Viral therapies for glioblastoma and high-grade gliomas in adults: a systematic review

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OBJECTIVE High-grade gliomas (HGGs) inevitably recur and progress despite resection and standard chemotherapies and radiation. Viral therapies have emerged as a theoretically favorable adjuvant modality that might overcome intrinsic factors of HGGs that confer treatment resistance.

METHODS The authors present the results of systematic searches of the MEDLINE and ClinicalTrials.gov databases that were performed for clinical trials published or registered up to July 15, 2020.

RESULTS Fifty-one completed clinical trials were identified that made use of a virus-based therapeutic strategy to treat HGG. The two main types of viral therapies were oncolytic viruses and viral vectors for gene therapy. Among clinical trials that met inclusion criteria, 20 related to oncolytic viruses and 31 to gene therapy trials. No oncolytic viruses have progressed to phase III clinical trial testing, although there have been many promising early-phase results and no reported cases of encephalitis or death due to viral therapy. Three phase III trials in which viral gene therapy was used have been completed but have not resulted in any FDA-approved therapy. Recent efforts in this area have been focused on the delivery of suicide genes such as herpes simplex virus thymidine kinase and cytosine deaminase.

CONCLUSIONS Decades of research efforts and an improving understanding of the immunomodulatory effects of viral therapies for gliomas are informing ongoing clinical efforts aimed at improving outcomes in patients with HGG. The available clinical data reveal varied efficacy among different virus-based treatment strategies.

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KEYWORDS glioblastoma; oncolytic viruses; oncolytic virotherapy; genetic therapy; immunomodulation

GLIOMASTOMA (GBM) is the most common primary brain malignancy¹ and is invariably fatal despite maximal safe resection, followed by adjuvant chemotherapy and radiation.² The median survival remains less than 15 months due to the infiltrative nature of these tumors (making complete resection impossible), sequestration behind the blood-brain barrier (BBB), immune-privileged status of the CNS, and eventual treatment resistance, thereby underscoring a need for novel treatments that extend survival.

Theoretically, viral therapies combat these characteristics through direct tumoricidal effects and generation of anticancer inflammatory responses that overcome the immunosuppressive high-grade glioma (HGG) and GBM microenvironments.³ The concept of virus therapies for cancer was established in 1912, when a woman's cervical cancer regressed after she received an attenuated rabies

vaccine;⁴ this connection between viral infection and cancer regression was anecdotally reported for several decades until a better understanding of viral genomics and engineering ushered in the modern era of viral therapies in the early 1990s. Martuza and colleagues engineered the first herpes simplex virus (HSV) mutants for oncolytic purposes in 1991,⁵ and these were shortly followed by an oncolytic adenovirus mutant in 1996.⁶ Since then, viral therapies have expanded to include dozens of different species and strains of viruses, which are broadly categorized into two overlapping strategies: replication-competent oncolytic viruses (OVs) and gene therapy viral vectors. Current clinical applications of these viruses are typically delivered into the tumor or resection cavity by stereotactic injection, free-hand injection, or convection-enhanced delivery (CED), bypassing the BBB.^{7–10}

OVs are replication-competent viruses that either are

ABBREVIATIONS Ad5 = human adenovirus serotype 5; BBB = blood-brain barrier; CAR = coxsackie and adenovirus receptor; CDA = cytosine deaminase; CED = convection-enhanced delivery; eIF2 α = eukaryotic initiation factor; GBM = glioblastoma; HGG = high-grade glioma; HSV = herpes simplex virus; HSV-tk = HSV thymidine kinase; MTD = maximum tolerated dose; OS = overall survival; OV = oncolytic virus; PFS = progression-free survival; PPE = preproendothelin.

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genetically engineered to selectively target tumor cells while sparing normal cells or are naturally onco-selective. Inside a tumor cell, the OV replicates, causing tumor cell lysis and subsequent release of viral progeny that may further infect surrounding tumor cells. Tumor cell lysis also releases tumor neoantigens that stimulate both the innate and adaptive immune system.^{11,12} This multiplication of the initial dose is unique to OVs among all forms of medical treatments and theoretically requires a lower inoculum compared to replication-incompetent vectors.

Alternatively, gene therapy viral vectors can be used to deliver genetic material specifically into tumor cells and to harness cellular transcription and translation machinery to produce desired proteins. These products may convert nontoxic prodrugs to toxic forms, or may function as immunomodulatory proteins and cytokines that activate the immune system against tumor cells.

Although early viral therapies fell squarely into the oncolytic or gene therapy categories, progress in viral technology has created numerous viruses that have characteristics of both groups to take advantage of the relative benefits of both strategies. OVs such as DNX-2440 and M032 transduce immune stimulatory genes (*OX40L* and *IL-12*, respectively) into infected tumor cells to promote antitumor immune responses in the tumor environment.¹³ The gene therapy vector Toca 511 is replication competent, allowing it to expand its tumoricidal reach beyond the initial injection dose and volume.^{14–16}

More than 2 decades of clinical trials with virus-based therapies have yielded promising early results. This systematic review was performed to provide a comprehensive overview of completed and ongoing clinical trials investigating viral therapies for HGG in adults.

Methods

Search Strategy and Study Selection

The systematic search of the MEDLINE database was performed on July 15, 2020. MeSH headings were queried to identify all clinical study citations indexed under “glioma” or “brain neoplasm” and including at least one of the following MeSH headings: “viruses,” “oncolytic viruses,” “oncolytic virotherapy,” “genetic therapy,” “vaccines,” or “immunomodulation.” Keyword searches were performed to identify any relevant studies that were not captured in the MeSH heading search due to incomplete or alternative MeSH indexing. Keywords used included “glioma,” “glioblastoma,” “viral therapy,” “oncolytic virus,” “gene therapy,” and “viral.” Publication types were limited to “clinical trial” or “clinical study” and their subtypes. Ongoing or completed studies without published results were identified on clinicaltrials.gov via keyword searches using the same keywords as above. Additional relevant studies were identified during the assessment of full texts and were added to the review.

Studies collected from the search were screened individually and included in the final review only if 1) the study investigated the administration of virus therapy to treat adult gliomas, and 2) the study was designed as a prospective clinical trial, using human subjects. Because treatment of GBMs and HGGs faces very similar chal-

lenges, and many clinical trials have included both patients with GBM and those with HGG, trials including patients with HGG were included in this literature review.

Data Collection

Data were collected from these studies individually by the investigators, with consensus regarding data reached by at least 3 authors. Variables collected from these studies included the following: type and dose of virus and drugs administered, experimental design and treatment groups, primary and secondary outcome measures, safety data, and survival outcomes.

Results

A total of 51 completed clinical trials investigating viral therapies for gliomas were identified using the search and selection algorithm¹⁷ in Fig. 1. Additionally, 16 ongoing trials were identified within published manuscripts or through searches on clinicaltrials.gov. Among clinical trials that met inclusion criteria, 20 were related to OVs and 31 to gene therapy trials. The accumulation of clinical trials on viral therapies for HGG over time is depicted in Fig. 2. Those viruses that share properties of both oncolytic and gene therapy vectors are discussed below in the category that they more closely resemble and/or alongside their respective siblings and predecessors.

Oncolytic Viruses

Currently, most available clinical data regarding OVs for GBMs and HGGs are from phase I trials; no trial has progressed to phase III. Most trials have been performed for recurrent or progressive rather than newly diagnosed HGG, except for one completed and two ongoing trials (ISRCTN70044565, NCT03072134). Serious adverse events such as encephalitis or death have not been reported. A comprehensive summary of completed and ongoing OV trials can be found in Tables 1 and 2, respectively.

HSV-1

HSV type 1 (HSV-1) is a large, double-stranded DNA human pathogen. It is perhaps the best studied of the OVs, given its neurotropism, safety profile, and genomic modifiability. The HSV-1 genes for neurovirulence and for oncolysis are distinct, allowing for genetic control that selects for oncolysis while eliminating untargeted nonspecific neurovirulence.⁷ The first iteration of HSV-1 investigated for glioma therapy involved a mutated HSV thymidine kinase (*HSV-tk*) gene, *dlstpK*, which attenuated neurovirulence but rendered the virus insensitive to acyclovir and ganciclovir, thus precluding its use in clinical trials.⁵ This early work, however, led to the development of HSV-1 mutants that exhibited reduced neurotoxicity while retaining their oncolytic ability. The first generation of clinical oncolytic HSV-1 mutants (HSV1716) had deletions of both copies of the viral $\gamma_134.5$ gene, which is essential for neurovirulence by dephosphorylating eukaryotic initiation factor (eIF2 α) and restoring protein synthesis. Later-generation HSV-1 mutants built on the $\gamma_134.5$ deletions by adding further mutations. For example, disruption

FIG. 1. PRISMA 2009 study selection flow diagram.

of *UL39* (which encodes ICP6, ribonucleic reductase) prevents reactivation of DNA replication machinery in post-mitotic cells (e.g., neurons)⁷ and increases viral sensitivity to acyclovir,¹⁹ while DNA replication persists in actively dividing tumor cells.

Three phase I trials investigating HSV1716 have been completed for 33 total patients with HGG, demonstrating the following: 1) feasibility of stereotactic intratumoral inoculation;²⁰ 2) viral replication in tumor cells;²¹ and 3) safety of peritumoral resection cavity injections.¹⁸ Treatment with virus was well tolerated without serious adverse events, and a survival of 14–25 months was observed in 7 patients (5 recurrent, 2 newly diagnosed). In one trial, 12 patients received intratumoral inoculation followed by tumor resection 4–9 days later. Assays for viral replication within tumor cells were positive at inoculation sites in 10 patients and at distal tumor sites in 4 patients.

G207 is a second-generation, $\gamma_134.5$ -deleted HSV-1 with inactivated *UL39*. The safety profile is improved by inactivation of the *UL39* gene. However, neurovirulence and replicative ability are further attenuated, thus requiring higher doses of G207 to reach infective and oncolytic levels similar to those of HSV1716. Three phase I trials have been conducted for 38 total patients and have demon-

strated the safety of intratumoral and peritumoral resection cavity injections, tolerability of 2 injections in a week, HSV replication in tumor cells, and synergistic effect of concurrent radiation.^{22–24} Similar to the HSV1716 trials, the maximum tolerated dose (MTD) was not achieved, with or without radiation the following day. Radiographic regression and pathological evidence of viral replication and antitumor activity were observed in the first two trials. Additionally, use of G207 in conjunction with radiation showed potential for clinical response, with a median overall survival (OS) of 7.5 months. Two trials using G207 for pediatric brain tumors are ongoing.

Additional strategies to improve oncolytic HSV-1 mutants currently under clinical investigation include the following: deletion of the *ICP47* gene to increase major histocompatibility complex class I antigen presentation and enhance viral replication (G47 Δ , JPRN-UMIN000002661, JPRN-UMIN000015995);^{25,26} transduction of the *IL-12* gene to help activate type 1 cell-mediated anticancer immune activity (M032, NCT02062827);¹³ and reinsertion of one copy of $\gamma_134.5$ under a nestin promoter to overcome host-cell hyperphosphorylation of eIF2 α , which leads to decreased protein synthesis and poor $\gamma_134.5$ -deleted virus growth (rQNestin34.5v.2, NCT03152318).^{27,28}

FIG. 2. Graph showing cumulative viral therapy trials for HGG over time. Curves plotted by publication year, or if completed but results not yet published, by study completion year. Additionally, ongoing trials that have not yet been published were plotted above or below the completed trials curves based on the start date listed on the official clinical trial registry record (clinicaltrials.gov or WHO International Clinical Trials Registry Platform). GT = gene therapy virus.

Adenovirus

Human adenovirus serotype 5 (Ad5) is a human pathogen that normally infects human cells using the coxsackie and adenovirus receptor (CAR), a step that is necessary for internalization and subsequent infectivity of the virus. However, CAR expression is markedly reduced on glioma tumor cells, and various genetic modifications that allow Ad5 to selectively infect tumor cells in a CAR-independent fashion have been studied. These “conditionally replicating adenoviruses” (CRAds) are attenuated by E1A and E1B gene deletions. DNX-2401 (previously Delta-24-RGD) has a 24–base pair deletion in the Ad5 E1A gene, which inhibits E1A protein binding to retinoblastoma protein. Thus, DNX-2401 selectively replicates in tumor cells that harbor retinoblastoma mutations. In a phase I dose-escalation trial for intratumoral injection of DNX-2401, 37 patients with recurrent HGG received either a single intratumoral inoculation (group A, $n = 25$) or intratumoral inoculation followed by resection and peritumoral injections (group B, $n = 12$). In group A, tumor reduction occurred in 18 patients, with a median OS of 9.5 months, and 5 patients survived more than 3 years. Group B had a median OS of 13.0 months, and 2 patients survived for 2 years.²⁹ Trials investigating CED of DNX-2401 (phase I/II, NCT00805376), and synergies of intratumoral DNX with temozolomide (phase I, NCT01956734) and with interferon-gamma (phase Ib, NCT02197169) have been completed, but results are not yet published. A phase II trial investigating intratumoral DNX-2401 followed by intravenous pembrolizumab (CAPTIVE/KEYNOTE-192, NCT02798406) and a phase I trial evaluating the feasibility and safety of intraarterial delivery of allogeneic

bone marrow–derived mesenchymal stem cells³⁰ loaded with DNX-2401 (NCT03896568) are ongoing. DNX-2440 is a newer virus based on DNX-2401, but additionally transduces the OX40 ligand (*OX40L*) gene. Expression of *OX40L* on tumor cell surfaces activates T cells and generates antitumor immune response. DNX-2440 is currently undergoing a phase I trial (NCT03714334).

Other OVs

Reoviruses, paramyxoviruses, parvoviruses, and poliovirus have all demonstrated safety without MTDs in phase I trials, of which some are currently in the phase II trial stage (Tables 1 and 2).

Gene Therapy

Viral vectors can deliver various types of cytotoxic and/or immunostimulatory genes to tumor cells, the most common anticancer strategy being “suicide” genes that express enzymes that convert a systemically delivered prodrug to active cytotoxic compounds.³¹ Summaries of completed and ongoing clinical trials in which viral gene therapy is being used are provided in Table 3 and Table 4, respectively.

Thymidine Kinase

The best-studied suicide gene is *HSV-tk*, the enzyme product of which converts systemically delivered ganciclovir or acyclovir to monophosphate forms, which are then converted to triphosphate forms by cellular kinases. The triphosphate forms inhibit DNA synthesis, resulting in S-phase delay, G2M-phase arrest, and subsequent initiation of tumor cell apoptosis.^{32,33}

The phase III GTI-115 study published in 2000 evaluated a retroviral vector delivering HSV-tk in newly diagnosed patients with HGG.³⁴ All patients received standard radiation after tumor resection. Additionally, half ($n = 124$) of the 248 patients were randomized to receive peritumoral resection cavity injections of retroviral vector–producing cells derived from murine fibroblasts at the time of tumor resection, followed by daily ganciclovir for 2 weeks starting on postoperative day 14. There were no differences in median progression-free survival (PFS) between the experimental and control groups (180 vs 183 days) or median OS (365 vs 354 days).

The ASPECT phase III trial (EudraCT 2004-000464-28) completed in 2008 used adenoviral vectors (sitimagene ceradenovec) expressing HSV-tk.³⁵ All 236 patients underwent resection, and 119 patients were randomized to receive peritumoral resection cavity injections of adenovirus, followed by daily intravenous ganciclovir for 14 days starting on postoperative day 5. Patients who received adenovirus demonstrated improvement in the primary outcome measure of composite of time to death or reintervention (corrected for temozolomide use) (308 vs 268 days), although there was no difference in the secondary endpoint, OS (497 vs 452 days). The investigators noted that increased adenovirus-neutralizing antibodies (3, 6, and 12 months after surgery) were associated with increased efficacy of treatment, potentially suggesting a bystander effect of adenovirus enhancement of an antitumor immune response.

TABLE 1. Completed HGG OV trials

Authors & Year	Trial Phase	Pathology	Virus	Delivery & Dosing	No. Treated (vs control)	Results	Median OS (mos)
HSV-1							
Harrow et al., 2004 ¹⁸	I	Recurrent HGG (4 GBM, 2 grade III) & newly diagnosed GBM (6)	HSV1716: ICP34.5-deleted HSV	Injection into resection cavity walls of up to 10 ⁶ PFU after resection	12 pts	No cases of HSV encephalitis, no Tx-related toxicities	3/12 pts alive 15–22 mos post-Tx
Papanastasiou et al., 2002 ²¹	I	Recurrent HGG (11 GBM, 1 grade III)	HSV1716: ICP34.5-deleted HSV	Stereotactic intratumoral injection of 10 ⁵ PFU	12 pts	No Tx-related AEs, no HSV encephalitis, histological evidence of viral replication in tumors	>7 mos
Rampling et al., 2000 ²⁰	I	Recurrent HGG	HSV1716: ICP34.5-deleted HSV	Stereotactic intratumoral injection of up to 10 ⁵ PFU	9 pts, dose escalation	No HSV encephalitis, no AEs, no reactivation of latent HSV	4/9 pts alive 14–24 mos post-Tx
Markert et al., 2014 ²⁴	I	Recurrent GBM	G207: ICP6-inactivated & ICP34.5-deleted HSV	Intratumoral injection before resection & injection into resection cavity walls totaling 1.15 × 10 ⁹ PFU, followed by 5 Gy focal radiation	9 pts	No DLT, MTD not achieved, evidence of antiangioma activity	7.5
Markert et al., 2009 ⁴⁰	Ib	Recurrent GBM	G207: ICP6-inactivated & ICP34.5-deleted HSV	Intratumoral injection before resection & injection into resection cavity walls totaling 1.15 × 10 ⁹ PFU	6 pts	1 pt w/ transient fever, delirium, hemiparesis, resolved w/ dexamethasone; no cases of HSV encephalitis, no Tx w/ acyclovir required; radiographic & neuropathological evidence of antitumor activity & viral replication	6.6
Markert et al., 2000 ²²	I	Recurrent HGG (16 GBM, 5 grade III)	G207: ICP6-inactivated & ICP34.5-deleted HSV	Stereotactic intratumoral injection of up to 3 × 10 ⁹ PFU	21 pts, dose escalation	No cases of HSV encephalitis, no serious AEs “unequivocally” Tx related	6.2
JPRN-UMIN000002661 ⁴¹	I/II	Recurrent GBM	G47Δ: ICP6-inactivated, ICP34.5-deleted, & ICP47-deleted HSV	Stereotactic intratumoral injection & repeat injection 5–14 days following initial inoculation	9 planned dose escalation; 12 planned at maximum safe dose	Completed but results not yet published	
Adenovirus							
Chiocca et al., 2004 ⁴²	I	Recurrent HGG (17 GBM, 7 grade III)	ONYX-015: E1B- & E3-deleted adenovirus	Intratumoral injection of up to 10 ¹⁰ PFU into area around resection cavity	24 pts dose escalation	No serious Tx-related AEs, MTD not achieved	6.2
Lang et al., 2018 ²⁹	I	Recurrent HGG	DNX-2401 (Delta-24-RGD): E1A-deleted adenovirus w/ RGD-4C expression	1) single intratumoral injection up to 3 × 10 ¹⁰ vp; 2) intratumoral injection through implanted catheter up to 3 × 10 ⁸ vp, followed by resection & injection around the resection cavity up to 3 × 10 ⁸ vp	25 w/ Tx only; 12 treat-resect-treat	No DLT, no MTD identified, 2/37 had Tx-related AEs (grade 1–2); tumor reduction in 72% of pts (group 2)	9.3 (group 1); 13.0 (group 2)

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TABLE 1. Completed HGG OV trials

Authors & Year	Trial Phase	Pathology	Virus	Delivery & Dosing	No. Treated (vs control)	Results	Median OS (mos)
<i>Adenovirus (continued)</i>							
NCT01582516	I/II	Recurrent GBM	DNX-2401 (Delta-24-RGD): E1A-deleted adenovirus w/ RGD-4C expression	Intratumoral CED of up to 10 ¹¹ vp	20 enrolled, dose escalation	Completed but results not yet published	
NCT01956734	I	Recurrent GBM	DNX-2401: E1A-deleted adenovirus w/ RGD-4C expression	Intraparenchymal injection of 3 × 10 ¹⁰ vp, followed by oral temozolomide 14 days after injection		Completed but results not yet published	
NCT02197169	Ib	Recurrent GBM	DNX-2401: E1A-deleted adenovirus w/ RGD-4C expression	Group 1: single intratumoral injection of virus Group 2: intratumoral injection of virus & interferon gamma	37 enrolled	Completed but results not yet published	
NCT03072134	I	Newly diagnosed HGG	NSC-CRAD-S-pk7: neural stem cells transfected w/ adenoviral vector expressing tumor-specific surviving promoter & a fiber protein polylysine modification (pk7)	Biopsy or resection followed by intratumoral or intraparenchymal injection of neural stem cells loaded w/ virus	13 enrolled	Completed but results not yet published	
<i>Reovirus</i>							
Samson et al., 2018 ⁴³	Ib	Recurrent HGG	Reolysin: reovirus	IV infusion of 10 ¹⁰ TCID ₅₀	6 pts	Reovirus RNA widely detected in tumor cells, increased levels of PD-1 & PD-L1 in treated pts	NR
Kicziński et al., 2014 ⁴⁴	I	Recurrent HGG	Reolysin: reovirus	Intratumoral CED over 72 hrs of up to 10 ¹⁰ TCID ₅₀	15 pts	No serious AEs related to Tx, 1 grade 3 AE (convulsions) possibly related to Tx; antiangioma activity seen in some pts	4.6
Forsyth et al., 2008 ²³	I	Recurrent HGG	Reolysin: reovirus	Stereotactic intratumoral injection of up to 10 ⁹ TCID ₅₀	12 pts dose escalation	No serious AEs, MTD not achieved	4.8
<i>Paramyxovirus</i>							
Freeman et al., 2006 ⁴⁵	I/II	Recurrent GBM	NDV-HUJ: lentogenic NDV	1) IV inpatient dose escalation to 11 BIU + 3 cycles of 55 BIU; 2) 3 cycles of IV dosing at 11 BIU + 2 doses of 11 BIU weekly	6 dose escalation; 8 constant dose	Minimal toxicity; MTD not achieved; 1 pt w/ complete response	7.3
NCT00390299	I	Recurrent HGG	MV-CEA: carcinoembryonic antigen-expressing measles virus	1) resection cavity injection up to 10 ⁷ TCID ₅₀ ; 2) biopsy followed by intratumoral catheter delivered virus up to 10 ⁷ TCID ₅₀ , followed by resection 4 days later & injection of up to 10 ⁷ TCID ₅₀	Arm 1: 10 pts; arm 2: 13 pts	No MTD or serious AEs	Arm 1: 11.8; arm 2: 11.4

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TABLE 1. Completed HGG OV trials

Authors & Year	Trial Phase	Pathology	Virus	Delivery & Dosing	No. Treated (vs control)	Results	Median OS (mos)
Parvovirus							
Geletneký et al., 2017 ⁴⁶	I/II	Recurrent GBM	Parvovirus01: H-1 parvovirus	1) intratumoral injection up to 5 × 10 ⁹ PFU followed by resection & injection around resection cavity; 2) IV infusions up to 10 ⁹ PFU for 5 days prior to resection & injection around resection cavity	12 w/ intratumoral injection, 6 w/ IV dosing	No dose-dependent AEs, no DLT, no MTD	15.3
Poliovirus							
Desjardins et al., 2018 ⁴⁷	I	Recurrent GBM	PVSR1PO: recombinant non-pathogenic polio-rhinovirus chimera	Intratumoral CED over 6.5 hrs of up to 10 ¹⁰ TCID ₅₀	61 pts	1 DLT (intracranial hemorrhage after catheter removal) at highest dose; 19% of pts had Tx-related AEs grade 3 or higher; OS at 24 mos improved compared to historical controls	12.5

AE = adverse event; BIU = 10⁹ 50% egg infectious dose; DLT = dose-limiting toxicity; IV = intravenous; NDV = Newcastle disease virus; NR = not reported; PFU = plaque-forming units; pts = patients; TCID₅₀ = 50% tissue culture infectious dose; Tx = treatment; vp = viral particles.

Two phase II trials using HSV-tk demonstrated promising results.^{36,37} The first (NCT00589875) enrolled 48 newly diagnosed patients with HGG and 134 matched standard-of-care controls at a separate institution. The 48 study patients received peritumoral resection cavity injections of an adenoviral vector expressing *HSV-tk* (aglatimagene besadenovec) at the time of tumor resection, followed postoperatively with valacyclovir.³⁶ Outcome measures that were significantly improved for the treatment group included median OS (17.1 vs 13.5 months) and median PFS (8.1 vs 6.5 months). Investigators noted that patients who underwent gross-total resection benefited most from the gene therapy, with median OS values of 25.0 and 16.9 months for those in the experimental and control groups, respectively. In contrast, there was no survival benefit for those receiving subtotal resection (13.5 vs 12.5 months), which investigators posited was due to the immunosuppressive capabilities of remaining tumor that may mask the effect of gene-mediated cytotoxicity or viral-mediated immune stimulation. The authors further suggested that the addition of checkpoint inhibitors may be important in overcoming this immunosuppression in patients receiving subtotal resection.

The other phase II trial (NCT00870181) randomized 43 patients with recurrent HGG to receive intraarterial cerebral injections of an adenoviral vector expressing HSV-tk, followed by ganciclovir in addition to standard of care (n = 22; 14 GBM, 8 WHO grade III) or by standard of care alone (n = 22; 18 GBM, 4 WHO grade III).³⁷ Patients receiving adenovirus had increased PFS at 6 months (54.5% vs 13.6%, respectively), median PFS (29.6 vs 8.4 weeks, respectively), and median OS (45.4 vs 14.3 weeks, respectively) compared to controls.

Cytosine Deaminase

Another gene therapy strategy involves delivery of cytosine deaminase (CDA), followed by systemic administration of 5-fluorocytosine (5-FC). In transduced cells, CDA converts nontoxic 5-fluorocytosine into toxic 5-fluorouracil (5-FU), resulting in tumor cell death. Toca 511 (vocimagene amiretrorepvec) is a replicating retroviral vector expressing CDA, which, in contrast to replication-deficient vectors, could infect tumor cells more extensively over time and increase expression levels of the CDA enzyme, and had shown promising early-phase results in recurrent HGG. In completed phase I dose-escalation trials, stereotactic administration of Toca 511 intratumorally (NCT01156584)¹⁵ and into peritumoral resection cavity walls (NCT01470794)¹⁶ demonstrated favorable safety and survival profiles (median OS 11.9–13.6 months). Durable and complete responses were noted in 6 of 53 patients receiving peritumoral injections. Despite promising early-phase results and a number of ongoing or unpublished phase I trials investigating Toca 511, the randomized, open-label phase III trial (NCT02414165) of 271 patients failed to meet its primary outcome of OS compared to standard of care (11.1 months vs 12.2 months, HR 1.06; p = 0.62) and demonstrated no significant difference in any secondary endpoints between the two arms.¹⁴ Full peer-reviewed phase III data have not yet been published, but the failure of the phase III trial effectively ended other investigation into Toca 511.

TABLE 2. Ongoing HGG OV trials

Identifier	Trial Phase	Pathology	Virus	Design	Primary Outcomes
HSV-1					
JPRN-UMIN000015995	II	Recurrent or residual GBM	G47A: ICP6-inactivated, ICP34.5-deleted, & ICP47-deleted HSV	Constant dose stereotactic intratumoral administration	Efficacy, 1-yr OS
NCT02062827 ¹³	I	Recurrent HGG	M032: oncolytic HSV-1 expressing <i>IL-12</i>	Dose escalation of intratumoral administration	Safety & tolerability
NCT03657576	I	Recurrent HGG	C134: γ 34.5-deleted, <i>hCMV-IRS1</i> -expressing chimeric HSV-1	Dose escalation of intratumoral administration	Safety & tolerability
NCT03152318	I	Recurrent HGG	rQNestin34.5v.2: replication-competent <i>ICP6</i> - & <i>ICP34.5</i> -deleted HSV-1 w/ <i>ICP34.5</i> gene reinserted under control of nestin promoter	Group 1: dose escalation of intratumoral administration Group 2: dose escalation of intratumoral administration w/ preop cyclophosphamide	Safety & tolerability
Adenovirus					
NCT02798406	II	Recurrent GBM	DNX-2401: <i>E1A</i> -deleted adenovirus w/ <i>RGD</i> -4C expression	Dose escalation of intratumoral delivery of virus w/ IV pembrolizumab	Objective response rate
NCT03896568	I	Recurrent HGG	DNX-2401: <i>E1A</i> -deleted adenovirus w/ <i>RGD</i> -4C expression	Group 1: dose escalation of intraarterial delivery of allogeneic bone marrow-derived human mesenchymal stem cells loaded w/ DNX-2401 Group 2: dose escalation of intraarterial administration pre- & postresection of tumor	MTD, local & systemic toxicity, capacity of Tx to target & deliver DNX-2401 to tumor
NCT03714334	I	Recurrent GBM	DNX-2440: oncolytic adenovirus expressing <i>OX40L</i> & <i>RGD</i> -4C	Dose escalation of stereotactic intratumoral delivery	Safety & tolerability
Reovirus					
ISRCTN70044565	I	Newly diagnosed GBM	Reolysin: reovirus	Dose escalation of IV administration of reovirus & GM-CSF	Safety & tolerability
Poliovirus					
NCT02986178	II	Recurrent GBM	PVSR1PO: recombinant nonpathogenic polio-rhinovirus chimera	Constant-dose intratumoral CED	24-mo OS

GM-CSF = granulocyte-macrophage colony-stimulating factor.

TABLE 3. Completed HGG gene therapy trials

Authors & Year	Trial Phase	Pathology	Intervention	No. Treated	Results	Median OS (mos)
HSV-tk						
Westphal et al., 2013 ³⁵	III	Newly diagnosed, untreated GBM	Adenoviral vector expressing HSV-tk	119 (vs 117 control)	Improved time to death or reintervention, but no improvement in OS	16.3
Rainov, 2000 ³⁴	III	Newly diagnosed, untreated GBM	Retroviral vector expressing HSV-tk	124 (vs 124 control)	No improvement in PFS or OS	12
Wheeler et al., 2016 ³⁶	II	Newly diagnosed HGG (43 GBM, 5 grade III)	Adenoviral vector expressing HSV-tk	48 (vs 134 external control)	Improved OS, w/ greatest improvement in those pts w/ minimal residual disease after resection	17.1 (25 mos in pts w/ GTR)
Ji et al., 2016 ³⁷	II	Recurrent HGG (32 GBM, 12 grade III)	Intraarterial cerebral infusion of adenoviral vector expressing HSV-tk	27 (vs 26 control)	Improved PFS at 6 mos, PFS, & OS	10.5
Immonen et al., 2004 ⁴⁸	II	Recurrent GBM	Adenoviral vector expressing HSV-tk	17 (vs 19 control & 36 historical controls)	Improved time to death or reop, & in OS	14.4
Colombo et al., 2005 ⁴⁹	I/II	Recurrent GBM	Retroviral vector expressing HSV-tk & IL-2	7 stereotactic injection; 4 open resection & injection	No serious AEs; evidence for tumor transduction as well as local & systemic Th1 cell cytokine elevation	7.5
Prados et al., 2003 ⁵⁰	I/II	Recurrent GBM	Retroviral vector expressing HSV-tk	30	16/30 pts w/ serious AEs	8.4
Voges et al., 2003 ⁵¹	I/II	Recurrent GBM	Liposomal vector bearing HSV-tk	8	No serious AEs; significant Tx responses in 2/8 pts	6.5
Shand et al., 1999 ⁵²	I/II	Recurrent GBM	Retroviral vector expressing HSV-tk	28	No serious AEs	8.6
Klatzmann et al., 1998 ⁵³	I/II	Recurrent GBM	Retroviral vector expressing HSV-tk	12	No serious AEs; significant Tx response in 4/12 pts & complete response in 1	6.8
Ram et al., 1997 ⁵⁴	I/II	Recurrent HGG (9 GBM, 2 grade III)	Retroviral vector expressing HSV-tk	11	Limited gene transfer detected, responses only in small tumors	NR
Chiocca et al., 2019 ⁵⁵	I	Recurrent HGG (28 GBM, 3 grade III astrocytoma)	Ad-RTS-hil-12: adenoviral vector expressing hil-12, w/ pre- & postop administration of oral veledimex (oral activator of IL-12)	31	No dose-limiting toxicities; cytokine release syndrome severity & frequency correlated w/ veledimex dose & reversed w/ discontinuation	12.7 (in group receiving 20 mg veledimex, determined to be superior dose)
Chiocca et al., 2011 ⁵⁶	I	Newly diagnosed GBM	Adenoviral vector expressing HSV-tk	13	No dose-limiting toxicities, 2 serious AEs possibly related to Tx	12.4
Chiocca et al., 2008 ⁵⁷	I	Recurrent HGG (10 GBM, 1 grade III)	Adenoviral vector expressing human interferon beta	11	1/11 w/ dose-limiting toxicity; Tx at highest doses was associated w/ tumor cell apoptosis & necrosis	4.1
Cornetta et al., 2006 ⁵⁸	I	Recurrent & newly diagnosed HGG (7 GBM, 2 pontine glioma, 1 grade III)	Retroviral vector expressing MGMT	10	No serious AEs	14.8
Lang et al., 2003 ⁵⁹	I	Recurrent HGG	Adenoviral vector expressing p53	15	No dose-limiting toxicity; transduction of p53 was successful but only w/in a short distance (5 mm) from the injection site	9.9
Germano et al., 2003 ⁶⁰	I	Recurrent HGG	Adenoviral vector expressing HSV-tk	11	No serious AEs or dose-limiting toxicities	25.9

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TABLE 3. Completed HGG gene therapy trials

Authors & Year	Trial Phase	Pathology	Intervention	No. Treated	Results	Median OS (mos)
<i>HSV-tk (continued)</i>						
Smitt et al., 2003 ⁶¹	I	Recurrent HGG	Adenoviral vector expressing HSV-tk	14	No serious AEs or dose-limiting toxicities	4
Sandmair et al., 2000 ⁶²	I	Recurrent (9 GBM, 4 grade III) & newly diagnosed (9 GBM) HGG	Adenoviral vector expressing HSV-tk or retroviral vector expressing HSV-tk	8 retrovirus; 7 adenovirus; 7 control	4/7 pts receiving adenovirus had significant increase in anti-adenovirus antibodies, 2/14 pts w/ increased seizure frequency, otherwise no AEs possibly related to gene therapy	7.4 (retrovirus), 15.0 (adenovirus)
Trask et al., 2000 ⁶³	I	Recurrent HGG (10 GBM, 3 grade III)	Adenoviral vector expressing HSV-tk	13	CNS toxicities (confusion, hyponatremia, seizures) observed at highest dose (2×10^{12} vp, 2 pts); no evidence of Tx-related toxicity in second highest dose (2×10^{11} vp)	9.4
Qureshi et al., 2000 ⁶⁴	I	Recurrent HGG	Retroviral vector expressing HSV-tk	6	No AEs related to Tx	NR
Palù et al., 1999 ⁶⁵	I	Recurrent GBM	Retroviral vector expressing HSV-tk & IL-2	4	No AEs related to Tx	9.3
Colombo et al., 1997 ⁶⁶	I	Recurrent GBM	Retroviral vector expressing HSV-tk & IL-2	4	No AEs related to Tx	NR
Puumalainen et al., 1998 ⁶⁷	I	HGG	Retroviral & adenoviral vectors expressing beta-galactosidase	10 (2 control)	Gene transfer successful in all pts w/o AEs; adenoviruses were more efficient than retroviruses	NR
<i>CDA</i>						
Cloughesy et al., 2019 ¹⁴	II/III	Recurrent HGG	Toca 511: retroviral replicating vector expressing CDA	271	No significant difference in OS btwn pts receiving Toca 511 & standard of care alone; no difference in secondary endpoints	11.1 (Toca 511 arm) vs 12.2 (standard of care)
Cloughesy et al., 2018 ¹⁶	I	Recurrent HGG (46 GBM, 10 grade III)	Toca 511: retroviral replicating vector expressing CDA	56	6/53 pts w/ complete, durable responses (5/23 pts receiving phase III eligible doses w/ complete, durable responses); improved OS	11.9 (14.4 mos in phase III eligible subgroup)
Cloughesy et al., 2016 ¹⁵	I	Recurrent HGG (37 GBM, 8 grade III)	Toca 511: retroviral replicating vector expressing CDA	45 (84 external controls treated w/ lomustine)	1/27 serious AE related to Toca 511; successful transduction of tumor cells; increased OS compared to control	13.6
NCT01985256	I	Recurrent HGG	Toca 511: retroviral replicating vector expressing CDA	17	Completed but results not yet published	
NCT02576665	I	Recurrent HGG, brain metastases, or lymphoma	Toca 511: retroviral replicating vector expressing CDA	21	Completed but results not yet published	
<i>Anti-angiogenic virus</i>						
Cloughesy et al., 2018 ³⁹	III	Recurrent GBM	VB-111: adenoviral vector expressing <i>PPE</i> & <i>Fas-TNFR1</i> chimera	256	No improvement in OS w/ combination VB-111 & bevacizumab over bevacizumab alone	6.8
Brenner et al., 2016 ³⁸	II	Recurrent GBM	VB-111: adenoviral vector expressing <i>PPE</i> & <i>Fas-TNFR1</i> chimera	46	Improved OS w/ combination VB-111 & bevacizumab over historical controls of bevacizumab alone	13.6

GTR = gross-total resection.

TABLE 4. Ongoing HGG gene therapy trials

Identifier	Trial Phase	Pathology	Virus	Design
NCT02598011	Ib	Newly diagnosed HGG	Toca 511: retroviral replicating vector expressing <i>CDA</i>	Peritumoral resection cavity injections of Toca 511 w/ dose escalation of Toca FC, in combination w/ standard-of-care chemoradiation & temozolomide
NCT01811992	I	Newly diagnosed HGG	Ad-hCMV-tk & Ad-hCMV-Flt3L: adenoviral vectors delivering <i>HSV1-tk</i> & Flt3L	Dose escalation of peritumoral resection cavity injections
NCT03679754	I	Recurrent GBM	Ad-RTS-hil-12: adenoviral vector expressing <i>hil-12</i>	Peritumoral resection cavity injections, w/ oral veledimex (expansion substudy of NCT02026271)
NCT03636477	I	Recurrent GBM	Ad-RTS-hil-12: adenoviral vector expressing <i>hil-12</i>	Peritumoral resection cavity injections, w/ nivolumab & oral veledimex (expansion substudy of NCT02026271)
NCT00031083	I	Recurrent HGG	BG00001: adenoviral vector expressing interferon beta	Dose escalation of intratumoral injection (study suspended)
NCT03294486	I/IIa	Recurrent GBM	TG6002: <i>TK</i> - & <i>RR</i> (ribonucleotide reductase)-deleted vaccinia viral vector expressing <i>FCU1</i>	Phase I: dose escalation of IV administration; phase IIa: IV administration at dose recommended based on phase I results
NCT04406272	II	Recurrent GBM	VB-111: adenoviral vector expressing <i>PPE</i> & <i>Fas-TNFR1</i> chimera	Experimental arm A: IV VB-111 before & after resection; experimental arm B: IV VB-111 after resection; control arm: placebo & standard-of-care resection

Angiogenesis

VB-111 is a nonreplicating, E1-deleted adenoviral vector expressing a modified murine preproendothelin (PPE) promoter and a proapoptotic Fas-chimera transgene composed of tumor necrosis factor receptor-1 and the intracellular signaling domain of Fas. This construct provides strong cell death signaling with targeting specificity to angiogenic vessels in tumor tissue. Phase II data (NCT01260506)³⁸ demonstrated that in patients with recurrent GBM, those who received VB-111 both prior to and following further progression and who also received concurrent bevacizumab after further progression (i.e., they had been primed with VB-111 for bevacizumab treatment) had significantly increased PFS (90 days) and OS (414 days) compared to those in the other two groups. The other groups were composed of patients who did not receive VB-111 concurrently with bevacizumab and patients who did receive VB-111 concurrently with bevacizumab but not prior to bevacizumab therapy (PFS 60 vs 63 days, respectively; OS 223 vs 142 days, respectively). However, the phase III trial did not include the priming group that demonstrated improved PFS and OS in the phase II trial. Thus, the phase III trial failed to demonstrate survival benefit of VB-111 in conjunction with bevacizumab over bevacizumab alone (NCT02511405).³⁹

Discussion

The future of virus-based therapies for GBM will probably see progression of the viral strategies discussed here into later-phase trials, and newer oncolytic and gene therapy strategies entering the early-phase clinical trial pipeline. Since the first clinical trial examining viruses for HGG treatment published more than 2 decades ago, the number of virus candidates and studies has drastically increased. As viral technology progresses, more viruses are being created that share attributes and capabilities of previously

strictly oncolytic or gene therapy viruses. OV loaded with genes such as *OX40L* and *IL-12*¹³ augment and expand the antitumor response already generated by tumor cell death. Replication-competent gene therapy vectors carrying suicide genes^{14–16} spread beyond their injection volume and provide an effective dose higher than their initial inoculum. Although there are many approaches to viral therapy development, those harnessing the full potential of virus replication and viral gene delivery together may overcome the challenges that either strategy faces alone (such as limitations in virus spread and immune sequestration and suppression by tumor cells).

Owing to much of the early research, viral therapies are increasingly recognized as well tolerated with few adverse side effects. In the majority of early-phase trials MTDs were not achieved, and when toxicities were noted, most were related to the underlying disease, such as headache, seizure, or progressive neurological deficit. Potential systemic responses to viral infection, such as fever and malaise, were mild and resolved spontaneously. Additionally, studies in which virus was injected into the “normal” brain parenchyma surrounding the resection cavities did not show an increased rate of adverse events or toxicities such as cerebritis, suggesting that nonspecific viral infection of normal brain parenchyma or direct toxicity of the injection is not a frequent event.

Although several delivery methods have been studied, no single method has been standardized. Intratumoral injection and peritumoral resection cavity injections are the simplest and most studied routes of administration, offer direct inoculation of the affected tissue, and are well tolerated. However, they are constrained by their invasive nature, single dose, limited penetration and distribution of virus (especially with replication-deficient viruses), potential for reflux out along the injection tract, and (in the case of free-hand and/or peritumoral injections) lack of unifor-

mity of administration. CED allows for continuous infusion of virus and a theoretically increased volume of tissue covered, but remains limited by its invasive technique. CED is a mechanism of interest to both neurosurgeons and engineers, and optimization of current CED components is subject to ongoing investigation. Systemic administration of virus could bypass the need for neurosurgical intervention but faces the challenges of crossing the BBB and possible destruction by the systemic immune system, for which cell-based delivery of virus is being developed. Replication-competent virus strategies may have more applications given typical single-dosing parameters.

Over the course of these studies, investigators have gained increased understanding of the immune response to virus administration that leads to additional antitumor effects. HGGs produce highly immunosuppressive tumor microenvironments, with high levels of transforming growth factor- β , IL-10, and prostaglandin E2, and these lesions recruit immunosuppressive T-regulatory lymphocytes and M2-phenotype macrophages. Many studies have shown that viral infection of the tumor generates local and systemic inflammatory responses, despite the immunosuppressed nature of patients with HGG, as evidenced by increased numbers of local T lymphocytes, natural killer cells, macrophages, and circulating Th1 cell-related cytokines. Additionally, most of these studies have been performed in patients with recurrent HGG, who already had received systemic therapies that further immunosuppression, such as chemotherapies, radiation, and corticosteroids. Future studies must clarify the relationship between the type of virus administered and immune response generated, and whether there are optimal combinations for certain tumors or patients.

Finally, although checkpoint inhibitors have been effective in the management of melanoma and other immunogenic malignancies, preliminary studies have not shown a definitive benefit of checkpoint inhibitors alone for primary brain malignancies. Recent preclinical studies in HGG have shown that viral therapies may augment the response to checkpoint inhibition. Thus, the combined use of viral therapy and checkpoint inhibitors is of particular interest for continued clinical investigation.

Conclusions

Viral therapies for HGG have been subject to trials for more than 2 decades, without a novel standard-of-care treatment. However, recent work with oncolytic and gene therapy viruses has benefited from improved understanding of synergistic effects with standard chemotherapies, radiation treatments, and immunomodulatory therapies. A number of ongoing trials continue the push toward finding a broadly applicable and efficient therapy for patients with HGG.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Wang. Acquisition of data: all authors. Analysis and interpretation of data: all authors. Drafting the article: all authors. Critically revising the article: all authors. Reviewed submitted version of manuscript: all authors. Approved the final version of the manuscript on behalf of all authors: Wang. Administrative/technical/material support: Elder. Study supervision: Wang, Elder.

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