitor and GM‐CSF), the proportions of tumor‐infiltrating $CD8^+$ and $CD4^+$ T cells significantly increased, while the proportions of Tregs and MDSCs decreased, indicating that the armed virus effectively remodeled the immunosuppressive

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microenvironment[20]. Qiao et al. adopted a strategy of loading adoptively transferred OT-IT cells with oncolytic VSV, and found that virus loading not only enhanced the expression of T cell activation markers CD44 and CD25 and effector functions, but also promoted T cell accumulation at the tumor site. More importantly, this combination therapy was dependent on the participation of host $CD8^+$ T cells, indicating that OVs can synergize with adoptive T cell therapy to collectively reverse immunosuppression[108].

In summary, OVs remodel the immunosuppressive microenvironment of glioma through a triple mechanism: (i)OVs infection induces ICD in tumor cells, releasing DAMPs and tumor-associated antigens, promoting dendritic cell maturation and antigen cross-presentation, and initiating tumor-specific adaptive immune responses; (ii)shifting TAMs from the M2 to the M1 phenotype in glioma, attenuating the sources of inhibitory cytokines such as IL-10 and TGF-β while recruiting $CD8^+$ T cell infiltration; and (iii)promoting the recruitment and activation of $CD8^+$ cytotoxic T lymphocytes and $CD4^+$ helper T cells through remodeling of the chemokine network, while partially attenuating the immunosuppressive function of Tregs. Through the coordinated interplay of ICD elicitation, TAM repolarization, and T cell activation, gliomas are transformed from immunologically "cold" lesions into "hot" tumors endowed with appreciable immune responsiveness, thus establishing a permissive microenvironmental platform that paves the way for subsequent combinatorial therapeutic interventions.

4 Targeted Delivery Strategies for OVs in Glioma

The effective delivery of OVs to intracranial neoplasms stands as one of the foremost challenges confronting glioma therapy. The BBB, as the innate guardian of the CNS, restricts passage to lipophilic small molecules. OVs particles, however, generally range from tens to hundreds of nanometers in diameter, precluding their passive diffusion across this barrier to reach the brain parenchyma[8, 109]. Clinically, conventional OVs therapy relies heavily on intracranial injection. However, this approach is difficult to apply in patients with deep-seated or multifocal gliomas and cannot adequately cover the invasive front of tumors, thus limiting its broad clinical application[9]. Although systemic intravenous administration can overcome this limitation, it faces even more complex challenges. Intravenously administered viruses must not only traverse the BBB before reaching the brain tumor, but also contend with clearance by neutralizing antibodies in the circulation and sequestration by the mononuclear phagocyte system, resulting in extremely low effective viral concentrations at the tumor site[11]. Furthermore, intravenously administered viruses are subject to rapid clearance by the reticuloendothelial system, including the liver, further reducing the viral load that reaches the brain tumor[10]. Even in the context of direct intratumoral administration, both pre-existing and de novo-induced neutralizing antibodies are capable of infiltrating the intracranial tumor milieu and neutralizing viral particles, thereby profoundly compromising treatment outcomes[110]. The expression density of viral entry receptors on neoplastic cells critically governs OVs infectivity. Thompson and colleagues demonstrated that the oncolytic activity of herpes simplex virus G207 exhibits a highly significant negative correlation with CD111 receptor levels, and that diminished receptor abundance directly engenders primary resistance of tumor cells to OVs infection[13].

To overcome the core delivery bottlenecks of the physical BBB barrier and insufficient targeting, researchers have in recent years developed a variety of innovative strategies.Based on their primary delivery mechanisms, these strategies can be classified into five categories:(1)active targeting, which relies on ligand-receptor-mediated transcytosis;(2)biomimetic cell-membrane camouflage, which uses natural cell membranes to confer immune evasion and targeting;(3)cell-based carrier delivery, which employs living cells as migratory vehicles;(4)physical targeting, which utilizes external physical forces or anatomical routes to bypass the BBB; and(5)exosome-like particle delivery, which leverages cell-derived nanovesicles to deliver viral signals. Each category has distinct features and applicable scenarios, although they may also be combined in practice. The following subsections will discuss these strategies in detail.

4.1 Active Targeting Strategies

Active targeting approaches functionalize viral particles or nanocarriers with ligands including antibodies, peptides, and aptamers that specifically recognize and engage receptors upregulated on the BBB or neoplastic cells, thus enabling receptor-mediated transcytosis. He and colleagues engineered a nanocapsule designated EV71@AM, employing the recombinant OVs EV-A71-miR124T as the core template and utilizing in situ free-radical polymerization as the fabrication methodology. Specifically, in sodium bicarbonate buffer, acrylamide and N-methacrylamide (APMAAm) monomers were electrostatically adsorbed onto the viral particle surface. Subsequently, two bis-acrylate-modified crosslinking peptides-the ANG peptide and the MMP-2 substrate peptide-were introduced. Free-radical polymerization was then initiated with ammonium persulfate and TEMED to form a polymer shell encapsulating the virus, with an average particle size of approximately 41 nm(**Fig.3a**). The core mechanism of this delivery system operates at two levels. First, receptor-mediated transcytosis (RMT)-the ANG peptide, serving as a high-affinity ligand for low-density lipoprotein receptor-related protein 1 (LRP-1), binds to LRP-1 highly expressed

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on BBB endothelial cells and glioma cell membranes, triggering clathrin‐dependent endocytosis to form endocytic vesicles that traverse the endothelial cells and are subsequently released into the brain parenchyma via exocytosis, achieving active BBB crossing. Compared with adsorption‐mediated transcytosis (AMT) that relies on electrostatic interactions, this approach offers higher tissue selectivity and lower nonspecific background. Second, tumor microenvironment‐responsive release and viral amplification, the MS peptide contains a specific MMP‐2 cleavage sequence (VPLGVRLPGVPLGVRTK), and MMP‐2 expression is significantly higher in glioma tissues than in normal brain tissue. Upon arrival of the nanocapsules at the tumor site, MMP‐2 cleaves the MS crosslinking peptides, leading to disintegration of the polymer shell. The released EV‐A71‐miR124T then enters glioma cells through its natural receptor, utilizes the host translational machinery to replicate, lyses tumor cells, and releases progeny viruses and tumor‐associated antigens, initiating in situ immune activation[14].

Chen et al. utilized oncolytic herpes simplex virus type II (OH2) as the core, employed the abundant amino groups on the viral capsid proteins to introduce PEGylation via NHS‐PEG‐Mal linker chains, and further covalently conjugated RVG29‐Cys peptide to the PEG chain termini through maleimide‐thiol coupling, thereby constructing the OH2‐PEG‐RVG nanoconjugate, which exhibited a particle size of approximately 193 nm and a polydispersity index reduced from 0.256 to 0.083, indicating markedly superior dispersity compared with bare virus(**Fig.3b**). The core mechanisms of this delivery system encompass two aspects. First, receptor‐mediated transcytosis across the BBB. RVG29 peptide functions as a high‐affinity ligand for the nicotinic acetylcholine receptor, which is abundantly expressed on brain microvascular endothelial cells. Upon receptor engagement, the conjugate is internalized predominantly via clathrin‐dependent endocytosis, forming endosomal vesicles that traverse the endothelial monolayer and are released into the brain parenchyma through exocytosis, thereby enabling active BBB penetration. Although RVG29 may also engage the GABA receptor system in the transport process, the clathrin‐mediated pathway remains dominant, implicating the endolysosomal trafficking route as a key determinant of viral particle transcytosis. Second, PEGylation confers immune shielding and extended circulation kinetics. The PEG corona effectively conceals antigenic determinants on the OH2 capsid, markedly diminishing recognition and neutralization by pre‐existing antiviral antibodies. This approach substantially prolongs viral bloodstream residence time in BALB/c mouse models, while concurrently attenuating nonspecific deposition in the liver, spleen, and kidneys, and minimizing reticuloendothelial system‐mediated clearance. Leveraging the synergistic combination of RVG29‐directed active targeting and PEGylation‐mediated immunological shielding, OH2‐PEG‐RVG achieved efficient BBB penetration and tumor‐selective accumulation in orthotopic brain tumor models, resulting in marked tumor growth inhibition and extended survival of tumor‐bearing mice, while preserving BBB integrity and eliciting no discernible CNS behavioral aberrations. This platform thus establishes a safe and efficacious chemical modification strategy for the intravenous administration and active intracranial delivery of OVs[111].

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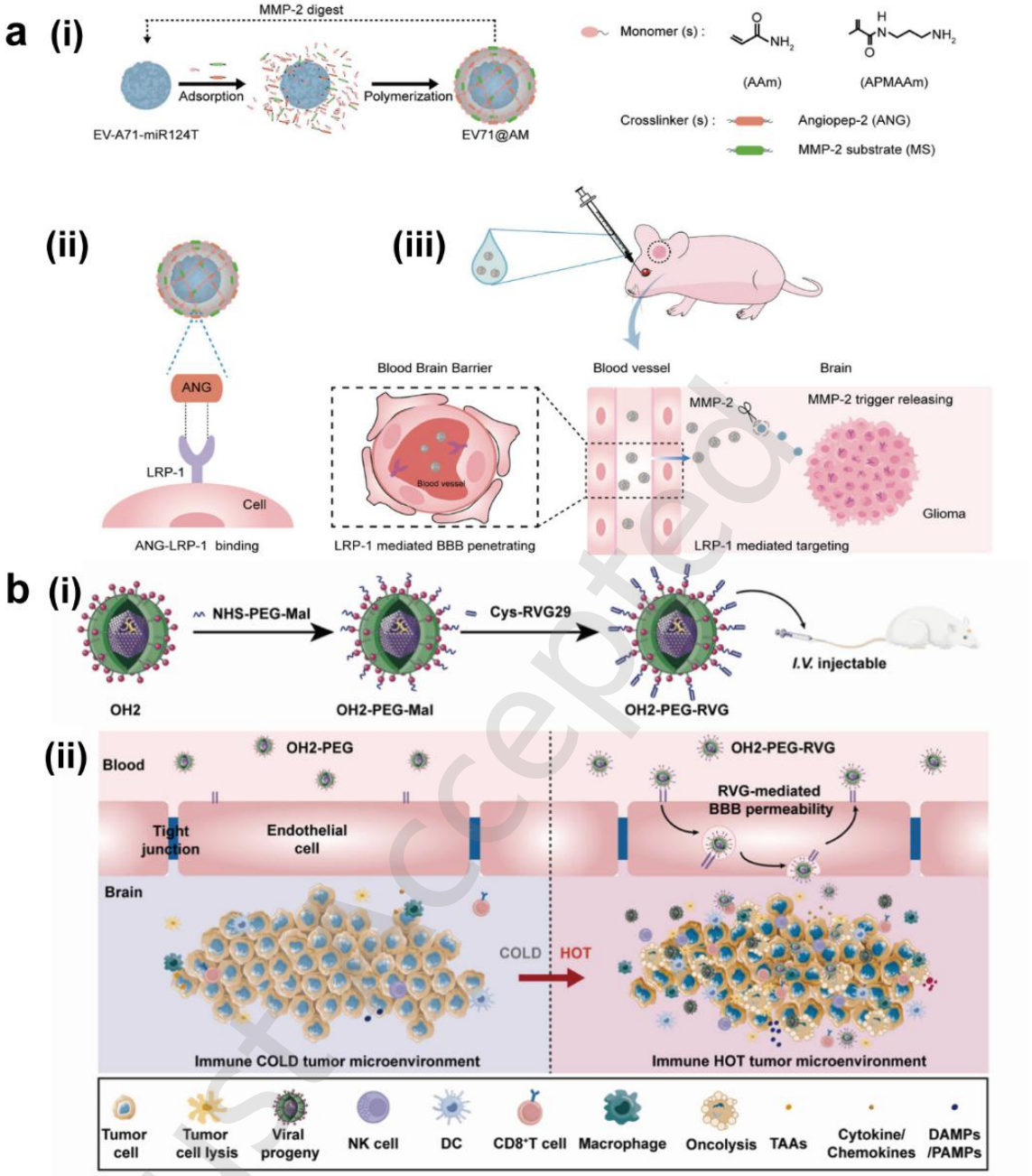


Figure 3 Active targeting strategies for OVs delivery based on nanocapsules and surface chemical modification.a.Schematic design of nanocapsules encapsulating OVs for glioma therapy.(i)A schematic illustrating the design and synthesis of EV71@AM nanocapsules.(ii)) ANG on the nanocapsules interaction with LRP-1 receptor.(iii) Receptor-mediated transcytosis across the BBB and MMP-2-triggered viral release in the tumor microenvironment.Copy right 2025 John Wiley and Sons[14].b.Generation and characterization of nanoparticles.(i)Schematic of OH2- PEG- RVG synthesis(ii)RVG29-mediated BBB penetration via nAchR binding and the antitumor mechanism.Copy right 2025 Elsevier[111].

Shin et al. utilized the serotype 5 adenovirus- based oncolytic adenovirus Delta- 24- RGD as the backbone, replaced the hypervariable regions of its hexon protein with the corresponding sequences from the rare serotype 43 through molecular cloning, and constructed the chimeric oncolytic adenovirus Delta- 24- RGD- H43m, while retaining the two key modifications, the 24- bp deletion in the E1A region and the RGD- 4C motif insertion in the fiber protein. The principal objective underlying the design of this chimeric construct was to circumvent pre- existing neutralizing antibody- mediated recognition while preserving both viral replicative capacity and oncolytic potency. Given that the hexon hypervariable loops serve as the dominant antigenic determinants of adenovirus and the primary targets of neutralizing humoral immunity, substitution of the wild- type Ad5 hexon hypervariable domains with those from Ad43, a serotype with a seroprevalence of less than 5% in the human population, resulted in a marked alteration of surface antigenic architecture. Homology modeling predicted a folded conformation distinctly different from that of wild- type Ad5, enabling effective evasion of anti- Ad5 antibody recognition and

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neutralization, while leaving viral structural integrity and infectivity intact[12].

Niu et al. employed the oncolytic adenovirus H101 as the core, utilized hydrophobic interactions between cholesterol molecules and the viral surface, and through simple co- incubation, self- assembled cholesterol onto the viral particle surface, constructing the cholesterol- modified oncolytic adenovirus OA@Cho. The fundamental design rationale underpinning this delivery platform is the use of cholesterol to govern the in vivo protein corona profile, thus conferring upon the virus both BBB- penetrating and glioma- targeting capabilities. Mechanistically, the cholesterol modification- directed active enrichment of the ApoE protein corona arises from the fact that cholesterol, as a core component of lipoproteins, can actively adsorb apolipoprotein E via hydrophobic domain interactions in the circulation. The ApoE adsorbed onto the viral surface serves as an endogenous ligand, binding to LDLR- related protein 1(LRP1) highly expressed on BBB endothelial cells and glioma cell membranes, triggering receptor- mediated transcytosis and achieving active BBB crossing and glioma- targeted accumulation, thus providing a simple and efficient surface modification strategy for systemic administration and intracranial active delivery of OVs[112]. Based on a similar principle, Ge et al. conjugated H101 with a modified β- amyloid peptide Aβ- am, which exhibits reduced neurotoxicity, via PEG coupling, constructing OA@Aβ- am nanoparticles. The Aβ- am peptide can likewise actively enrich plasma ApoE to form a protein corona in the circulation, which subsequently mediates BBB transcytosis and glioma targeting through the interaction between ApoE and LRP1. In contrast to the cholesterol- based approach, Aβ- am modification provides the viral surface with a better- defined ApoE- binding interface and superior ApoE recruitment efficiency. This platform likewise exhibited potent tumor growth inhibition and extended survival in the GL261 orthotopic glioma model. Moreover, when combined with anti- PD- 1 checkpoint blockade, it achieved further augmentation of antitumor activity. Collectively, these two investigations substantiate a versatile strategy whereby surface chemical modification is employed to modulate protein corona composition, thereby engaging the endogenous ApoE- LRP1 axis to accomplish active BBB penetration. This paradigm offers a transferable design framework for brain- targeted OVs delivery(**Fig.4**)[113].

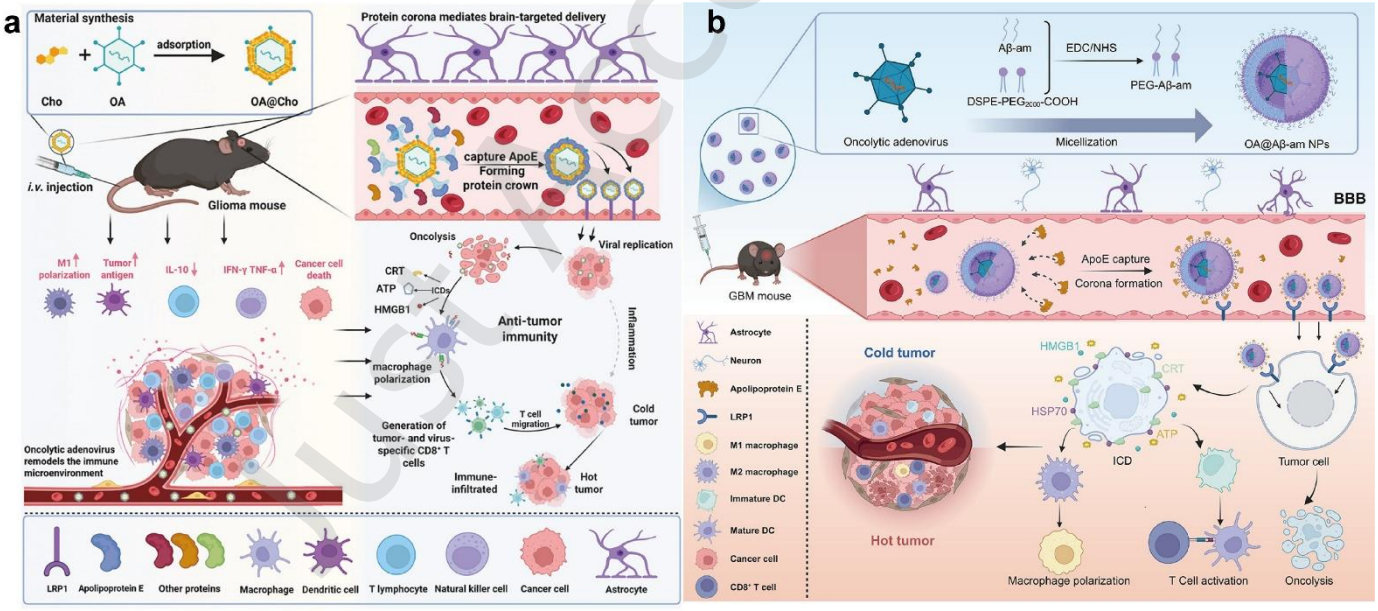


Figure 4 Oncolytic adenovirus brain delivery systems based on protein corona- mediated active targeting strategies. a.OA@Cho self-assembles from oncolytic adenovirus and cholesterol, adsorbs plasma ApoE to form a protein corona, and mediates LRP1-dependent transcytosis across the BBB for targeted glioma delivery.Copy right 2025 Elsevier[112]. b.OA@Aβ-am nanoparticles are formed by assembly with PEG-Aβ-am, enrich ApoE, and similarly achieve LRP1-mediated BBB penetration and glioma targeting.Copy right 2025 Elsevier[113].

4.2 Biomimetic Cell- Membrane Camouflage

Cell- membrane biomimetic camouflage strategies involve coating viral particles with extracted cell membranes, leveraging membrane surface proteins to achieve immune evasion and targeting functions. Tumor cell membranes exploit the principle of homologous targeting, whereby adhesion molecules and antigens on the tumor cell membrane surface can mediate specific recognition and uptake of nanoparticles by homologous tumor cells, achieving a homing effect. Meanwhile, tumor cell membranes carry abundant tumor- associated antigens, which can serve as a source of antigens to induce antitumor immune responses. Jia et al. utilized membranes from NSCs and GBM cells to separately encapsulate oncolytic

adenoviruses, constructing two biomimetic viruses designated NCM-Ad and GCM-Ad[15]. Mechanistically, the cell-membrane shell acts as a physical barrier that effectively shields the antigenic epitopes of viral capsid proteins, rendering the coated viral particles unrecognizable to anti-adenovirus neutralizing antibodies. In pre-immunized mouse models, this successfully evaded clearance by the reticuloendothelial system and significantly reduced nonspecific accumulation in the liver and spleen. NSC membranes, leveraging their naturally occurring chemokine receptors and adhesion molecules, actively traverse the BBB and home to intracranial glioma foci through receptor-ligand interactions. GBM cell membranes, in turn, harness homologous targeting mechanisms, achieving homing to cognate tumor cells through mutual recognition of membrane surface antigens and adhesion molecules. Both strategies effectively enriched viruses in brain tumor regions rather than normal brain tissue in vivo. Moreover, the membrane cloaking strategy redirected viral entry from the conventional CAR-dependent route toward mechanisms involving membrane fusion or surface-specific interactions, thereby markedly improving infection efficiency in glioma cells characterized by low CAR expression. In orthotopic glioma mouse models, intravenously administered NSC-membrane-coated viruses exhibited superior BBB penetration and tumor targeting compared with GBM-membrane-coated viruses. Furthermore, when the capsid-dual-modified oncolytic adenovirus A4/k37 was further encapsulated within NSC membranes, this biomimetic virus outperformed both naked virus and GBM-membrane-coated virus in prolonging the survival of tumor-bearing mice, with viral localization restricted to tumor tissues without invading normal brain parenchyma, thereby providing a safe and efficient biomimetic platform for intravenous administration and brain-targeted delivery of OVs(**Fig.5**). Biomimetic strategies employing different cell-membrane sources each offer distinct advantages, allowing for the selection of optimal formulations based on therapeutic objectives and tumor microenvironment characteristics, or even the construction of hybrid membranes to integrate multiple targeting functions.

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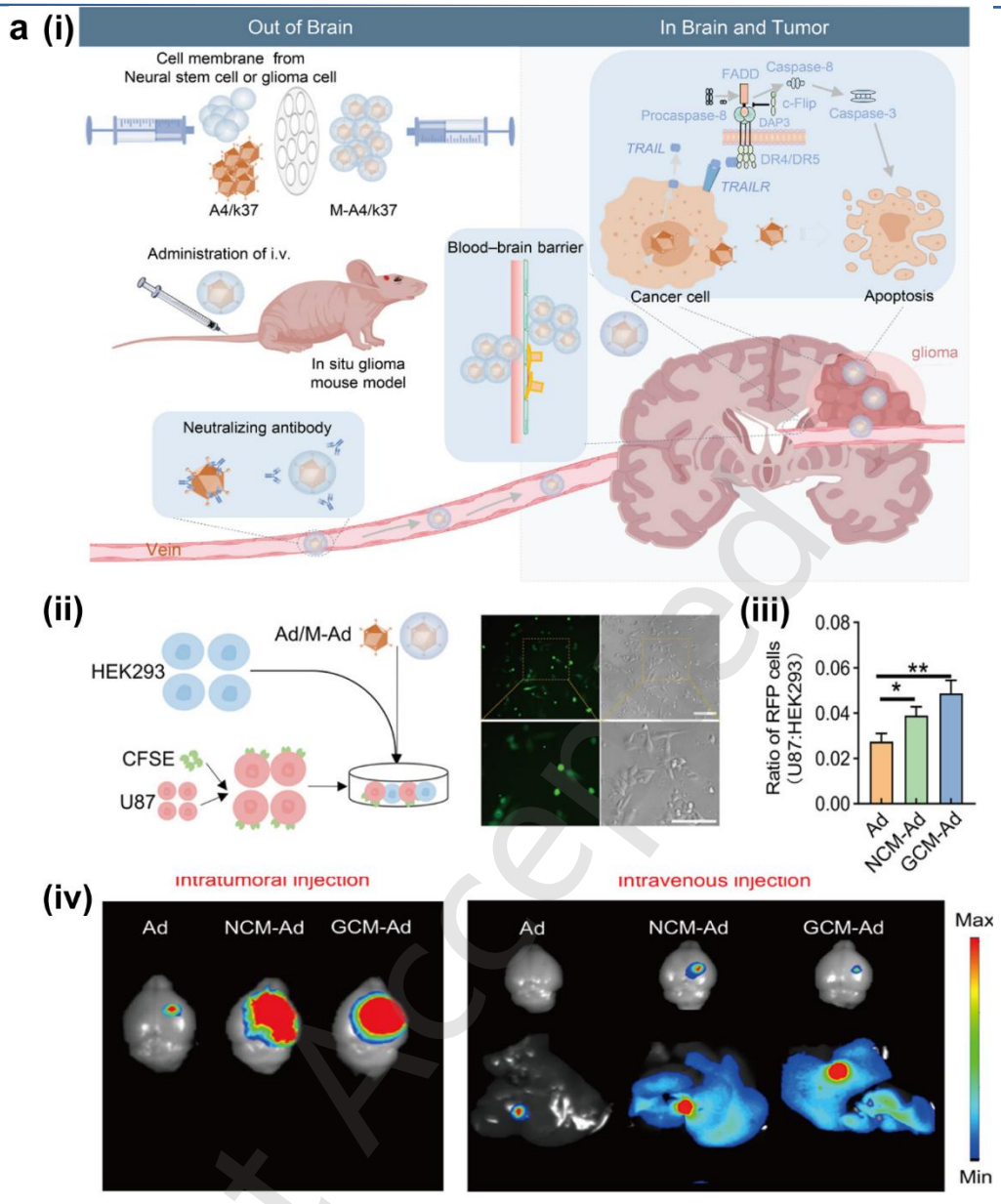


Figure 5 Construction and targeted delivery validation of cell membrane-coated oncolytic adenoviruses. a.(i) Design and Mechanism of a Cell Membrane-Coated Oncolytic Adenovirus for GBM Treatment.(ii) Co-culture of U87 and HEK293 cells for targeted infectivity analysis.(iii) Ratio of RFP positive cells (U87:HEK293) 24h post-Ad or -M-Ad infection measured by flow cytometry.(iv)Virus-mediated RFP expression in brain tissue 48h after intratumoral and intravenous injection in orthotopic U87 tumor-bearing mice.Copy right 2023 American Chemical Society[15].

4.3 Cell- Based Carrier Delivery Strategies

In contrast to non- living biomimetic membrane cloaking approaches, living cell- based delivery platforms employ intact, actively migrating cells as natural vehicles to guide viral transport. Ahmed et al. utilized the oncolytic adenovirus CRAd- S- pk7 as the core, leveraged the intrinsic tumor- tropic migratory capacity of NSCs to serve as living carriers for virus loading, and achieved targeted delivery and intratumoral dissemination of the virus in orthotopic glioma mouse models. The design of this delivery platform was predicated upon the observation that NSCs are permissive to CRAd- S- pk7 infection while displaying enhanced viability relative to glioma cells, thereby affording the NSCs adequate time to home to neoplastic lesions prior to viral- mediated cytolysis.Mechanistically, NSCs express chemokine receptors including CXCR4, c- Met, and VEGFR2 on their surface, enabling them to respond to chemokine gradients secreted by gliomas and directionally migrate to tumor foci. Upon loading with CRAd- S- pk7, NSCs exhibit upregulated CXCR4 and VEGFR2 expression, conferring markedly augmented migratory activity relative to their uninfected counterparts. After reaching the tumor site, NSCs loaded with viruses continuously produce and release progeny virions within the tumor, with the NSC- loaded group maintaining higher viral titers and longer persistence compared with the naked virus group. NSCs in the brain parenchyma are capable of attenuating virus- elicited host inflammatory responses, a phenomenon potentially mediated by NSC- derived

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upregulation of the anti- inflammatory cytokine IL- 10 following infection. IL- 10, in turn, curtails IL- 6- driven astrocyte proliferation, thus alleviating viral- induced neuroinflammation and tissue injury.The NSC- CRAd- S- pk7 cohort exhibited a markedly extended median survival relative to the naked virus group, with a subset of animals surviving in excess of 100 days. In pre- immunized murine models, the NSC- loaded group retained its therapeutic superiority, validating that the living cell carrier effectively protected the virus from host humoral immune neutralization[114].

Chao and colleagues employed human induced pluripotent stem cell (iPSC)- derived neural progenitor cells (NPCs) as delivery vehicles for the chimeric poxvirus CF17, and successfully accomplished targeted viral transport and tumor growth inhibition in orthotopic murine models established from patient- derived GSCs(**Fig.6a**). The construction of this carrier system was based on the susceptibility of human NPCs to CF17 infection. At an appropriate multiplicity of infection (MOI), NPCs can effectively carry the virus while maintaining sufficient viability, providing the prerequisite for NPC migration toward tumors and subsequent viral release. Human NPCs are endowed with intrinsic glioma- tropic migratory properties, allowing them to home to neoplastic lesions upon intracranial or intraventricular administration.Following CF17 loading, the virus itself does not impair the migratory capacity of the carrier NPCs. Virus- loaded NPCs remain capable of effectively reaching tumor regions and releasing the virus.CF17 remains refractory to the intrinsic resistance pathways operative in GSCs. It potently eliminates patient- derived GSCs in vitro, demonstrating robust cytolytic activity regardless of whether it is administered directly or shuttled through NPC carriers[115].

Similarly, Chai et al. utilized the oncolytic adenovirus YSCH- 01, which carries the L- IFN gene, as the core therapeutic payload, employed human NSCs as living cell carriers, and delivered the virus to intracranial glioma lesions via the intracerebroventricular (ICV) injection route(**Fig.6b**). ICV injection enables the virus to be broadly distributed throughout the CNS via cerebrospinal fluid (CSF) circulation, overcoming the limitation of intratumoral injection in covering the invasive tumor front. NSCs, serving as cellular carriers, can actively transport the virus to tumor sites through their tumor- tropic migratory capacity.Following successful loading of YSCH- 01, NSCs retained their stemness marker expression and chemokine receptor levels, laying the foundation for targeted migration and viral release in vivo. ICV injection allows NSC- OV to reach various regions of the ventricular system via CSF circulation. Subsequently, through chemokine receptors such as CXCR4 and CCR2 on the NSC surface that respond to tumor- secreted chemokines, the NSCs directionally migrate to glioma lesions within the brain parenchyma, while simultaneously serving as a physical barrier that protects the virus from clearance by pre- existing neutralizing antibodies in the bloodstream and CSF[116].

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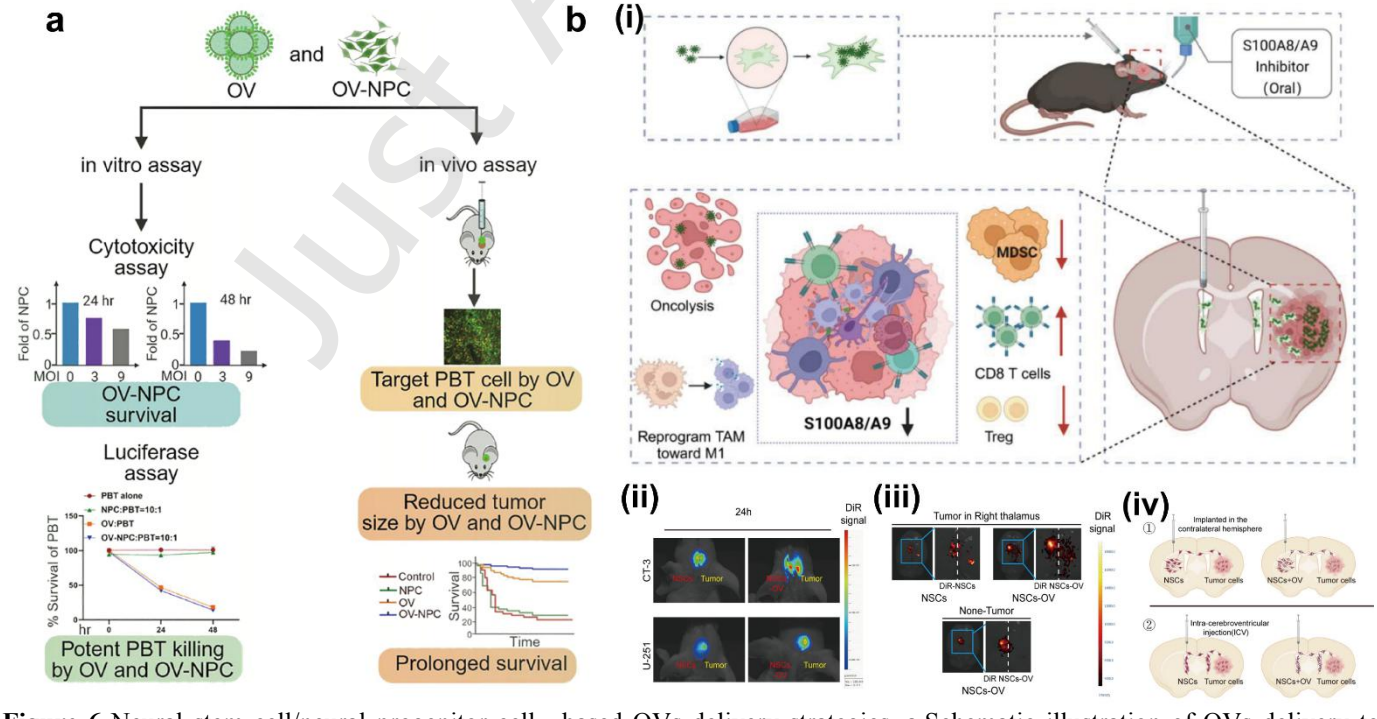


Figure 6 Neural stem cell/neural progenitor cell- based OVs delivery strategies. a.Schematic illustration of OVs delivery to glioma using human pluripotent stem cell- derived neural progenitor cells.Adapted from ref.[115] under the terms of the Creative Commons Attribution (CC BY 4.0) License.b.Neural stem cell-mediated OVs delivery via intracerebroventricular (ICV) administration for glioma targeting and validation.(i) NSCs-OV therapy enhances tumor targeting and viral persistence

via ICV injection, reducing immune clearance and OV-induced toxicity. (ii) In vivo tracking of DiR-labeled NSCs and NSCs-OV in CT-3 and U-251 glioma-bearing mice. (iii) Fluorescence imaging of DiR-labeled NSCs and NSCs-OV in a tumor localized to the right thalamus. (iv) Experimental models for evaluating NSCs-OV targeting and efficacy. Copy right 2025 BMJ Publishing Group Ltd[116].

Jazowiecka‐Rakus et al. utilized the pro‐apoptotic oncolytic myxoma virus vMyx‐M011L‐KO/EGFP, in which the M011L gene has been knocked out, as the core therapeutic agent, and employed adipose‐derived stem cells (ADSCs) as living cell carriers for virus loading. ADSCs can be efficiently infected by the virus in vitro and support viral replication. ADSCs possess an inherent tumor‐homing capacity, enabling them to directionally migrate to intracranial glioma lesions following intravenous administration, while simultaneously serving as a physical barrier that protects the virus from host immune system clearance during migration. In vitro BBB transwell assays revealed that ADSCs laden with virus could negotiate the endothelial monolayer established by bEnd.3 cells and subsequently infect glioma cells residing below the barrier, thus substantiating the potential of ADSCs to ferry OVs across the BBB(**Fig.7a**). Following their arrival at neoplastic lesions, ADSCs sustain the ongoing release of progeny virions, thereby achieving local viral amplification and dissemination within the tumor microenvironment. Moreover, as a consequence of M011L gene ablation, vMyx‐M011L‐KO/EGFP lacks the capacity to suppress host cell apoptotic signaling. Following infection, it engages the mitochondrial death pathway to trigger the caspase cascade, thereby selectively inducing apoptotic cell death in glioma cells. Adipose‐derived stem cells display a measure of resilience against this pro‐apoptotic stimulus, enabling them to undergo viral‐mediated lysis only after the completion of their delivery function.Adipose‐derived stem cells can be conveniently harvested from autologous adipose tissue and possess low immunogenicity. These attributes endow this platform with considerable promise for translation to systemic administration paradigms[117].

T cells‐based delivery platforms have garnered substantial attention in recent years. Qiao et al. loaded oncolytic VSV onto the surface of antigen‐specific $CD8^{+}$T lymphocytes, exploiting the inherent tumor‐tropic homing ability of T cells to direct viral transport to neoplastic lesions. Notably, virus loading further potentiated T cells activation and augmented their effector functionality[108]. On this basis, Chen and colleagues utilized an oncolytic adenovirus encoding CRISPR‐Cas9 directed against the PDL1 gene (eOA) as the core therapeutic component. The virus was encapsulated within tumor cell membrane vesicles that displayed T cell‐specific antigens, and the resulting membrane‐cloaked virus was then physically anchored onto T cell surfaces via antigen‐receptor engagement, yielding an OVs‐T cell chimera designated ONCOTECH (T‐M@eOA). This platform was conceived to accomplish targeted OVs delivery upon intravenous administration by capitalizing on T cell tumor‐homing properties, while concurrently counteracting microenvironmental immunosuppression through Cas9‐driven PDL1 gene disruption. The membrane‐encapsulated virus is tethered to the T cell surface via cognate recognition between the T cell receptor and the surface‐presented antigens. Importantly, this anchoring approach leaves T cell proliferation, activation, and migration unimpaired. Once T cells reach neoplastic lesions and engage tumor antigens, the membrane‐cloaked virus detaches from the T cell surface via competitive displacement and membrane fusion processes, and is then taken up by tumor cells. After internalization into neoplastic cells, eOA drives the expression of Cas9 and PDL1‐targeting sgRNA, effecting PDL1 gene ablation in both tumor cells and tumor‐infiltrating immune populations, with consequent downregulation of PD‐L1 surface expression on tumor cells(**Fig.7b**). Treatment with T‐M@eOA triggers ICD of tumor cells, fosters dendritic cell maturation and cross‐presentation, mobilizes systemic antitumor immunity, and achieves potent tumor growth inhibition along with extended survival in tumor‐bearing murine models[118].

Tang et al. constructed the chimeric adenovirus OAd5/F35 [E2F1], which ingeniously exploits the fact that conventional Ad5 infects cells via the CXADR receptor, whereas macrophages lack CXADR expression and are naturally resistant to Ad5. Accordingly, the researchers replaced the Ad5 fiber protein with that of Ad35, generating the Ad5/F35 chimeric virus, which enables efficient infection of both macrophages and glioma cells through the CD46 receptor, highly expressed on both cell types. Concurrently, the virus carries the E1A gene driven by the E2F1 promoter, exploiting the RB pathway inactivation characteristic of glioma cells to achieve tumor‐selective replication, while in macrophages the virus infects but does not replicate, ensuring the safety of the carrier cells. Macrophages, as the most abundant immune cell population in the glioma microenvironment, possess inherent tumor‐tropism and can actively infiltrate glioma tissues and penetrate tumor spheroids. Upon arrival at the tumor site, the virus exploits the E2F1 promoter to initiate E1A transcription in RB‐mutated glioma cells, achieving tumor‐specific replication and lysis of tumor cells, while replication is restricted in normal cells and macrophages, ensuring therapeutic safety.Virus‐infected macrophages polarize toward an M1‐like phenotype within the microenvironment, and this microenvironmental remodeling further potentiates

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antitumor immune responses(**Fig.7c**)[119].

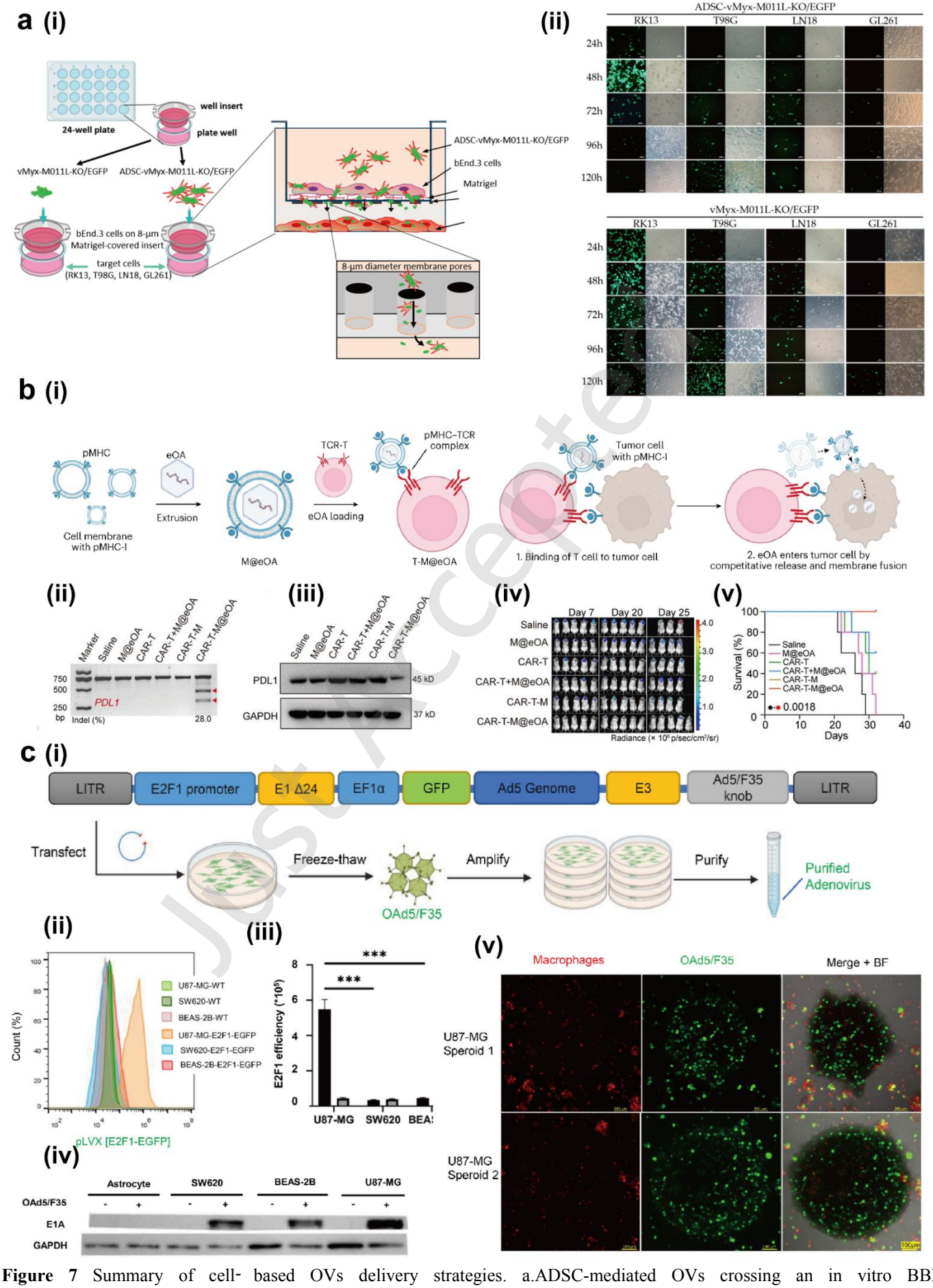


Figure 7 Summary of cell- based OVs delivery strategies. a.ADSC-mediated OVs crossing an in vitro BBB

model.(i)Schematic of the in vitro BBB model.(ii)ADSCs carrying OVs achieve comparable target cell infection to free virus after multiple administrations, demonstrating effective BBB crossing and viral delivery. Adapted from Ref. [117] under the terms of the Creative Commons Attribution (CC BY 4.0) License. b. Construction of the OVs-T cell chimera and its antitumor efficacy in glioblastoma.(i) T cell-anchored virus delivery with Cas9-mediated PDL1 editing. (ii) T7E1 cleavage assay verifying PDL1 editing in tumor tissues. (iii) Western blot showing downregulated PD-L1.(iv) Bioluminescence imaging showing glioma growth inhibition. (v) Survival curves showing prolonged median survival.Copy right 2024 Springer Nature[118]. c.Macrophage-mediated delivery. (i) OAd5/F35[E2F1] with tumor-selective replication. (ii) Confocal microscopy showing viral delivery to tumor cells. (iii) Flow cytometry showing enhanced tumor cell infection. (iv) 3D tumor spheroid model showing virus-loaded macrophages penetrate spheroids and deliver virus to intratumoral cells.Copy right 2025 Elsevier[119].

4.4 Physical Targeting Strategies

Physical targeting modalities bypass the need for biological recognition, instead employing external physical forces or locoregional delivery techniques to directly transport viral therapeutics to intracranial neoplastic lesions. Convection-enhanced delivery (CED) utilizes sustained positive-pressure perfusion to circumvent the BBB, enabling direct viral administration into both the tumor core and its adjacent infiltrative margins. Souza and colleagues employed CED for intratumoral administration of the oncolytic HSV-1 G207 in pediatric patients afflicted with diffuse intrinsic pontine glioma (DIPG), with the Phase I trial reporting a reassuring safety profile[120, 121]. MVR-C5252, an oncolytic HSV-1 engineered to express PD-1 antibody and IL-12 developed by ImmVira, was administered via CED for the treatment of recurrent high-grade glioma. Among 5 patients, no serious adverse events (AEs) or dose-limiting toxicities (DLTs) were observed, with only grade 1-2 fatigue, flu-like symptoms, and cognitive impairment reported[122]. The strategy of CED combined with craniotomy has also been applied in retroviral gene therapy for multifocal recurrent GBM [123].

Focused ultrasound combined with microbubble technology can transiently and reversibly open the BBB, providing a temporary passage for intravenously administered viruses. Bhoopathi et al. devised a two-step delivery platform leveraging focused ultrasound in tandem with dual microbubbles (FUS-DMB). In the first step, empty microbubbles were administered intravenously, followed by application of focused ultrasound to the cerebral target zone. The microbubbles oscillated or collapsed violently within the acoustic field, generating mechanical forces that temporarily loosened the tight junctions of brain microvascular endothelial cells, thus achieving reversible BBB opening without concomitant parenchymal injury. Within the temporal window of BBB permeability, a second intravenous infusion of oncolytic adenovirus-loaded microbubbles was performed. These microbubbles had been surface-opsonized with complement to mask viral epitopes, thereby minimizing immune recognition and subsequent elimination. Following arrival at the cerebral target, a second focused ultrasound pulse triggered the cavitational disruption of the payload-bearing microbubbles, resulting in focal viral release within the tumor microenvironment. The liberated Ad.5/3-CTV engages its E1A genetic program to achieve tumor-restricted replication, while concurrently expressing and secreting the MDA-7/IL-24 therapeutic cytokine, which exerts dual antitumor activity, directly triggering apoptotic cell death in infected neoplastic cells and mediating bystander killing of uninfected neighboring tumor cells. This two-step paradigm synergistically combines physical BBB disruption with viral immune shielding and spatiotemporally controlled release, thereby successfully accomplishing systemic administration and intracranial tumor-selective delivery of the oncolytic adenoviral payload(**Fig.8a**)[124].

The nasal-to-brain delivery route represents another physical strategy to bypass the BBB, leveraging the unique anatomical connections between the nasal cavity and the brain to directly deliver therapeutic agents to the CNS via the olfactory nerve pathway. This study constructed a nanovaccine (EphA2 HBc@CaP NVs) based on hepatitis B core antigen virus-like particles (HBc VLPs) surface-displaying the glioma-associated antigen EphA2, and further coated the particle surface with a calcium phosphate (CaP) shell through biomineralization. This CaP shell not only significantly enhanced the adhesion of the vaccine to the nasal mucosal surface, but also prolonged its retention time within the nasal cavity, delaying mucociliary clearance. The CaP shell facilitated the uptake of the nanovaccine by nasal mucosal epithelial cells and assisted in its traversal of the epithelial barrier, after which it traveled along the olfactory nerve to the olfactory bulb (OB), and then progressively infiltrated deeper brain regions and glioma tissues(**Fig.8b**). This strategy effectively circumvents systemic exposure and hepatic first-pass metabolism associated with drug delivery via the bloodstream, providing a highly efficient and low-toxicity physical delivery paradigm for glioma immunotherapy[125].

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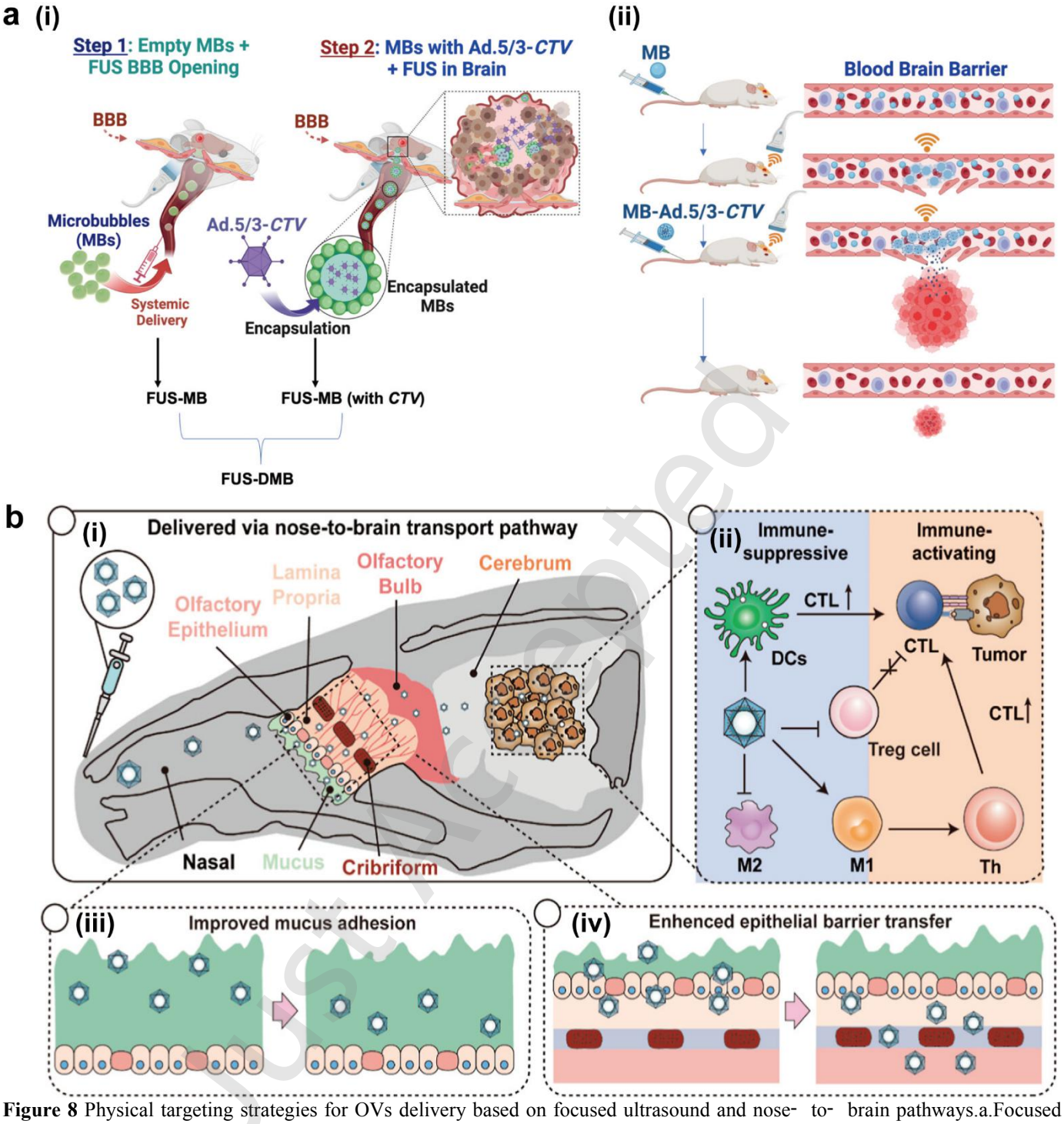


Figure 8 Physical targeting strategies for OVs delivery based on focused ultrasound and nose- to- brain pathways.a.Focused ultrasound combined with double microbubble strategy.(i)Schematic of the two-step focused ultrasound-double microbubble delivery strategy.(ii)Released virus replicates in tumor cells and secretes MDA-7/IL-24, eliminating tumors via direct killing and bystander effects. Copy right 2024 John Wiley and Sons[16].b.Biomimetic virus-like nanoparticles for glioma immunotherapy via the nose-to-brain pathway.(i) Intranasal EphA2HBc@CaP nanovaccines achieve mucosal absorption, epithelial crossing, and olfactory nerve-mediated brain delivery with glioma infiltration. (ii) The CaP shell enhances mucosal adhesion and prolongs nasal retention.(iii) The CaP shell promotes epithelial cell uptake and improves epithelial barrier crossing efficiency.(iv) The nanovaccine remodels the immunosuppressive TME by increasing M1-like TAMs and reducing Tregs, eliciting robust anti-glioma immunity.Copy right 2025 American Chemical Society[125].

4.5 Exosome- Like Particle Delivery

Exosomes and exosome- like vesicles have emerged as promising alternative delivery vehicles for OVs, endowed with low immunogenicity, intrinsic tropism, and the capacity to encapsulate a broad spectrum of bioactive payloads. Harat et al. utilized oncolytic HSV- 1 to infect GSCs, inducing the secretion of exosome- like particles (designated EVOVs) that function as replication- incompetent vaccine- like immune activators. Although these particles lack replicative virus, they are enriched with virus- derived pathogen- associated molecular patterns (PAMPs) that effectively activate both innate and adaptive antitumor

immunity. This design circumvents the safety risks associated with live viruses while preserving the ability to deliver viral immunostimulatory signals. As cell-derived vesicles, EVOVs inherently possess the capacity to target GSCs and immune cells, enabling efficient uptake by target cells and delivery of virus-derived immunostimulatory signals. Compared with direct injection of free viruses, EVOVs enable intracellular delivery of immune-activating signals without relying on the expression of viral receptors[126]. This study represents a novel strategy in which replication-incompetent virus-like particles remodel the glioma immune microenvironment through targeted delivery of viral immune signals, offering an important direction for safety improvement in oncolytic virotherapy.

Each of the delivery strategies described above has its own characteristics, and they differ significantly in terms of targeting mechanisms, vector materials, routes of administration, and clinical translatability. For the convenience of readers in systematically comparing the underlying principles, strengths, and shortcomings of these strategies, the essential characteristics of the aforementioned delivery platforms are consolidated in **Table 1.**

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Table 1 Comparison of Combination Delivery Technologies for OVs

Strategy Category	Representative Approach	Core Principle	Advantages	Limitations	Main References
Active Targeting	ANG peptide modification	Binds to LRP-1 receptor to mediate transcytosis	High specificity, intravenous administration feasible; nanocapsules protect virus	Receptor expression heterogeneity; particle size optimization required	[14]
	RVG29 peptide modification	Binds to nicotinic acetylcholine receptor to cross BBB	Efficient BBB penetration	May induce immunogenicity; PEGylation required for prolonged half-life	[111]
	RGD-4C peptide modification	Targets integrin αvβ3/αvβ5 to enhance glioma cell infection	Evades pre-existing neutralizing antibodies; enhances viral replication and distribution	Variable integrin expression levels; complex chimeric virus engineering	[12]
	Cholesterol modification (ApoE enrichment)	Surface cholesterol actively adsorbs ApoE, crosses BBB via LDLR	Utilizes endogenous protein corona for precise brain targeting	Protein corona composition affected by individual variability	[112]
	Aβ-m peptide modification (ApoE enrichment)	Modified β-amyloid peptide actively enriches ApoE, binds to LRP1	Dual targeting of BBB and glioma	Stability of ApoE corona formation requires optimization	[113]
Biomimetic Cell-Membrane Camouflage	Tumor cell membrane	Homologous targeting; immune evasion; carries tumor-associated antigens	Enhanced homing; induces antitumor immunity	Limited membrane sources; low encapsulation efficiency	[15, 127, 128]

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	NSC membrane	Utilizes homing-related receptors for targeting and BBB penetration	NSC membrane can actively cross BBB	Immunogenicity risk from allogeneic cell membranes	[15]
	NSCs	Tumor-tropic migration	Active BBB crossing; uniform distribution in infiltrative regions	Allogeneic immune rejection risk; scalable production challenges	[114-116]
	ADSCs	Tumor-tropic migration	Can cross BBB; delivers high viral copy numbers	Mechanism not fully elucidated	[117]
Cell-Based Carriers	T cells	Natural tumor homing of cells	Synergistically enhances T cell function; can knock out immunosuppressive genes	Virus may damage T cells	[108, 118]
	Macrophages	Natural tumor tropism; active infiltration into glioma	Natural tumor tropism; tumor-selective viral replication in glioma cells	Complex chimeric virus engineering	[119]
	Convection-enhanced delivery	Sustained positive-pressure perfusion to bypass BBB	Large distribution volume; direct delivery to brain parenchyma and infiltrative margins	Invasive; requires stereotactic catheter placement; may induce inflammation	[120, 122, 123]
Physical Targeting	Focused ultrasound + microbubbles	Transient, reversible BBB opening	Non-invasive; repeatable; applicable to multiple drug types	Equipment-dependent; may induce neuroinflammation	[16, 124]

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	Nose-to-brain pathway	Bypasses BBB via olfactory nerve through nasal cavity	Non-invasive; avoids systemic exposure and hepatic first-pass metabolism	Small administration volume; requires particle size and mucosal adhesion optimization	[125]
Exosome-like particles	Exosome-like particles	Virus infects cells, which release exosome-like particles containing virus-derived immune activation signals	High safety; activates innate and adaptive immunity	No viral replication, may require multiple administrations; low yield	[129]

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In summary, the convergence of innovative delivery strategies, including active targeting, biomimetic cell- membrane camouflage, cell- based carriers, physical targeting, and exosome- like particles, has provided diversified solutions for overcoming the BBB and addressing insufficient tumor targeting. Each strategy possesses its own advantages and limitations. Active targeting can achieve receptor- mediated transcytosis with high specificity, but is constrained by the heterogeneous expression of ligands and their cognate receptors. Cell- membrane camouflage and cell- based carrier strategies endow viruses with immune evasion and tumor- homing capabilities. However, the scalable manufacturing and quality control of living cell carriers remain challenging. Physical targeting strategies can transiently open the BBB and are applicable to a broad range of therapeutic agents, but they are highly equipment- dependent and may elicit neuroinflammatory responses. Exosome- like particles combine low immunogenicity with inherent targeting capacity, yet their low production yield and suboptimal loading efficiency require further improvement.

Future research should focus on the synergistic integration of multiple strategies. For example, modifying active targeting ligands onto the surface of cell- membrane biomimetic cloaking particles, or utilizing focused ultrasound to assist cell- based carriers in crossing the BBB, thereby achieving additive or synergistic effects in delivery efficiency. Concurrently, the personalized selection of optimal delivery regimens based on the patient's tumor molecular subtype, BBB integrity, and immune microenvironment characteristics will be a critical direction for achieving precision OVs therapy. Furthermore, the establishment of standardized manufacturing protocols, systematic assessment of long- term safety, and optimization of clinical translation pathways remain core tasks in advancing the field from the laboratory to clinical application. With the interdisciplinary convergence of materials science, viral engineering, and precision medicine, OVs delivery technologies hold promise for delivering meaningful survival benefits to patients with glioma.

5 OVs Combination Therapy

Despite successful delivery of OVs to intracranial tumors via the aforementioned strategies, OVs therapy still faces three core obstacles:(i) while activating antitumor immunity, the host adaptive immune response also generates antiviral neutralizing antibodies and virus- specific T cells that rapidly clear the virus, making it difficult to sustain durable immune activation[110, 130]; (ii) the glioma microenvironment harbors complex feedback inhibitory mechanisms, such as the upregulation of immune checkpoint molecules including PD- L1 on tumor cells in response to inflammatory stimuli, and the persistence of suppressive cell populations including TAMs and MDSCs, that rapidly drive newly recruited T cells into an exhausted state[97, 131]; and (iii) the high heterogeneity of gliomas, particularly the non- permissiveness of GSCs to OVs, allows a subset of tumor cells to escape viral attack, serving as a source of subsequent recurrence[18, 115]. Therefore, monotherapy with OVs alone is insufficient to achieve durable tumor control. Combining OVs with other therapeutic modalities to synergistically enhance immune responses, reverse immunosuppression, and overcome delivery barriers has become an inevitable direction for breaking through the current efficacy bottleneck[18, 132]. This review summarizes the core features and comparative aspects of OVs combination strategies derived from diverse research systems, and further categorizes their clinical translation stages, providing important reference value for optimizing combination regimen design and facilitating subsequent clinical translation(**Table 2**).

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Table 2 Major Combination Therapeutic Strategies for OVs

Combination Strategy		Core Mechanism	Advantages	Limitations	Clinical Translation Stage	Main References
Immune Checkpoint Blockade	G47Δ-mIL12 + anti-PD-1 + anti-CTLA-4	IL-12 activates immunity + PD-1/CTLA-4 dual blockade reverses T cell exhaustion	Cures majority of mice; induces long-term immunological memory	Triple combination toxicity unknown; requires immunocompetent model validation	Preclinical	[133]
TAMs Modulation	ARF1 knockdown	ARF1 knockdown amplifies cGAS/STING activation, promotes TAM M1 polarization, enhances $CD8^+$ T responses	Precisely targets TAM intracellular signaling	Complex delivery; not yet clinical	Preclinical only	[100]
Metabolic Modulation	Cholesterol efflux (ApoA1)	ApoA1 promotes cholesterol efflux, reduces 7-ketocholesterol, restores TAM phagocytosis and antigen presentation	Targets unique metabolic axis; combinable with statins	Durability of metabolic remodeling unknown	Preclinical only	[78]

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Combination Strategy		Core Mechanism	Advantages	Limitations	Clinical Translation Stage	Main References
Conventional Therapy	IDO/kynurenine pathway inhibition	Inhibits IDO to relieve tryptophan depletion and kynurenine-mediated immunosuppression	Relieves feedback immunosuppression induced by OVs	Limited efficacy as monotherapy; requires ICI combination	Preclinical only	[134]
	Chemotherapy	Induces ICD; reduces Tregs/MDSCs	Compatible with standard of care; overcomes TMZ resistance	TMZ may antagonize certain OV immune effects	Phase I trial reported	[130, 135-137]
	Radiotherapy	Induces ICD; increases vascular permeability to promote viral spread	Clinically mature; enhances viral distribution	High doses may damage vasculature	Phase I trial reported	[121]
	Photodynamic therapy	Generates ROS to directly kill tumor cells and release DAMPs	Reduces OVs dose requirement	Limited light penetration depth	Preclinical only	[138, 139]
Adoptive Cell Therapy	CAR-T/TCR-T/NK + OVs	CAR-T/TCR-T/NK serve as viral carriers for targeted delivery; OV	Addresses CAR-T infiltration deficiency and	Low infiltration efficiency in solid tumors; antigen loss	Preclinical/early stage	[140-146]

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Combination Strategy	Core Mechanism	Advantages	Limitations	Clinical Translation Stage	Main References
	armed with IL-15/IL-21 or antigens supports immune cell persistence and function, overcoming antigen heterogeneity	antigen heterogeneity; flexible combination of carriers and targets	leading to resistance		

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5.1 Combination with Immune Checkpoint Inhibitors

Although OV infection can induce T cell infiltration and activation, the feedback-induced upregulation of immune checkpoint molecules such as PD-L1 in the glioma microenvironment rapidly drives infiltrating T cells into an exhausted state, characterized by reduced effector molecule secretion and diminished proliferative capacity, thereby severely compromising the therapeutic efficacy of OVs. Therefore, combining OVs with immune checkpoint inhibitors (ICIs) to block the PD-1/PD-L1 or CTLA-4 signaling pathways can restore T cell function and thereby enhance antitumor immune responses. However, in the context of gliomas with profound immunosuppression, single-agent ICI therapy often yields limited efficacy. Saha et al. systematically evaluated the combination strategy of the oncolytic herpes simplex virus G47Δ-mIL12 with immune checkpoint inhibitors in immunocompetent mouse models derived from GSCs. G47Δ-mIL12 alone or single-agent ICI only modestly extended survival. The dual combination of G47Δ-mIL12 with anti-CTLA-4 showed some efficacy, but yielded only a few long-term survivors. However, the triple combination of G47Δ-mIL12 with anti-PD-1 and anti-CTLA-4 antibodies cured the majority of tumor-bearing mice in both glioma models, and the cured mice developed long-term immunological memory capable of resisting tumor rechallenge(**Fig.9**)[133].

In addition to systemic administration, direct loading of ICIs onto OVs vectors enables local intratumoral delivery. Wang et al. constructed the armed vaccinia virus VV-iPDL1/GM, which expresses the PD-L1 inhibitor (iPDL1) and GM-CSF. This virus can systematically engage and inhibit PD-L1 on both tumor cells and immune cells, inducing robust neoantigen-specific T cell responses and effectively suppressing the growth of distant, non-injected tumors in melanoma and colon cancer models[20]. This strategy delivers ICIs locally to the tumor microenvironment, with the potential to reduce systemic immune-related adverse events. Given that gliomas also exhibit features such as PD-L1 overexpression and an immunosuppressive microenvironment, this paradigm, in which viral vectors are used to locally produce immunomodulatory factors, offers a transferable conceptual framework for addressing the immunotherapeutic challenges of glioma.

Beyond PD-1/PD-L1 and CTLA-4, other immune checkpoint receptors, including LAG-3, TIM-3, TIGIT, and VISTA, are also overexpressed in glioma and contribute to T cell exhaustion[69-72]. LAG-3 is co-expressed with PD-1 on exhausted T cells and Tregs, and its co-blockade can synergize with OV-induced immune activation. TIM-3 is upregulated on glioma-infiltrating T cells; upon binding to Galectin-9, it triggers T cell apoptosis and fosters Treg proliferation. TIGIT, expressed on both T and NK cells, inhibits effector functions upon engagement with CD155. VISTA is highly expressed on glioma cells, TAMs, and MDSCs, and its expression is associated with unfavorable clinical outcomes. OVs can upregulate these checkpoints as an adaptive resistance mechanism, thereby limiting the durability of OV-induced immunity. Therefore, combining OVs with blockade of LAG-3, TIM-3, TIGIT, or VISTA may further reverse immunosuppression and enhance antitumor efficacy, representing a promising direction for future combination strategies.

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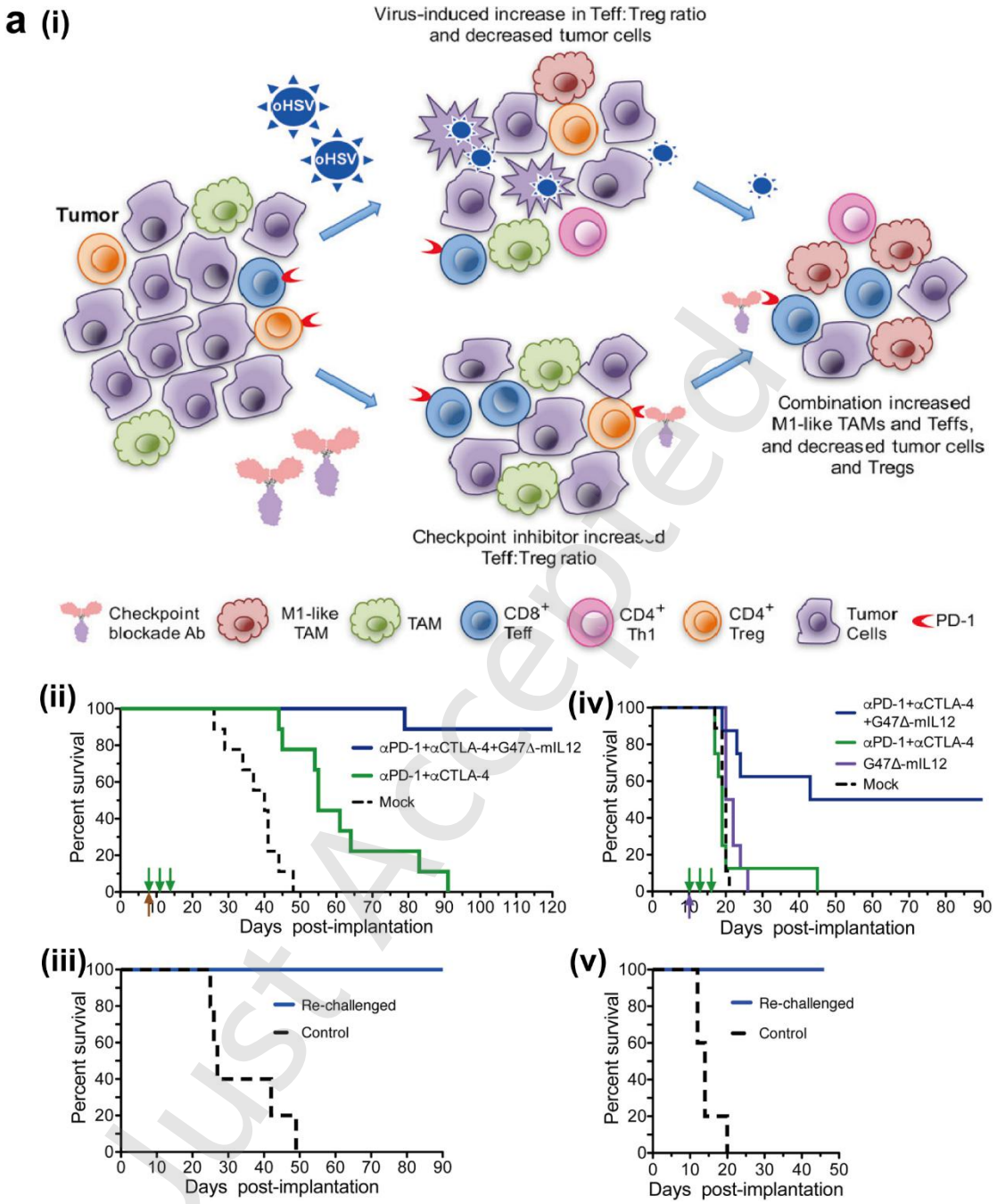


Figure 9 Synergistic antitumor efficacy of triple combination therapy with OVs and dual immune checkpoint inhibitors in glioma.(i)Schematic of triple combination therapy.(ii) Survival curves in the 005 glioma model.(iii) Re-challenge of cured mice in the 005 model.(iv) Survival curves in the CT-2A glioma model.(v)Re-challenge of cured mice in the CT-2A model.Copy right 2017 Elsevier[133].

5.2 Combination with TAMs Modulation

ADP- ribosylation factor 1 (ARF1) is a key negative regulator of the cGAS/STING pathway. ARF1 suppresses STING signaling by maintaining mitochondrial homeostasis to reduce mitochondrial DNA release and by mediating retrograde transport of activated STING from the Golgi apparatus. Cao et al.

combined intracellular signal reprogramming of TAMs with oncolytic virotherapy by incorporating ARF1 knockdown elements into an oncolytic adenoviral vector under the control of the macrophage-specific CD68 promoter to achieve TAM-targeted knockdown. Following this approach, virus infection-induced cGAS/STING pathway activation was markedly amplified, TAMs transitioned to a pro-inflammatory state, intratumoral $CD8^+$ T cell infiltration increased, and tumor-antigen-specific immune responses were significantly enhanced.In GL261 and CT2A glioma models, TAM-specific ARF1 knockdown combined with OVs significantly suppressed tumor growth and prolonged the survival of tumor-bearing mice. When ARF1 knockdown was simultaneously applied to both TAMs and tumor cells, the antitumor efficacy was further augmented (**Fig.10**). Cured mice in rechallenge experiments completely resisted subsequent challenge with the same tumor, indicating that this strategy successfully induced long-term immunological memory[100]. This study achieved synergistic enhancement between TAM intracellular signal reprogramming and oncolytic virotherapy, providing a precision strategy for TAM modulation at the genetic editing level.

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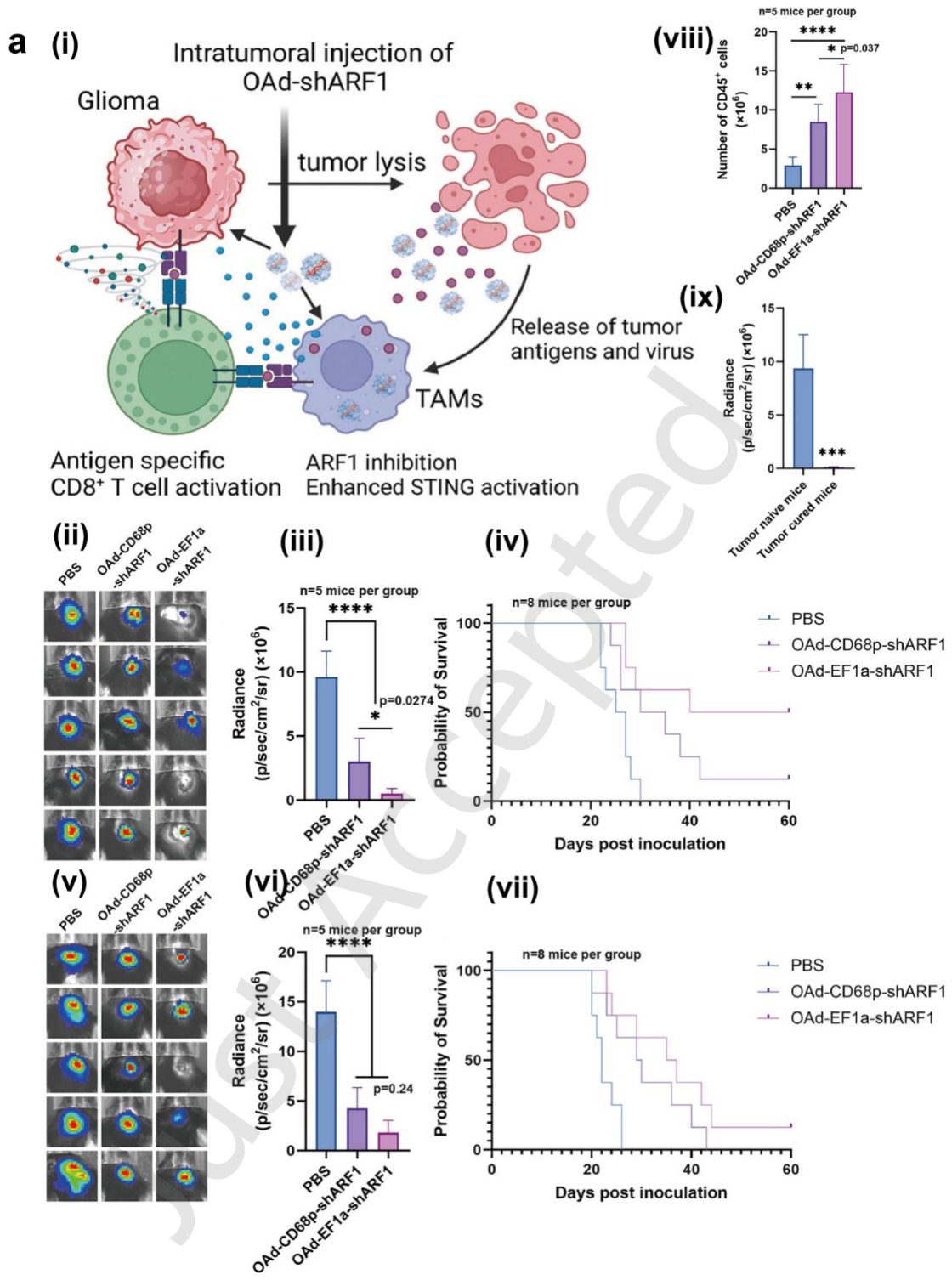


Figure 10 OVs combined with TAMs modulation for glioma therapy.a.Mechanism and antitumor efficacy of TAMs-specific ARF1 knockdown combined with oncolytic adenovirus.(i)Schematic illustration(ii)Tumor growth curves of treatment groups in the GL261 orthotopic glioma model(iii)Quantitative analysis of tumor bioluminescence.(iv)Survival curves of treatment groups.(v)Tumor growth curves in the CT2A glioma model.(vi)Tumor growth curves in the CT2A glioma model.(vii)Survival curves in the CT2A model.(viii)Quantification of tumor- infiltrating immune cells.(ix)Re-challenge experiment of cured mice.Copy right 2025 Elsevier[100].

5.3 Combination with Metabolic Modulators

The glioma microenvironment harbors multiple metabolic aberrations, including cholesterol enrichment, lactate accumulation, and kynurenine

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buildup from tryptophan metabolism. These metabolites not only directly impair immune cell functions but also promote immunosuppression through modulation of signaling pathways. Although OVs can partially reverse metabolic suppression by inducing inflammatory responses, combination with metabolic modulators can further enhance therapeutic efficacy.

Cholesterol metabolic dysregulation in the glioma microenvironment constitutes one of the key drivers of immunosuppression. Wang et al. systematically delineated the co-dependency features of cholesterol metabolism in glioma. Glioma cells rely on exogenous cholesterol uptake, establishing a cholesterol reservoir in the tumor interstitial fluid, which leads to aberrant cholesterol accumulation in infiltrating monocyte-derived TAMs. Cholesterol undergoes oxidation in TAMs to generate 7-ketocholesterol, which impairs TNF signaling by inhibiting mitochondrial translation, thereby attenuating TAM phagocytic function while upregulating the expression of "don't eat me" receptors including Siglec-10 and PD-1, culminating in a phagocytic fragility phenotype. Based on this mechanism, the research team incorporated the apolipoprotein A1 (ApoA1) gene into an oncolytic adenovirus (AdV-ApoA1). ApoA1 promotes cholesterol efflux from TAMs, reduces 7-ketocholesterol levels, restores mitochondrial protein synthesis and TNF signaling activity, thereby reinstating TAM phagocytic function and enhancing antigen presentation. In GL261 and G422 orthotopic glioma models, a single intratumoral injection of AdV-ApoA1 achieved a 42% complete response rate. In an immune-reconstituted humanized GBM model, the complete response rate reached 71%(**Fig.11**). Mechanistically, AdV-ApoA1 treatment promoted the formation of immunological synapses between TAMs and $CD8^+$ T cells, increased the secretion of IFN-γ and granzyme B by tumor-infiltrating $CD8^+$ T cells, and simultaneously reduced the expression of immune checkpoints including PD-1, LAG-3, and TIGIT. Cured mice in rechallenge experiments exhibited specific immune memory against the same tumor type, but showed no cross-protection against non-syngeneic tumors[78].

The IDO/kynurenine axis constitutes another viable therapeutic target for metabolic microenvironment reprogramming. IDO is widely overexpressed in glioma and suppresses effector T cell function while promoting Treg differentiation through tryptophan depletion and kynurenine production[147-150]. Tsuboi et al. found that following oncolytic HSV infection of GSCs, activation of the cGAS-STING pathway significantly upregulated IDO expression in co-cultured monocyte-derived dendritic cells, leading to extensive infiltration of IDO^+ $CD11c^+$ dendritic cells in the glioma microenvironment. IDO, through tryptophan depletion and kynurenine generation, suppresses effector T cell function and promotes Treg differentiation, thereby establishing a negative brake on OV-elicited immune responses. To interrupt this feedback inhibition, the team combined the IDO inhibitor Indoximod with oncolytic HSV in the 005-OVA GSC-derived immunocompetent mouse model. The combination therapy not only significantly suppressed tumor growth and extended survival, but more importantly synergistically enhanced dendritic cell maturation, upregulated the co-stimulatory molecules CD80 and HLA-DR, increased secretion of pro-inflammatory cytokines including IL-12, and effectively induced antigen-specific $CD8^+$ T cell activation[134]. These findings indicate that feedback activation of the IDO pathway during OVs therapy is a critical factor limiting efficacy, and that combining IDO inhibitors can relieve this metabolic immunosuppression, providing direct evidence for OVs combination with metabolic modulation.

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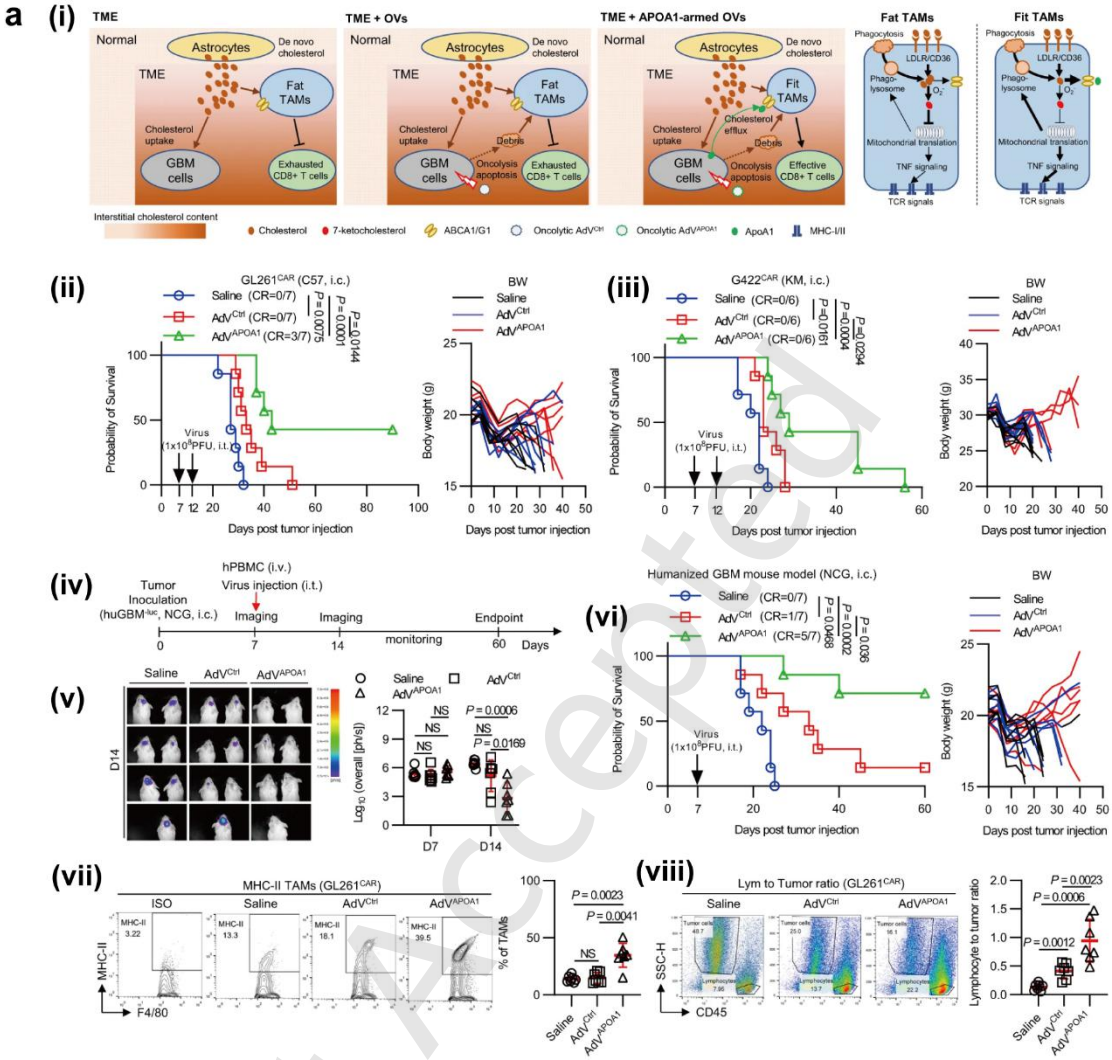


Figure 11 OVs combined with metabolic modulation for glioma therapy.a.Oncolytic adenovirus expressing ApoA1 enforces cholesterol efflux to remodel immunometabolic microenvironment and antitumor immunity in glioblastoma.(i)Schematic illustration of cholesterol metabolism reprogramming in the glioma TIME.(ii, iii) Survival curves in GL261CAR and G422CAR orthotopic glioma models. (iv–vi) Tumor growth (iv), survival (v), and IVIS monitoring (vi) in a humanized immune-reconstituted GBM model. (vii, viii) Flow cytometry of MHC-II on TAMs (vii) and tumor-infiltrating lymphocyte-to-tumor cell ratio (viii) after AdV-ApoA1 treatment.Adapted from Ref.[78] under the terms of the Creative Commons Attribution 4.0 International License.

5.4Combination with Other Conventional Therapies

Combination with chemotherapy: low- dose cyclophosphamide (CPA) can transiently deplete or suppress Tregs and MDSCs, thereby attenuating host innate and adaptive immune clearance of OVs and prolonging the viral replication window within tumors.Fulci et al. found in an orthotopic glioma model that following oncolytic HSV injection, NK cells and $CD68^+$ microglia/macrophages rapidly infiltrate the tumor and release IFN- γ, reducing the virus- infected area to less than 10% within 72 hours. In contrast, cyclophosphamide pretreatment suppressed the infiltration of these immune cells and IFN- γ production, increasing the virus- infected area to approximately 80%, significantly enhancing

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viral replication capacity, and prolonging the survival of tumor‐bearing animals[130].

Temozolomide (TMZ) is a standard chemotherapeutic agent for glioma that induces ICD in tumor cells, releasing DAMPs including HMGB1 and ATP, and synergizes with OV‐induced ICD(Fig.12a)[135]. Liikanen administered oncolytic adenovirus in combination with low‐dose TMZ and low‐dose CPA to 17 patients with chemotherapy‐refractory advanced solid tumors. The results demonstrated a favorable safety profile, with 67% of evaluable patients achieving disease control. Tumor biopsies revealed autophagy induction, HMGB1 release, and tumor‐specific T cell responses, providing direct human evidence for the triple combination in glioma therapy[151]. TMZ resistance represents a major challenge in the clinical management of glioma, and OVs may offer a novel avenue to overcome this obstacle. Foreman et al. reported that the double‐gene‐deleted vaccinia virus vvDD (lacking thymidine kinase TK and viral growth factor VGF) is capable of effectively killing TMZ‐resistant human brain tumor stem cells. The mechanism underlying this efficacy is that vvDD replication is independent of the tumor cell DNA repair status, and it can bypass TMZ‐induced resistance signaling by engaging distinct cell death pathways. Therefore, the combination of OVs with TMZ can not only synergistically amplify the ICD effect but also holds promise for overcoming primary or acquired resistance of gliomas to TMZ[136].

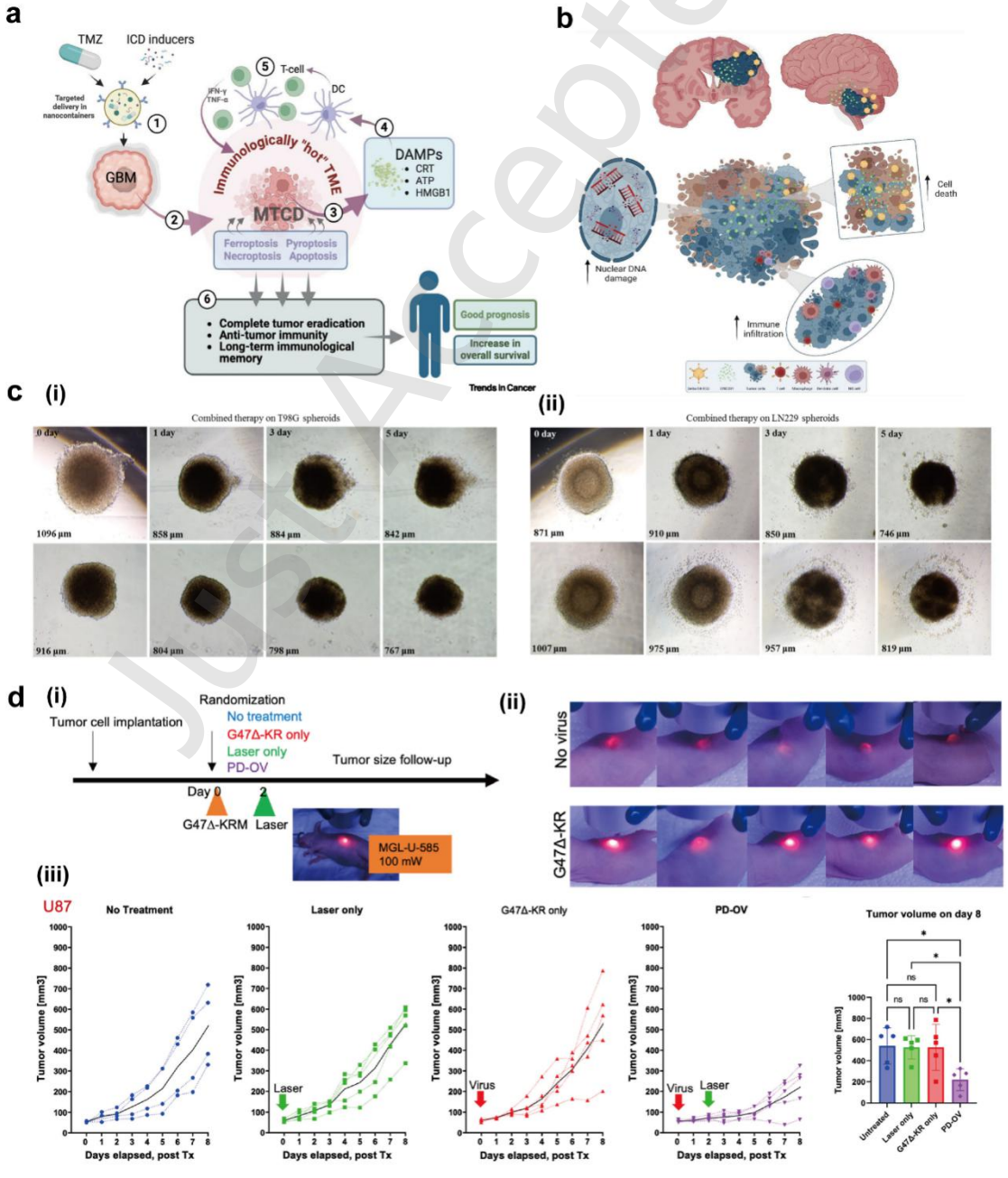
In addition to traditional chemotherapeutics, the integration of oncolytic adenoviruses with small‐molecule targeted therapies has emerged as a promising strategy. ONC201 is a small‐molecule agent that selectively targets dopamine receptor D2 (DRD2) and casein kinase 1α (CK1α), and has shown single‐agent activity in pediatric high‐grade glioma (pHGG) and diffuse intrinsic pontine glioma (DIPG). Investigators evaluated the efficacy of the oncolytic adenovirus Delta‐24‐RGD in combination with ONC201 for the treatment of pHGG and diffuse midline glioma (DMG). The study found that the combination treatment did not affect the viral replication capacity of Delta‐24‐RGD, and exhibited synergistic or additive cytotoxic effects in both in vitro and in vivo models. Mechanistically, the combination treatment exacerbated nuclear DNA damage while sustaining the metabolic perturbations and mitochondrial damage induced by each single agent(Fig.12b). In tumor‐bearing mouse models, Delta‐24‐RGD/ONC201 combination therapy significantly prolonged survival and, in murine DMG models, remodeled the tumor microenvironment toward a pro‐inflammatory phenotype[137].

Combination with radiotherapy: by eliciting DNA damage and ICD, radiotherapy potentiates the immunogenicity of neoplastic cells, establishing a synergistic interplay with oncolytic virotherapy[152]. Furthermore, radiotherapy may facilitate intratumoral viral spread through disruption of the tumor vasculature and enhanced vascular permeability[153]. Clinically, Markert and colleagues performed a Phase I study evaluating oncolytic HSV‐1 G207 in combination with radiotherapy, which included 9 patients with recurrent GBM. All patients were administered intratumoral G207, with radiotherapy initiated on the following day. The results showed that 6 patients achieved radiographic responses, with a median survival of 7.5 months, and no dose‐limiting toxicities were observed[121].

Combination with photodynamic therapy (PDT): PDT utilizes photosensitizers to generate ROS upon irradiation with light of specific wavelengths, directly killing tumor cells while inducing ICD and remodeling the tumor microenvironment, thereby synergizing with OVs.Nazarenko and colleagues integrated vesicular stomatitis virus (VSV) with protoporphyrin IX in a sequential treatment paradigm, wherein neoplastic cells were first exposed to protoporphyrin IX‐based PDT and subsequently challenged with VSV infection. The synergistic mechanism of this strategy is primarily based on two levels. ROS generated by PDT directly damage tumor cell DNA and interfere with its repair systems, while concurrently altering cellular redox homeostasis, significantly enhancing the susceptibility of damaged cells to subsequent viral infection. In addition, PDT compromises tumor vascular integrity and enhances vessel permeability, thereby facilitating improved viral distribution and penetrative capacity within neoplastic lesions. In T98G and LN229 glioma cell lines and 3D tumor spheroid models, the sequential regimen achieved complete cell elimination, whereas

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neither PDT nor virus alone could attain comparable effects. Moreover, the combination allowed the use of lower doses of both virus and photosensitizer(Fig.12c)[138]. Shimizu et al., in turn, directly incorporated the gene encoding the photosensitizing protein KillerRed into the clinical- grade oncolytic herpes simplex virus G47Δ, generating G47Δ- KR and achieving integrated unification of PDT and oncolytic virotherapy. Following viral infection of tumor cells, KillerRed expression is driven to the cell membrane, where it is activated upon irradiation with 585 nm amber light to generate superoxide and other ROS. These ROS not only directly damage the cell membrane and organelles but also synergize with viral replication to induce tumor cell death(Fig.12d). Simultaneously, PDT can remodel the tumor microenvironment, suggesting that PDT may further amplify virus- induced immune responses. This strategy encodes the photosensitizing protein within the viral genome, achieving tumor- specific expression of the photosensitizer such that only virus- infected tumor cells express KillerRed and generate phototoxicity, thereby circumventing the nonspecific accumulation in normal tissues associated with systemic photosensitizer administration in conventional PDT, and providing a precision- based paradigm for the deep integration of PDT and oncolytic virotherapy[139].



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Figure 12 OVs combined with conventional therapies for glioma.a.Strategy for inducing mixed cell death to shape TMZ. Copy right 2025 Elsevier[135].b. Oncolytic adenovirus Delta-24-RGD combined with small-molecule drug ONC201 for glioma therapy. Copy right 2024 Oxford University Press[137]. c.Synergistic antitumor efficacy of vesicular stomatitis virus combined with protoporphyrin IX-mediated photodynamic therapy in glioma. (i) Sequential PDT followed by VSV infection completely cleared T98G 3D spheroids by day 5. (ii) LN229 3D spheroids similarly achieved complete clearance.Adapted from Ref.[138](CC BY 4.0).d.In vivo antitumor efficacy of KillerRed-expressing oncolytic herpes simplex virus G47Δ-KR combined with amber light irradiation in glioma. (i) Experimental design. (ii) Tumor volume curves in the U87 model. (iii) Tumor volume curves in the IOMM-Lee malignant meningioma model.Copy right 2023 Elsevier[139].

5.5 Combination with Adoptive Cell Therapy

Combination with adoptive cell therapy: Zhang et al. utilized B7‑H3‑targeted CAR‑T cells as living carriers for oncolytic herpes simplex virus, achieving virus‑specific delivery to glioma lesions through CAR‑mediated antigen recognition(**Fig.13a**). Following HSV loading, CAR‑T cell viability and function remain unimpaired in the short term, while viral infection itself can enhance the effector functions of CAR‑T cells, suggesting a potential synergistic mechanism between the two modalities.Upon arrival at the tumor, CAR‑T^{+} HSV cells release progeny HSV, which further infects and lyses adjacent tumor cells, achieving synergy between CAR‑T‑mediated direct killing and viral oncolysis[140]. On this basis, Fang and colleagues utilized CD70‑directed CAR‑T cells as live delivery vehicles for third‑generation oncolytic adenoviruses, loading OAd‑GFP or IL‑15‑expressing TS‑2021 onto CAR‑T cells via centrifugal infection, thus accomplishing precision‑targeted viral transport to glioma lesions. OAd is capable of restricted replication within CAR‑T cells without inducing immediate cytolysis, thereby preserving carrier cell integrity while enabling viral delivery to neoplastic sites. CAR‑mediated antigen engagement secures the tumor‑selective nature of viral delivery. CD70 CAR‑T‑OAd‑GFP cells transfer the virus to underlying tumor cells exclusively in the presence of CD70 expression on target cells, while exhibiting no delivery to CD70‑negative human astrocytes(**Fig.13b**)[141].

Chalise and colleagues employed the cancer‑specific monoclonal antibody LpMab‑2 to generate third‑generation CAR‑T cells (Lp2‑CAR‑T) targeting podoplanin, and subsequently combined these cells with the third‑generation oncolytic herpes simplex virus G47Δ, conducting a systematic investigation into the challenges posed by glioma heterogeneity and the potential off‑target toxicity of CAR‑T therapies against normal tissues (**Fig.13c**). The conventional anti‑podoplanin antibody NZ‑1 cross‑reacts with podoplanin expressed in normal tissues, carrying inherent toxicity liabilities. LpMab‑2, by contrast, specifically recognizes cancer‑type podoplanin characterized by aberrant glycosylation in gliomas, and exhibits no reactivity against podoplanin‑positive normal mesothelial cells, thus achieving an improved safety profile without compromising antitumor efficacy. Glioma antigenic heterogeneity poses a fundamental challenge for single‑target CAR‑T therapies, as podoplanin‑negative or antigen‑lost neoplastic subclones are capable of evading CAR‑T cell recognition. The incorporation of G47Δ effectively circumvents this constraint. G47Δ infects and lyses tumor cells in a podoplanin‑independent manner, thus complementing Lp2‑CAR‑T activity, CAR‑T cells eliminate podoplanin‑expressing tumor cells and elaborate IFN‑γ, whereas G47Δ clears antigen‑negative escape subclones. The combination regimen markedly augmented IFN‑γ production and achieved enhanced in vitro tumor cytolysis with accelerated kinetics. In orthotopic glioma subcutaneous xenograft models, the Lp2‑CAR‑T/G47Δ combination achieved markedly superior tumor growth inhibition and survival extension relative to either monotherapy, with 50% of treated mice attaining long‑term survival[142].

OVs can also enhance CAR‑T cell recognition through arming with tumor antigens. Using vesicular

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stomatitis virus as the backbone, the HER2 gene was inserted into the viral genome via reverse genetics to construct a novel OVs designated OVV-03. Following infection of glioma cells, OVV-03 drives efficient HER2 antigen expression on the cell surface. Flow cytometry revealed that in multiple glioma models including U87, SHG140, and SHG141, HER2 expression in OVV-03-infected cells peaked at 24 hours post-infection in an MOI-dependent manner, whereas the control virus OVV-00 exhibited no such effect. The upregulation of HER2 expression provides HER2-targeted CAR-T cells with more abundant antigen recognition targets, thereby enhancing CAR-T cell recognition and killing of tumor cells(**Fig.13d**)[143].

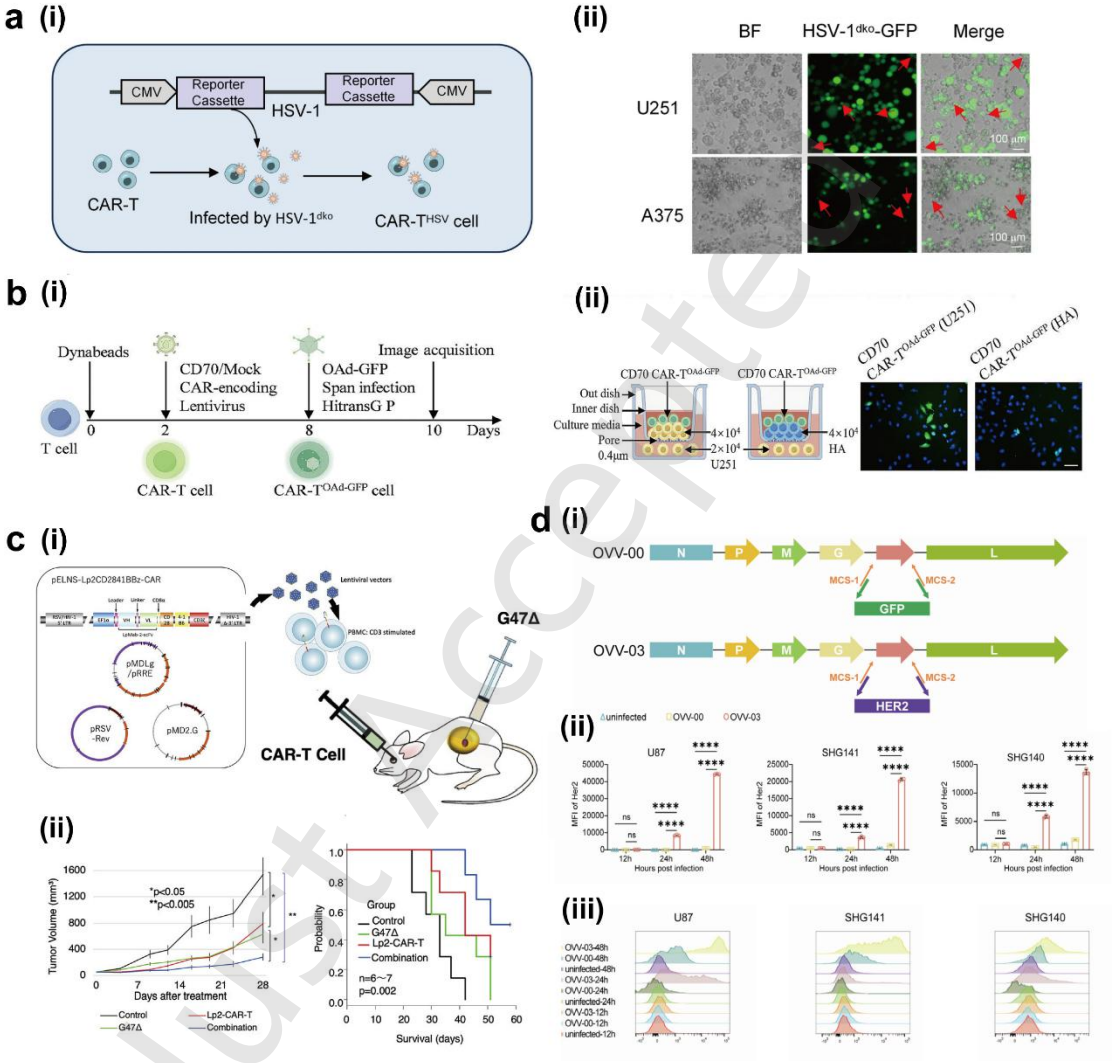


Figure 13 Combination strategies with adoptive cell therapy to enhance OVs efficacy against glioblastoma.a.Systemic delivery of oncolytic HSV by B7-H3 CAR-T cells.(i)Schematic: B7-H3 CAR-T cells loaded with HSV are intravenously administered,synergizing CAR-T killing with viral oncolysis.(ii)CAR-T cell viability remained stable.Adapted from Ref. [140] under CC BY 4.0 International License. b.Precise delivery and autocrine support by CD70 CAR-T cells loaded with third-generation oncolytic adenovirus TS-2021.(i)Schematic of preparation.(ii)Antigen-specific delivery validation.Adapted from Ref. [141] under CC BY 4.0 International License. c.Cancer-specific Lp2-CAR-T combined with clinical-grade oncolytic HSV G47Δfor glioma therapy.(i)Schematic illustration.(ii)G47Δ infects and lyses tumor cells independently of podoplanin expression.Adapted from Ref. [3]under CC BY 4.0 License. d.OVV-03 enhances CAR-T cell therapy.(i)Schematic of OVV-03 construction.(ii)Flow cytometry showed OVV-03 infection of glioma cells led to MOI-dependent HER2 surface upregulation.(iii)HER2 expression peaked at 24h post-infection, confirming

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efficient antigen delivery to enhance HER2 CAR-T recognition.Copy right 2026 Taylor & Francis[143].

Li et al. developed a multimodal immunotherapy platform that combines an oncolytic poxvirus encoding dual antigens, CD19t and EGFRvIII (designated OVDual), with bispecific CAR‐T cells targeting both CD19 and EGFRvIII (BiCAR‐T), aiming to address the antigenic heterogeneity and immunosuppressive microenvironment of gliomas. Following infection of tumor cells, the OVs delivers both CD19 and EGFRvIII antigens onto the cell surface, forcing the presentation of tumor antigens that are otherwise absent or heterogeneously expressed. BiCAR‐T cells, through dual recognition by CD19 CAR and EGFRvIII CAR, specifically kill OVDual‐infected glioma cells, whereas control T cells lack this effect. In orthotopic glioma xenograft models, OVDual combined with BiCAR‐T significantly inhibited tumor growth and extended survival compared with either agent alone(**Fig.14**). Moreover, the platform is adaptable to BiCAR‐NK cells. BiCAR‐NK cells derived from human embryonic stem cells, in combination with OVDual and OVmIL15/21, likewise achieved significant tumor growth inhibition and survival extension in vivo. This investigation exemplifies a multimodal paradigm integrating OVs‐mediated dual antigen delivery with bispecific CAR‐T/NK cellular therapies, providing a comprehensive framework to circumvent the obstacles of glioma antigenic heterogeneity and immune suppression[144].

The triple combination strategy of ROR1‐targeted CAR‐NK cells, IL‐21‐expressing oncolytic HSV (C021), and the IL‐15 receptor agonist NKTR‐255 demonstrated significant synergistic cytotoxicity against glioblastoma in vitro. ROR1 is upregulated in glioma stem‐like cells and serves as a critical factor in maintaining their tumorigenicity. The investigators first constructed IL‐21‐expressing oncolytic HSV (C021), and showed that C021‐infected glioma cells continuously secrete IL‐21 for at least 7 days. This combination strategy has been validated to effectively reduce tumor burden in neuroblastoma xenograft models, and in vivo studies for glioblastoma are currently ongoing[146]. Vazaios et al., in turn, explored a combination strategy of Vγ9Vδ2 TCR‐engineered T cells with OVs. These T cells recognize metabolic changes in tumor or virus‐infected cells by targeting BTN2A1 and BTN3A molecules. The investigators observed that infection of diffuse midline glioma cells with either oncolytic adenovirus Δ24‐RGD or reovirus R124 led to selective upregulation of BTN3A expression without affecting BTN2A1 levels. TEGs exhibited augmented cytolytic activity against OVs‐infected tumor cells, suggesting that oncolytic virotherapy enhances TCR‐engineered T cell recognition and effector function via the upregulation of target surface molecules[145].

In summary, OVs monotherapy for glioma faces multiple bottlenecks, including immune clearance, delivery barriers, re‐establishment of microenvironmental immunosuppression, and tumor heterogeneity, and combination strategies precisely address these challenges systematically from different dimensions. Combination with ICIs can reverse T cell exhaustion, converting the OVs‐induced suppressive microenvironment into a durable effector immune response. Combination with TAM modulation attenuates immunosuppression at the innate immune source, promotes M1 polarization, and restores macrophage phagocytic and antigen‐presenting functions. Combination with metabolic modulators directly intervenes in the metabolic‐immune axis within the microenvironment, relieving the suppressive effects of metabolites on T cells and macrophages. Combination with conventional modalities, including chemotherapy, radiotherapy, and photodynamic therapy, can generate mechanistically complementary cytotoxic effects with OVs through the induction of DNA damage and ICD. Combination with adoptive cell therapy, in turn, leverages OVs‐mediated microenvironmental remodeling, chemokine expression, and bispecific molecule production to significantly enhance the recruitment, activation, and persistence of effector T cells.

Multimodal combination strategies are not a simple

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superposition of individual mechanisms. Rather, they exert synergistic effects across multiple interconnected nodes, delivery, activation, exhaustion, metabolism, and immune evasion, progressively transforming glioma from a prototypical "cold" tumor into an immunologically active "hot" tumor, while simultaneously establishing the foundation for durable immunological memory. Therefore, systematic and personalized design of combination therapies represents the core direction for breaking through the efficacy bottleneck of OVs in glioma, and constitutes an essential pathway for advancing the field toward clinical translation.

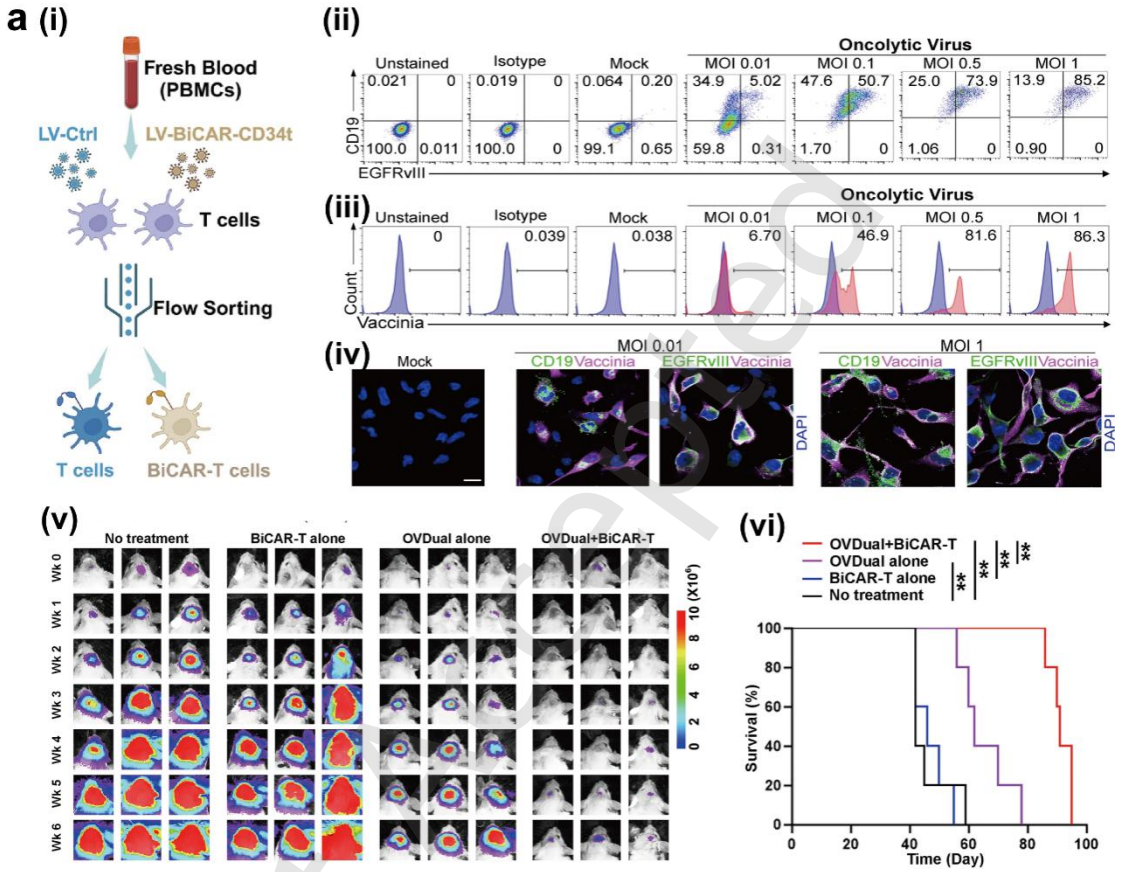


Figure 14 Preparation strategy and antitumor efficacy of dual-antigen OVs combined with bispecific CAR-T cells for glioblastoma therapy.(i)Schematic illustration of bispecific CAR-T cell generation.(ii)Flow cytometric quantification of dual antigen expression on GBM cells following OVDual infection.(iii)Flow cytometric quantification of vaccinia viral protein expression following OVDual infection.(iv)Confocal microscopy validation of dual antigen expression following OVDual infection.(v)In vivo bioluminescence imaging of tumor growth in an orthotopic GBM xenograft model across treatment groups.(vi)Survival curves of tumor-bearing mice across treatment groups.Adapted from Ref. [144] (CC BY 4.0).

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Conclusion and Outlook

In summary, OVs represent a promising immunotherapeutic modality for glioma, yet their clinical translation remains constrained by the BBB, systemic clearance, and the immunosuppressive TME. This review has systematically examined five categories of delivery strategies such as active targeting, biomimetic cell-membrane camouflage, cell-based carriers, physical targeting, and exosome-like particles, each with distinct advantages and limitations. To overcome the limitations of monotherapy, combination strategies integrating OVs with immune checkpoint blockade, TAM modulation, metabolic regulators, conventional therapies, and adoptive cell therapy have demonstrated synergistic potential by reversing immunosuppression, enhancing antigen presentation, and promoting durable antitumor immunity. Collectively, these approaches transform immunologically "cold" gliomas into "hot" tumors and provide a rational foundation for clinical translation.

Building on these conclusions, several key challenges and future directions merit further discussion. Although combination regimens based on OVs have shown encouraging synergistic efficacy in preclinical studies and early- phase clinical trials, their translation into routine clinical practice still faces multiple obstacles. Future research should first focus on the precise design of personalized combination regimens. Glioma exhibits high molecular and immune microenvironment heterogeneity, and different patients show significant differences in response to the same treatment regimen. Therefore, patient stratification should be performed based on indicators such as tumor mutational burden, PD- L1 expression level, $CD8^+$T cell infiltration density, and tumor- associated macrophage polarization status, so as to match the optimal combination strategy[132]. For example, for immunologically "cold" tumors, priority should be given to combination with TAM reprogramming or delivery- enhancing strategies, whereas for "hot" tumors that already have T cell infiltration but exhibit an exhausted phenotype, combination with immune checkpoint inhibitors may be more directly effective. Furthermore, the sequencing of CAR- T cell and OVs combination is of paramount importance, with computational models indicating that either simultaneous administration or OVs preceding CAR- T cell infusion may yield optimal therapeutic outcomes[19]. In the future, multi- omics integration and machine learning algorithms should be employed to establish a biomarker- driven stratification framework, truly achieving personalized precision immunotherapy.

Safety management, biomarker development, and delivery optimization constitute another critical dimension for advancing clinical translation. Combination with ICIs may increase the risk of immune- related adverse events, while combination with chemotherapy or radiotherapy may add myelosuppression and neurotoxicity. Therefore, a safety run- in period should be set in clinical trials, and the administration sequence and dose interval should be carefully determined. Meanwhile, developing reliable predictive biomarkers of efficacy is crucial. For example, dynamic changes in serum HMGB1, viral genome copy number in tumor tissue, IFN- γ- related gene signatures, and the degree of T cell receptor clonal expansion have all shown predictive potential and await validation in prospective trials[151]. In terms of delivery, the blood- brain barrier remains a core bottleneck. Strategies such as convection- enhanced delivery, neural stem cell/mesenchymal stem cell carriers, functionalized modification of nanoparticles, and viral capsid engineering each have their own advantages[14, 114, 121]. Looking forward, the field should move toward a trinity design that harmonizes active targeting, spatiotemporally controlled release, and immunotherapeutic synergy. One example is the construction of intelligent nanocarriers that respond to acidic pH or matrix metalloproteinase activity within the tumor microenvironment, thereby enabling programmed viral release.

Importantly, long-term immune memory and resistance to tumor rechallenge have been observed in several preclinical OVs-based combination studies. For example, mice cured by G47Δ-mIL12 plus dual immune checkpoint blockade, or by ARF1-knockdown oncolytic adenovirus, completely resisted subsequent tumor rechallenge[100, 133]. This indicates that OVs-based combination therapy can establish durable immunological memory, which is crucial for preventing glioma recurrence. Future studies should therefore evaluate memory T cell subsets and rechallenge resistance as key endpoints in both preclinical and clinical settings.

From a clinical translation perspective, the design of combination regimens must also address practical issues such as dose selection, dosing intervals, maintenance therapy, and toxicity-based discontinuation criteria. For OVs-based combinations, the optimal dose of OVs may depend on the route of administration, viral backbone, and pre-existing antiviral immunity, while the dose of the partner agent such as ICIs and chemotherapy may need to be adjusted to avoid overlapping toxicities. Dosing intervals should be carefully scheduled to allow sufficient viral replication and immune activation without causing excessive toxicity. Maintenance therapy with repeated low-dose OVs or partner agents may be considered to sustain immune memory and prevent recurrence. Moreover, clear discontinuation criteria based on treatment-related adverse events should be pre-defined in clinical protocols. These practical considerations warrant further investigation in future clinical trials.

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Finally, the challenges of manufacturing quality control, regulatory approval, and clinical trial design should not be overlooked. Large- scale production of OVs still suffers from significant batch- to- batch variability in titer, infectivity, and purity. Combination regimens involve multiple drugs, and their interactions, dosing intervals, and stability studies are far more complex than those for monotherapy. At present, neither the US FDA nor the European EMA has instituted a specific regulatory review channel for fixed- dose combination products comprising OVs and immune checkpoint inhibitors. Consequently, the majority of regimens remain under investigation via investigator- initiated trials. Given the low incidence of glioma and the strong heterogeneity among patients, traditional randomized controlled trials are often time- consuming. Adaptive trial designs combined with real- world evidence may accelerate clinical translation. In the future, priority should be given to promoting multicenter, randomized, blinded controlled trials, incorporating central imaging review and an independent data safety monitoring board, and including patient- reported outcomes, such as neurological function status, cognitive function, and fatigue scores, as key secondary endpoints, while systematically collecting tumor tissue and peripheral blood samples before and after treatment to establish a biobank, supporting subsequent studies on resistance mechanisms and biomarkers. With the continuous accumulation of basic research and clinical data, OVs- based combination immunotherapy is expected to become an important component of comprehensive glioma treatment, bringing real survival benefits to patients.

Data availability

Data will be made available on request.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

Yuxuan Liu: Writing-original draft, Formal analysis, Data curation. Yuejia Ding: Writing-original draft, Data curation. Jianshuang Chen: Writing-review & editing. Yi Xiao: Data curation. Jialei Wu: Data curation. Xiaozhou Mou: Methodology, Conceptualization. Shibing Wang: Funding acquisition, Methodology.Yangyang Li: Writing-review & editing, Conceptualization.Yunzhao Chen:Writing-review & editing, Methodology.

Use of AI statement

None.

Ethics Statement

Not applicable

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