

Multiple strategies to improve the therapeutic efficacy of oncolytic herpes simplex virus in the treatment of glioblastoma (Review)

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Abstract. Oncolytic viruses have attracted widespread attention as biological anticancer agents that can selectively kill tumor cells without affecting normal cells. Although progress has been made in therapeutic strategies, the prognosis of patients with glioblastoma (GBM) remains poor and no ideal treatment approach has been developed. Recently, oncolytic herpes simplex virus (oHSV) has been considered a promising novel treatment approach for GBM. However, the therapeutic efficacy of oHSV in GBM, with its intricate pathophysiology, remains unsatisfactory due to several obstacles, such as limited replication and attenuated potency of oHSV owing to deletions or mutations in virulence genes, and ineffective delivery of the therapeutic virus. Multiple strategies have attempted to identify the optimal strategy for the successful clinical application of oHSV. Several preclinical trials have demonstrated that engineering novel oHSVs, developing combination therapies and improving methods for delivering oHSV to tumor cells seem to hold promise for improving the efficacy of this virotherapy.

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1. Introduction

Glioblastoma (GBM) is the most common and fatal primary brain tumor, characterized by poor survival, with a median survival time of ~1-2 years (1,2). The current standard treatment for GBM includes maximal surgical resection followed by an effective combination of radiotherapy and chemotherapy (1). Despite this multimodal approach, due to the intricate pathophysiology of GBM, the prognosis and survival of patients remain poor (2). The development of resistance by tumor cells and the inability of drugs to effectively cross the blood-brain and blood-tumor barriers are often suggested as major impediments to therapeutic development (3). Thus, novel and effective therapies are urgently required to treat patients with GBM.

Herpes simplex virus type 1 (HSV-1), a common natural human pathogen, can cause serious disease, from asymptomatic viral shedding to fatal encephalitis and disseminated disease (4). The virus is a double-stranded DNA (dsDNA) virus containing a 152 kbp dsDNA molecule encoding ~80 proteins (4). The HSV genome encompasses two components, the long and short regions, both containing a unique region flanked by inverted repeat regions (terminal long/internal long and internal short/terminal short) (5). HSV genes can be divided into three groups according to the sequence of viral gene expression, namely immediate early, early and late genes (5). The products of immediate early genes regulate gene transcription, including the product of the ribosomal protein S23 gene, infected-cell polypeptide 47 (ICP47), which decreases major histocompatibility complex (MHC) class I expression in infected cells by inhibiting transporter associated with antigen presentation (6). The large subunit of ribonucleotide reductase (ICP6) encoded by the early gene, UL39, serves an essential role in HSV replication in noncycling cells (7). The product of the late gene γ 34.5, ICP34.5, is considered a major mediator of HSV neuropathogenicity, which enables the replication and propagation of viruses in the brain (8). HSV strains lacking ICP34.5 maintain their ability to replicate in tumor cells; however, they fail to effectively replicate in normal neurons (8).

HSV-1 has several features that make the development of novel oncolytic (o)HSVs possible using genetic engineering

techniques (9). For example, its large genome is stable and can be manipulated as multiple nonessential genes, including those responsible for pathogenicity, can be deleted, mutated or replaced with therapeutic transgenes, and its genome does not integrate (9). HSV replication is affected by several nucleotide-metabolizing enzymes, such as thymidine kinase, ICP34.5, ICP6 and ICP0 (6). Only rapidly proliferating tumor cells, which do not express the precursors for viral DNA synthesis found in normal cells, can promote HSV replication without the presence of nucleotide-metabolizing enzymes, explaining why oncolytic viruses (OVs) can target tumors (6). Engineered oHSVs can directly destroy tumor cells by selectively replicating in them (oncolysis), altering the tumor microenvironment and stimulating antitumor immune responses, which are the main mechanisms by which OVs kill cancer cells (10). Conversely, anti-HSV agents, including ganciclovir and acyclovir, can ensure the safety of oHSV in clinical use (6). The first genetically engineered HSV capable of undergoing selective replication and killing GBM cells was reported by Martuza *et al* in 1991, providing novel insights into the application of 'virotherapy' for treating GBM (11). It has been reported that oHSVs, which selectively infect and kill tumor cells, are effective in treating different types of cancer, including GBM, in preclinical and clinical trials (12).

The present review summarizes different strategies used in preclinical and clinical trials to improve the therapeutic efficacy of oHSVs in GBM. In addition, findings from completed clinical trials evaluating the potential use of oHSVs for GBM are discussed. Limitations of the current clinical use of oHSVs for GBM are highlighted to propose a promising future direction for promoting the clinical application of virotherapy.

2. Multiple strategies to improve the therapeutic efficacy of oHSV

Although oHSVs have exhibited promising results at the clinical stage, several studies have demonstrated that viruses alone are unlikely to completely cure GBM (6). In recent years, multiple strategies have tried to improve the efficacy of oHSVs in the treatment of GBM, including engineering novel oHSVs, developing combination therapies and increasing systemic delivery of oHSVs to tumor cells (Fig. 1).

Engineering novel oHSVs. As a biological antitumor agent for cancers, oHSV has gone through several generations of genetic manipulation. Traditionally, the main genetic engineering techniques used to generate HSV mutants include homologous recombination, cell transfection and bacterial artificial chromosome technology (6). Initially, genetically engineered viruses are constructed by deleting or mutating one or more of the HSV-1 genes (10). Conversely, there are double copies of multiple genes, such as ICP34.5, ICP4 and ICP0, which complicate the genetic manipulation of HSV-1 and limit the development of oHSVs but also provide more possibilities, such as oHSVs with a single copy of γ 34.5, ensuring the safety of normal nerve cells while maintaining the antitumor effect (8). Due to the development of bioengineering technology and the improved understanding of HSV-1 genes, additional novel oHSVs with improved efficacy and safety have been engineered (Table I).

Promoting the safety and viability of oHSVs. A series of trials demonstrated the safety and potential efficacy of mutants, such as HSV1716 (an ICP34.5 null mutant), G207 (deletion of both copies of the γ 134.5 gene locus and a lacZ insertion into the UL39 locus) and G47 Δ (generated from G207 with an additional deletion of the US12 gene) (13,14). The multifunctional viral protein, ICP34.5 plays a key role in the host interferon (IFN) response, which suppresses viral protein synthesis (8). Even in tumor cells, replication of oHSV without ICP34.5 is severely limited (14). To improve the replication of oHSV lacking γ 34.5, another mutant (rQNestin34.5) with greater replication and reproduction capacity and cytotoxicity was generated, in which one copy of the γ 34.5 gene was reinserted into the UL39-deleted, γ 34.5-deleted viral genome under the control of a tumor-specific promoter (15). The carboxyl-terminus of GADD34 exhibits sequence homology with the virulence factor ICP34.5 of HSV, which inhibits the neuropeptide Y receptor Y4-catalyzed dephosphorylation of eukaryotic initiation factor 2 $^{\alpha}$ (16). To further optimize the virus and decrease its potential neurotoxicity to normal nerve cells, Nakashima *et al* (17) generated a novel weakened HSV-1 strain (NG34) by replacing ICP34.5 with GADD34 based on rQNestin34.5. NG34 expressing human GADD34 was demonstrated to be significantly less neurovirulent in the brains of non-tumor-bearing HSV-1-susceptible mice and exerted considerable therapeutic potency in human GBM panels compared with rQNestin34.5 (17).

Increasing evidence suggest that pathophysiological hypoxia promotes the glioma stem-like cells (GSCs) phenotype and is associated with tumor progression and poor prognosis (18). However, recent study has revealed that the efficacy of oHSVs lacking γ 34.5 was diminished in hypoxic conditions (18). C130 and C134 are two types of chimeric HSV-1 with deletion of the γ 34.5 gene, which, however, express the human cytomegalovirus tRNA-Ser (anticodon TGA) 2-1 and insulin receptor substrate 1 genes, whose protein product counteracts with host protein kinase R (19). A study demonstrated that the cytotoxicity and viral recovery of C134 strain were significantly improved under both hypoxic and normoxic conditions compared with γ 34.5-deleted strains, and its ability to infect and kill CD133+ GSCs was similar to that of wild-type (WT) HSV-1 (18). A preclinical evaluation of C134 in mice and non-human primates confirmed its safety, thus providing a strong basis for further clinical trials (20).

Receptor mediated retargeted oHSVs. The majority of engineered OVs exhibit high safety at the expense of virulence (13). However, the ability of oHSVs to specifically infect tumor cells is not ideal. The solution to this problem is the development of an oHSV that specifically targets human GBM cells (21). It is currently known that HSV entry is a multistep process that requires the essential glycoproteins, gD, gH/gL and gB (22). gD is activated by interacting with its natural receptors, nectin 1 or HSV entry mediator (HVEM) (22). This is an essential interaction for the entry of HSV. In addition, gD activation is propagated to gH/gL and eventually to gB, a type of fusogenic glycoprotein that mediates the fusion of virus envelope with cell membrane (22). Thus, the re-targeting strategy for HSV can be implemented via modifying not only gD, but also gH (21).

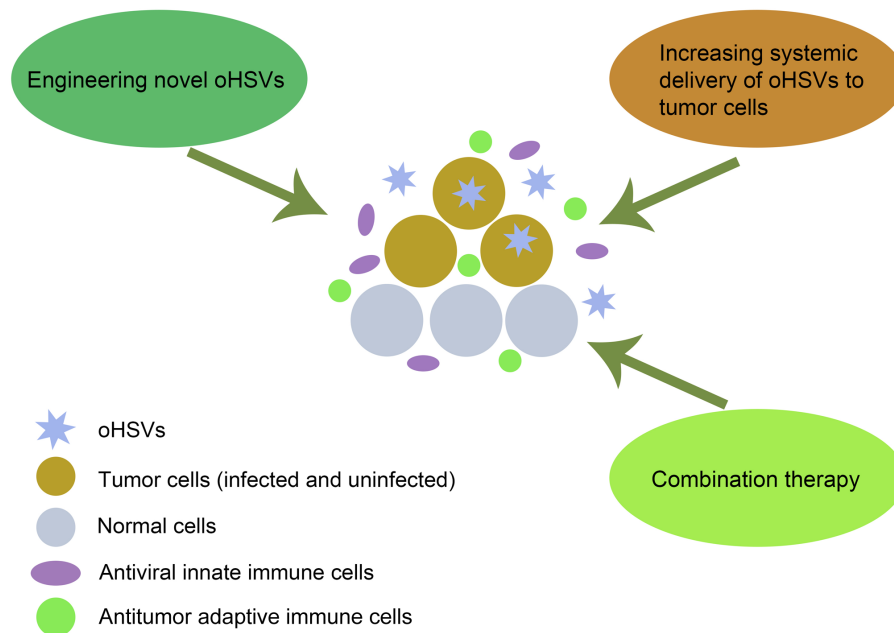


Figure 1. Schematic description of destruction mechanism of oHSVs destroying tumor cells and multiple strategies to improve its efficacy. oHSVs directly destroy tumor cells by selectively replicating in them and evade antiviral immunity, as well as stimulate antitumor immune responses, which are main mechanisms for oHSVs to kill cancer cells. Multiple strategies can improve the efficacy of oHSVs in the treatment of tumors, including engineering novel oHSVs, increasing systemic delivery of oHSVs to tumor cells and combination therapy. oHSV, oncolytic herpes simplex virus.

Human epidermal growth factor receptor 2 (HER2), a member of the EGFR family, is often overexpressed in GBM and other types of cancer (23). R-LM113 is a recombinant HSV strain generated by insertion of a single-chain variable fragment (scFv) specific for HER2 in the region encoding the viral envelope glycoprotein gD (24). This HSV is fully retargeted to HER2, which is frequently expressed in GBMs (25). A study demonstrated that mice injected with HER2-engineered GBM cells infected with R-LM113 can survive twice as long as mice injected with uninfected cells (25). EGFR is another attractive target given that it is frequently mutated or overexpressed in GBM (26). A study revealed that an EGFR-retargeted oHSV (containing the gB:NT allele, KNE) can efficiently and accurately enter cells expressing EGFR; this oHSV was generated by introducing human EGFR-specific scFv into mutant gD, which was demonstrated to be safe and effective in an orthotopic mouse model of human GBM (27). Mazzacurati *et al* (28) generated a new EGFR-retargeted virus strain (KGE-4:T124) by inserting four copies of the microRNA (miR)-124 identification sequence into the 3'-untranslated region of the ICP4 gene. Kge-4: T124 exhibited similar therapeutic effects and had significantly reduced neurovirulence in an orthotopic human GBM xenograft model (28). In addition, several cell surface proteins, such as folic acid receptor and CD44, are upregulated in tumor cells, broadening the potential for the development of retargeted oHSVs (28). Tropism manipulation through retargeting provides an avenue to increase cell-targeted viral infectivity and specificity; however, further clinical trials are required to verify the safety and efficacy of these novel engineered oHSVs.

Armed oHSVs carrying therapeutic genes. Although several types of oHSVs have achieved good results in preclinical or clinical trials, the results have demonstrated that it is difficult to completely eliminate tumors using the virus alone (6).

Some genes are not required for HSV viral replication, and their presence allows oHSVs to accommodate relatively large amounts of foreign DNA molecules (5). Thus, to enhance the oncolytic effect of HSV, several genes with therapeutic potential on tumors can be introduced on the basis of gene deletion or mutation to generate the so-called 'armed oHSVs'. These genes include immunomodulatory, tumor suppressor, antiangiogenic and prodrug-activating genes.

Beyond direct oncolysis, the efficacy of GBM virotherapy depends on the activation of antitumor immune responses. The immunosuppressive environment of GBM is a contributing factor to GBM development and progression (29). To overcome the immunosuppressive barriers in GBM, several immune-stimulating genes, such as interleukin (IL)-4, IL-12, Fms-related tyrosine kinase 3 ligand, immune checkpoint inhibitors, particularly cytotoxic T lymphocyte-associated antigen-4 and programmed death ligand 1, and immune stimulators, which can effectively inhibit or even kill tumor cells via antitumor immune responses, can be inserted into various oHSVs (30). Some preclinical trials demonstrated the safety and therapeutic efficacy of these armed viruses, such as oHSV-UL16 binding protein 3, G47Δ-tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and NG34scFv-programmed cell death 1 (31-33). In addition, treatment with OV-CDH1, an oHSV engineered to express CDH1 encoding E-cadherin (34), can substantially prolong the survival of GBM-bearing mice via the OV-CDH1-expressed E-cadherin, which in turn can interact with Killer cell lectin-like receptor G1 on natural killer (NK) cells, thus allowing evasion of NK cell-mediated cytotoxicity and improving viral spread (34).

The initiation of tumor neoangiogenesis and vasculogenesis play key roles in the GBM microenvironment (3). It has been reported that angiogenesis and vascular permeability stimulate tumor-associated macrophages and microglia,

Table I. List of typical oncolytic herpes simplex viruses associated with the treatment of glioblastoma.

Classification	Virus	Mutation characteristic	Stage	(Refs.)
Unarmed	Dlsptk	TK- (UL23)	P	(11)
	HSV1716	γ 34.5 ⁻	C (I)	(13,84-86)
	C101	γ 34.5 ⁻	P	(71)
	C130	γ 34.5 ⁻ , HCMV-TRS1 ⁺	P	(19)
	C134	γ 34.5 ⁻ , HCMV-IRS1 ⁺	C (I)	(18-20)
	MG18L	US3 ⁻ , UL39 ⁻ , LacZ ⁺	P	(47)
	G207	γ 34.5 ⁻ , UL39 ⁻ , LacZ ⁺	C (I)	(13,87-90)
	rHSVQ1	γ 34.5 ⁻ , UL39 ⁻ , EGFP ⁺	P	(71)
	NG34	γ 34.5 ⁻ , UL39 ⁻ , GADD34 ⁺	P	(17)
	G47 Δ	γ 34.5 ⁻ , US12 ⁻ , UL39 ⁻ , LacZ ⁺	C (I/IIa)	(13,91,92)
	rQNestin34.5	γ 34.5 ⁻ (one copy of the γ 34.5 ⁺), UL39 ⁻ , EGFP ⁺	C (I)	(15,17)
Retargeted	R-LM113	hHER2-retargeted	P	(24,25)
	R-115	hHER2-retargeted, mIL-12	P	(71)
	R-337	hHER2-retargeted, mIL-12	P	(22)
	KNE	EGFR-retargeted	P	(27)
	KGE-4: T124	EGFR-retargeted, ICP4 regulated by miR-124	P	(28)
Armed	HSV-VAE	γ 34.5 ⁻ , UL39 ⁻ , VAE ⁺	P	(35)
	HSV1-CD	γ 34.5 ⁻ , UL39 ⁻ , LacZ ⁺ , CD ⁺	P	(38)
	M032	γ 34.5 ⁻ , expressing hIL-12	C (I)	(30,71)
	M002	γ 34.5 ⁻ , expressing mIL-12	P	(30)
	OV-ChaseM	Expressing secreted ChaseM	P	(44)
	MGH2.1	γ 34.5 ⁻ , UL39 ⁻ , CPA ⁺ , CYP2B1 ⁺	P	(30,95)
	AdFlt3L	γ 34.5 ⁻ , LacZ ⁺ , expressing Flt3L	P	(30)
	oHSV-ULBP3	γ 34.5 ⁻ , miR-124 ⁺ , expressing ULBP3	P	(33)
	NG34scFvPD-1	γ 34.5 ⁻ , UL39 ⁻ , GADD34 ⁺ , scFVPD-1 ⁺	P	(32)
	OV-CDH1	γ 34.5 ⁻ , UL39 ⁻ , CDH1 ⁺ (encoding E-cadherin)	P	(34)
	G47 Δ -TRAIL	γ 34.5 ⁻ , US12 ⁻ , UL39 ⁻ , LacZ ⁺ , expressing TRAIL	P	(31)
	G47 Δ -IL12	γ 34.5 ⁻ , US12 ⁻ , UL39 ⁻ , LacZ ⁺ , expressing mIL-12	P	(30,45)
	G47 Δ -mAngio	γ 34.5 ⁻ , US12 ⁻ , UL39 ⁻ , LacZ ⁺ , expressing mAngio	P	(95)

P, preclinical; C, clinical; HCMV, human cytomegalovirus; HER2, human epidermal growth factor receptor 2; EGFR, epidermal growth factor receptor; miR, microRNA.

resulting in limited replication and spread of oHSVs in GBM cells (12). Zhu *et al* (35) demonstrated that an oncolytic HSV, namely γ 34.5⁻, UL39⁻, carrying an endostatin-angiostatin fusion gene (VAE) can significantly attenuate the activity of GSCs *in vitro*. In addition, the expression of exogenous VAE can inhibit human brain microvascular endothelial cell proliferation (35). Furthermore, the antitumor effects of G47 Δ -IL12 include activation of the innate and adaptive immune systems and inhibition of angiogenesis (30).

Prodrug-activating gene therapy can cause cell death by specifically converting a non-toxic prodrug into one or more cytotoxic metabolites (36). Yeast cytosine deaminase (CD) is a peculiar system that converts the nontoxic antifungal agent 5-fluorocytosine (5-FC) to the cytotoxic chemotherapeutic agent 5-fluorouracil (5-FU) (37). HSV1-CD combined with systemic 5-FC administration can increase the 5-FU concentration preferentially within tumors, thus potentially decreasing systemic side effects and increasing therapeutic efficacy (38).

Developing combination therapies. The hallmarks of GBM are closely associated with multiple changes in the tumor microenvironment, including the maintenance of proliferative signaling and the potential for replicative immortality, invasion and metastasis (39). Thus, improved treatment for GBM, with their multiple oncogenic pathways and refractory nature, requires a multimodal approach. Recent studies have reported that the combination of an oHSV and multiple anticancer modalities is often more effective than any single treatment alone for GBM, as with other malignancies (10,17).

Combining oHSVs with standard of care therapy for GBM. Both radiation and temozolomide (TMZ) chemotherapy are genotoxic, while oHSV infection also causes cellular DNA damage responses (40). Synergy between TMZ and oHSVs in GBM treatment occurs primarily through oHSV-mediated manipulation of the DNA damage response, a universal mechanism of cancer cell resistance to radiation and chemotherapy (40). A previous study suggested that TMZ combined with oHSV G207 and

G47Δ can kill human glioma cells by mediating the coordinated manipulation of DNA damage responses (40,41). Chondroitin sulfate proteoglycans (CSPGs), commonly overexpressed in GBM, serves a critical role in cell-cell and cell-extracellular matrix interactions. Increasing evidence suggest that their overexpression is closely associated with GBM cell proliferation and invasion (42). ChaseM is a mutant humanized version of the Chase ABC enzyme that can remove chondroitin sulfate glycosaminoglycans from CSPGs (43). Combination therapy with OV-ChaseM, an oncolytic HSV-1 expressing secreted Chase (44), and TMZ resulted in a notable synergistic increase in glioma cell death accompanied by enhanced apoptotic cell death (44). Recently, another study revealed that TMZ exerts a negative effect on oHSV immunotherapy for GBM in addition to its synergistic effect (45). Due to its negative effect on the number and activity of T cells and macrophages, which is closely associated with the therapeutic efficacy of G47Δ-IL12, TMZ cannot improve the overall survival of orthotopic tumor-bearing mice treated with G47Δ-IL12; instead, it eliminates certain beneficial effects of G47Δ-IL12 when both are administered concurrently (45). Therefore, understanding the effects of TMZ on different types of oHSVs in different GBM models is crucial for the clinical application of TMZ in combination with oHSVs. In addition, the combination of an oHSV with ionizing radiation exhibited synergistic therapeutic effects by inhibiting the repair of DNA double strand damage following treatment of GBM cells with ionizing radiation and by enhancing viral replication by exposure to ionizing radiation (46).

Combining oHSV with other anticancer agents. In addition to standard of care therapy, the combination of oHSV with other anticancer agents can also be promising for treating GBM. *In vivo*, the combination of MG18L, an oHSV containing a US3 deletion and LacZ insertion in the UL39 gene, and the PI3K/Akt inhibitors LY294002, triciribine, GDC-0941 and BEZ235, can significantly prolong the overall survival time of mice compared with mice treated with either single agent, achieving a long-term survival rate of 50% in GBM-bearing mice (47). Cheema *et al* (48) investigated a novel combinatorial approach of G47Δ with low-doses of etoposide and revealed that this combination can increase the survival of mice-bearing intracranial human GSC-derived tumors, while no significant adverse side effects were observed. In addition, the combination notably increased the sub-G₁ apoptotic population and significantly decreased the G₂-M phase population (48). It has been reported that the enhancing effect of antiangiogenic agents on the antitumor efficacy of OVs is associated with increased antiangiogenesis, viral spread, decreased macrophage populations and enhanced oncolytic effects (49,50). Combination treatment with bevacizumab and an oHSV exerted significant effects in a glioma-bearing model compared with those induced by either method alone (51). Another study suggested that bortezomib, a peptide-based reversible proteasome inhibitor, can cooperate with oHSV to treat tumors by increasing the expression of heat shock protein 90, which in turn can mediate the nuclear translocation of the oHSV polymerase enhancing viral replication (52). This combination therapy can also promote the activation of receptor interacting serine/threonine kinase 1 and c-JNK, resulting in tumor cell death and activation of the antitumor

immune response by upregulating IFN-γ and TRAIL in NK cells (52,53). In addition, the combination of an oHSV with a poly(ADP-ribose) polymerase inhibitor (PARP i), a new anticancer strategy, can sensitize GSCs to PARP i therapy, thus promoting DNA damage, cell death and apoptosis (54). Treatment of mice bearing either PARP i-sensitive or PARP i-resistant GSC-derived brain tumors with this combination therapy can markedly extend the median survival time compared with mice treated with either single agent (54).

OS2966 was the first clinical-ready humanized monoclonal antibody blocking integrin β1 (55). In a preclinical model of therapy resistant GBM, OS2966 exerted significant antitumor efficacy (55). A recent study has demonstrated that treatment with OS2966 attenuates the secretion of proinflammatory cytokines and IFN signaling in oHSV-treated tumor cells, and inhibits macrophage migration, resulting in enhanced oHSV replication (56). Furthermore, OS2966 can significantly inhibit the activation of the oHSV-induced focal adhesion kinase/Akt signaling pathway, thus increasing the cleavage of PARP and resulting in enhanced oHSV cytotoxicity (56). Notch signaling is highly active in GSCs, inhibiting cell differentiation and maintaining stem-like characteristics, thus contributing to GBM tumorigenesis and resistance to conventional therapies (57). Several γ secretase inhibitors (GSIs) blocking the Notch signaling have been used in clinical trials against different types of tumor, including glioma, and were demonstrated to exhibit antitumor properties by inhibiting or slowing tumor growth (58,59). Increasing evidence suggest that several viral infections activate the Notch signaling. For example, both oncolytic and WT HSV-1-infected glioma cells can activate the Notch signaling in the surrounding cells via miR-H16-mediated factors to inhibit hypoxia-inducible factor-1 downregulation (60,61). Thus, treatment of GBM with oHSV can further activate the Notch signaling, possibly promoting tumor growth (61). Notably, a recent study demonstrated that the combination of an oHSV with a Notch-blocking GSI can affect previously unresponsive GBM cells sensitive to GSIs, and demonstrated a therapeutic advantage over treatment with oHSV in two different models of intracranial glioma without affecting the safety profile of the virus *in vivo* (61). Trametinib, an orally administered mitogen-activated protein kinase (MEK) inhibitor, can be beneficial for the treatment of GBM by targeting the RAS-RAF-MEK-ERK signaling pathway; however, due to its poor central nervous system penetrance, the use of the MEK inhibitor for GBM is limited (62). A recent study suggested that oHSV therapy can significantly increase the blood-brain barrier penetration of trametinib and reverse the trametinib-mediated feedback reactivation of the MEK signaling (62). In addition, trametinib treatment decreases oHSV therapy-mediated inflammatory tumor necrosis factor-α secretion and enhances the T cell-dependent antitumor immunity (63). These findings suggest that more in-depth studies from different perspectives are required to support the application of oHSV in GBM treatment to provide additional benefits to patients with brain tumors.

Combining oHSVs with suppression of innate immunity. The antiviral immune response is the body's immunity against the virus, which can effectively resist infection and virus-mediated damage in the body (5). The prominent feature of tumor cells in immunology is the emergence of new antigen markers that

are not expressed by the corresponding normal cells, which trigger the immune system to eliminate these tumor cells (3). Antiviral and antitumor immune responses are important guarantees for the adaptation of the organism to changes and ensuring normal survival (10). oHSVs, with tumor selectivity and immunogenicity, are considered the ideal choice when combined with immunotherapy to improve the specificity and effectiveness of tumor treatment (64). When a virus infects the body, the activation of NK cells, macrophages and microglia can hinder viral replication and spread; this process has been recognized as a crucial defense mechanism against viruses (65). HSV can escape the host's immune response by various mechanisms, including inactivating immunoglobulin, inhibiting the production of cytokines/chemokines, blocking the maturation of antigen presenting cells, decreasing the expression of MHC class I in infected cells and inhibiting cytotoxic T lymphocyte mediated cell death (66). To prevent oHSV from replicating into normal cells and to adapt its function to the tumor microenvironment, a series of genes involved in host immune escape need to be deleted or mutated (6). Although an immunosuppressive state of the tumor microenvironment allows oHSVs to enter and replicate, the replication of oHSVs is not ideal (12). It is possible that inhibition of innate immune response can decrease the antiviral immunity and further ensure the replication of oHSVs in tumor cells. In addition, oHSVs in tumor cells can enhance the immunogenicity of tumor by promoting the infiltration of inflammatory cells (10). For example, G207 can induce systemic antitumor immunity by activating cytotoxic T lymphocytes (66). It has been reported that cyclophosphamide-induced inhibition of the systemic immune response and oxindole/imidazole-induced disruption of the STAT1/3-dependent oHSV barrier can substantially increase viral survival and propagation, eventually leading to neoplastic regression (67,68). In addition, transforming growth factor (TGF)- β can regulate innate immune responses by inhibiting the intracranial recruitment and activation of NK, macrophages and microglia (66). Han *et al* (69) demonstrated that combination therapy with TGF- β and oHSV significantly increases the survival of mice in both syngeneic and xenograft GBM models. Cellular communication network factor 1 (CCN1) plays a key role in coordinating the destruction and clearance of pathogens by enhancing the innate macrophage-mediated antiviral immune response (70). Anti-CCN1 antibody combined with oHSV exhibits a tendency to improve tumor control, which is associated with increased viral replication caused via suppression of the antiviral immune response (71). Several studies have suggested that innate immune cells, including NK cells, macrophages and microglia, not only contribute to the early elimination of oHSV, but also play an antitumor role in oncolytic therapy through different mechanisms of antitumor immune responses (10,66). However, the complex and apparently contradictory functions of combination therapies require careful thought and explanation. In addition, for all oHSV-based combination therapies, a series of problems caused by adverse reactions, long-term side effects and immune system dysregulation need to be resolved.

Increasing systemic delivery of oHSVs to tumor cells. Achieving systemic delivery of therapeutic viruses is the primary condition for the success of oHSV therapy. There are

some problems and defects that can limit the efficacy of oHSV delivery into tumor cells by either intratumoral or intravenous injection (71). The main reason for the poor efficacy of the direct injection into the resection cavity or into the tumor is that the cerebrospinal fluid and the secondary postoperative bleeding into the cavity may rinse out the injected virus (72). Systemic intravenous injection is considered the best method for clinical delivery of OV (71). There are still many obstacles that need to be addressed when trying to deliver OV specifically to tumors through intravenous injection, including poor penetration into tumors and rapid immune-mediated neutralization (73). The blood-brain barrier is the first barrier to intravenous injection of oHSV (74). To overcome this limitation, it has been reported that destroying the blood-brain barrier through a hypertonic mannitol solution and focused ultrasound can increase the number of viruses reaching the tumor site (73,74).

Cell carriers. To overcome the limitation of immune-mediated neutralization in the systemic delivery of OV, the use of cells as delivery vectors for OV seems to be a reliable method (75). Cell carriers can not only protect the virus from being neutralized by various immune cells in the blood, but also display inherent tumor tropism (75). Briefly, the OV is inserted into cell carriers, and when they target the tumor cells, OV is released to infect and destroy the tumor cells. Transformed cells, immune cells and stem cells can be used as delivery vehicles (75). Transformed cells were the first type of cell vectors used for oHSV delivery to tumors since they were more readily infected with OV compared with normal cells (76). The ability of transformed cells to locate specific anatomical and tumor sites is limited (75). In addition, the overall feasibility of immune cells is limited since they are expensive, and their clinical application is challenging. Factors released by mesenchymal stem cells (MSCs) are known to exert antitumor effects, which inhibits the proliferation of glioma cancer cells (77). In addition, MSCs have great potential for treating a variety of diseases associated with immune responses (77). It is highly attractive to use MSCs as carriers to extend the current therapeutic strategies. Several preclinical studies have demonstrated that stem cell-based cell carriers can deliver a variety of OV to tumors (75,78). Due to tumor specificity and given that different carrier cells may have different reactions with tumor cells, different cell carriers may be required to achieve tumor specific delivery (76). Although this approach is promising, there are still limitations and several obstacles for the therapeutic application of cell carriers, such as the cost, manufacturing and regulatory requirements (77,78).

Improving intratumoral delivery. The release of viral progeny from the killed solid cancer cells is an important mechanism for OV in destroying tumors (10). However, due to the uneven morphology and special physiological characteristics of tumors, the spread of chemotherapeutic drugs or OV between tumor cells is limited during the delivery phase (79). In addition to the transport of OV into the solid tumor, the method to improve the delivery of OV in solid tumor is another important factor for improving the therapeutic effect of OV (76). Neovascularization is considered as an important characteristic of tumors; however, these microvascular networks are abnormal and irregular (80). Previous studies have demonstrated that reconstructing the tumor vasculature and changing the tumor's pressure gradient

Table II. oHSV clinical trials for malignant glioma.

oHSV	Country	Stage	Target disease	Delivery	Trial number
HSV1716	UK	I	Recurrent high-grade glioma	Intratumoral/Peritumoral	NCT02031965
G207	US	I	Progressive and recurrent malignant glioma	Intratumoral injection	NCT00157703
G47Δ	Japan	II	Recurrent or residual glioblastoma	Intratumoral injection	UMIN000015995
rQNestin34.5	US	I	Recurrent high-grade glioma	Peritumoral	NCT03152318
C134	US	I	Recurrent high-grade glioma	Intratumoral injection	NCT03657576
M032	US	I	Progressive and current high-grade glioma	Intratumoral injection	NCT02062827

oHSV, oncolytic herpes simplex virus.

can improve perfusion, thus ameliorating drug penetration into the tumor (81,82). In addition, ultrasound and magnetic drug are also considered attractive and safe technologies (83).

3. oHSV clinical trials for GBM

Several clinical trials have assessed the effectiveness of oHSVs in treating a range of tumors, including GBM (84-86). HSV1716 is one of the ICP34.5 null mutants (13). The first study demonstrated the safety and feasibility of using a reproducible oHSV in human therapy (84). The results demonstrated that intratumoral injection of HSV1716 at doses of 10^3 - 10^5 plaque-forming units (pfu) for the treatment of nine cases of recurrent malignant glioma did not cause notable adverse reactions and reactivation of latent HSV (84). Furthermore, two separate phase I clinical trials completed by the same UK group revealed replication of HSV1716 without toxicity and supported the safety of delivering HSV1716 into the resection cavity (85,86). The most recent phase I trial of HSV1716 (NCT02031965) for recurrent high-grade glioma was terminated in 2016, and no report has been published (71).

G207 is another HSV-1 mutant with deletion of both copies of the γ 134.5 gene and lacZ insertion into the UL39 locus. Based on the promising results of preclinical trials, four phase I trials have been completed in the United States using G207 alone or in combination with radiation (87-90). The first trial commenced with 10^6 pfu dosage injected to a single enhancing location. This was completed with the 21st patient who had progressive or recurrent malignant gliomas despite being inoculated with standard therapy (3×10^9 pfu at five sites) (87). No toxic or serious adverse events, particularly HSV encephalitis, can be clearly attributed to G207, while radiological and neuropathologic evidence support the antitumor activity (87). In the following phase IB clinical trial, six patients with recurrent GBM each received two doses of G207, at a total dose of 1.15×10^9 pfu. This study demonstrated viral replication and antitumor activity of G207, and a good overall safety profile of multiple-dose administration of G207, including direct injection into the brain tissue surrounding the tumor resection cavity (88). The same group subsequently performed a phase I trial with G207, where nine patients with progressive and recurrent malignant glioma each inoculated with one dose of G207 at multiple sites of the tumor margin, followed by local treatment with 5 Gy

of radiation (NCT00157703) (89). This study supported the safety and potential clinical efficacy of combining G207 with radiation therapy for treating malignant glioma. Recently, this group further designed a phase I trial with continuous infusion of G207 via intratumoral catheters to treat progressive or recurrent malignant brain tumors (90). The results of this trial demonstrated that intratumoral catheter can be used as a potentially effective means of delivering OV, and that the frameless stereotactic technique was safe and feasible (90).

G47Δ, additional deletion of the ICP47 gene from G207, is currently the only third-generation oHSV to be evaluated in humans via a series of clinical trials completed in Japan (91). After the phase I-IIa clinical trial of G47Δ was completed in 2014, suggesting the safety of G47Δ injection into the human brain (UMIN000002661), a subsequent phase II clinical trial was performed in 2015 at the University of Tokyo to evaluate the efficacy of G47Δ in patients with recurrent or residual GBM. However, currently no results have been published (UMIN000015995) (92). These clinical trials adopt several strategies to improve efficacy, including engineering of novel oHSVs, developing combination therapies and improving the delivery efficiency of oHSVs into tumor cells. Thus, the combination of multiple strategies may remain the focus of follow-up research.

In addition to oHSVs, scientists from the Duke University completed another phase I clinical trial and announced promising results (93). According to this trial, 21% of a total of 61 patients with recurrent World Health Organization grade IV GBM, who were treated with intratumoral infusion of recombinant non-pathogenic polio-rhinovirus chimera, survived for >3 years (NCT01491893) (93). Several novel oHSVs, such as rQNestin34.5, C134 and M032, have exhibited encouraging antitumor efficacy and safety in the treatment of GBM in preclinical studies; thus, clinical trials or preparations for trials are underway. The clinical trials for treating malignant glioma with oHSVs are summarized in Table II.

4. Future perspectives

Due to the complex microenvironment and physiology of GBM, the development of highly effective and novel therapies for patients with GBM is difficult and slow. The continuous efforts and investigation of oHSV-based therapy in preclinical and clinical trials have provided valuable experiences and

optimistic results, offering hope for the successful clinical application of oHSVs for treating GBM. Initially, the main emphasis was on oHSV safety by means such as deletion of the ICP34.5 gene. Some novel oHSVs were evaluated for the treatment of GBM in clinical trials. In these trials, the use of these oHSVs in patients' brains was generally well tolerated, but no significant improvement in overall patient survival was observed. All OV's have the characteristics of the parental WT virus, and their overall antitumor efficacy is not ideal; thus, the development of novel viruses that are both safe and exhibit excellent antitumor effects, particularly in GBM, remains a top priority. Novel oHSVs with multigene mutations and armed with specific foreign genes require further investigation. In addition, given that the virus is easily neutralized by circulating antibodies when it enters the bloodstream, the most effective method for delivering oHSVs may not be intravenous infusion.

After penetrating tumor cells, the virus replicates and eventually triggers cell lysis, which releases new viral progeny that attack and kill neighboring cells (94). Tumor resection following viral inoculation is associated with a low virus replication rate that may indicate ineffective replication and limited transmission of oHSV in tumors, a limitation that can directly compromise its efficacy in GBM therapy (95). Modification of intratumoral physiology may solve the problem of poor tumor cell penetration. In addition, determining how to decrease antiviral immunity and allow the virus to induce stronger antitumor immunity deserves further investigation. Recently, additional oHSV-based treatment strategies, such as arming the virus with genes that encode proteins with therapeutic effects and combining oHSV with other agents, have been investigated (33,56). Thus, more clinical and preclinical trials are required to determine the best combination of multiple strategies to achieve unexpected results. Several challenges remain that require joint efforts in the clinical translation of oHSV therapy to improve the prognosis of patients with GBM.

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Authors' contributions

ZZ, JT, WZ, WX, YM, LC and JZ conceived and designed the present review. ZZ performed the literature review and drafted

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Competing interests

The authors declare that they have no competing interests.

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