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REVIEW

Oncolytic virus in gliomas: a review of human clinical investigations

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Gliomas remain one of the more frustrating targets for oncologic therapy. Glioma resistance to conventional therapeutics is a product of their immune-privileged milieu behind the blood-brain barrier, in addition to their suppressive effect on the immune response itself. Taking the lead from the growing success of immunotherapy for systemic cancers, such as lung cancer and melanoma, immunotherapeutics has emerged as a major player in the potential treatment of gliomas, with oncolytic viruses in particular showing significant promise as evidenced by the recent Breakthrough and Fast Tract Designations for PVSRIPO and DNX2401. This review serves as a useful and updated compendium of the completed human clinical investigations for several oncolytic viruses in the treatment of gliomas.

Key words: glioma, oncolytic virus, immunotherapy

INTRODUCTION

The goal of improving quality of life and prognosis for glioma patients remains the most pressing challenge in neuro-oncology. Standard of care for glioblastoma multiforme (GBM), the most aggressive form of glioma (WHO grade IV), was established in 2005 and includes surgical resection, radiation therapy, and chemotherapy. This regimen, known as the Stupp protocol, has yet to be significantly updated despite median overall survival (OS) of only 12-16 months, reflecting the exceptional resilience of GBM to current treatments.[1] Tumor treating fields (TTF) was approved in 2011 for recurrent GBM and in 2015 for newly diagnosed GBM. This antimitotic therapy, when added to the Stupp protocol, has shown an OS benefit of 5 months.[2] There has also been a proliferation of genetic markers in GBM that can indicate a more favorable prognosis, such as mutations in IDH1/2 and MGMT promoter methylation, but these have not yet led to targeted medical therapy.[3] GBMs remain among the most challenging oncologic entities to treat in the human body, as recent progress in extending median survival has been incremental over the last 5-10 years.

One promising area of therapeutic innovation for GBM has been immunotherapy. Taking the lead from the success of immunotherapy in other cancers, clinical trials for GBM treatment have increasingly incorporated immunotherapeutic strategies such as replication competent oncolytic viruses (OVs).[4-8] Viruses have also been used as vectors for gene therapy; however, this is outside the scope of this article. The mechanism of action of OVs was once thought to be limited to direct oncolysis of cancer cells. In fact, these viruses also have a significant immunomodulatory effect that boosts the immune system antitumor response.[4,5,9,10] To date, the only Food and Drug Administration (FDA)-approved oncolytic viral therapy is talimogene laherparepvec (T-Vec), which was approved in 2015 for metastatic melanoma.[9]

OVs for GBM have shown promise in early-stage trials as highlighted by recent FDA Fast Track Designation for two viruses, PVS-RIPO and DNX-2401, attenuated poliovirus and adenovirus, respectively.[11-13] Clinical trials have been limited to early stage and have not yet fulfilled the potential of their preclinical investigations.[14] OVs are particularly suited for combination therapy with immune checkpoint inhibitors (ICIs) resulting from their immunostimulatory effects. Recent trials have begun to incorporate such combinations.[10,15,16]

Numerous viruses like Newcastle disease virus (NDV), herpes simplex virus (HSV), reovirus, parvovirus, adenoviruses, and poliovirus have undergone clinical investigation for brain tumors as summarized in Table 1 and Figure 1. The goal of this review is to provide the most up-to-date compendium of existing data on OVs in glioma patients. Our

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Table 1. Summary of viruses used in clinical investigations to treat malignant gliomas

Viruses used in clinical investigation to treat malignant gliomas	Features	Natural host	Refs
Newcastle disease virus	Single-stranded, linear, RNA	Avian	8,14,18-20
Reovirus	Double-stranded, linear, DNA	Human	10,14,51
Parvovirus	Single-stranded, linear, DNA	Rat	14,59
Adenovirus	Double-stranded, linear DNA	Human	54,56,81
Poliovirus	Single-stranded, linear RNA	Human	45
Vaccinia virus	Double-stranded, linear DNA	Human	14
Herpes simples virus	Double-stranded, linear DNA	Human	15,35-38,43

hope is that neuro-oncologists and medical oncologists treating gliomas can use this review as a practical reference for understanding where the field currently stands with OV therapy.

Completed clinical trials

NDV. NDV is a spherical paramyxovirus with an avian natural host.[17] Its mechanism is via selective lysis of cancer cells and promotion of an antitumor inflammatory response. To date, there have been two types of NDV that have been used in glioma treatment: MTH-68/H, a mesogenic strain, and NDV-HUJ, a lentogenic strain[8,17-20] (Table 2). The safety and oncolytic effects of NDV are based on conditional replication in cancer cells and not in normal cells, which was demonstrated in 1988 and again in 2006.[21,22] Likewise, Lorence et al.[23] in 2007 proposed that cancer cells are more sensitive to NDV infection because cancer cells are generally defective in interferon (IFN) responses compared with a normal equivalent cell. Like MTH-68/H, NDV-HUJ also has been reported to rely on induction of apoptosis in glioma.[18] The mechanism for tumor regression after NDV has yet to be fully elucidated, though several pathways have been proposed: induction of apoptosis, direct tumor lysis, enhanced tumor-specific immune response, and cytokine release.[18,24] Cytokines such as tumor necrosis factor (TNF), IFNs, interleukin-6, and interleukin-10 have been recognized to enhance tumor immunity.[24] A more recent molecular pathway analysis describes NDV activating intrinsic death pathway, eIF2a kinase protein kinase R-like endoplasmic reticulum kinase and caspase 12, and TNF-related apoptosis inducing ligand.[17]

The MTH-68/H strain was developed from the Hertfordshire NDV strain and was first used in metastatic carcinoma in 1968.[25] Trials for central nervous system (CNS) tumors began in 1999.[8] In all three reports, MTH-68/H is administered intravenously (i.v.) in adult and pediatric patients with recurrent GBM and anaplastic astrocytoma (AA) refractory to surgery, radiation therapy, and chemotherapy and found to have a median OS of 3-5 years.[8,19,20] Csatary and Bakács[8] and Csatary et al.[20] reported two case series in 1999 and 2004 where adult and pediatric patients experienced survival of 3 years and 5-9 years, respectively, with MTH-68/H as their only form of therapy. The 2004 report also commented on increased efficacy with the i.v. dosing as compared with inhalational administration.[20] The group reported increased clinical efficacy with increased i.v. dosing and decreased frequency of administration. Wagner et al.[19] in 2006 later reported a case of a thalamic AA in a boy treated with i.v. and/or inhalational MTH-68/H and oral valproic acid. The tumor shrank to 15% of its original size, yet soon recurred in the 4th ventricle requiring surgery. This was the first study that demonstrated abundant

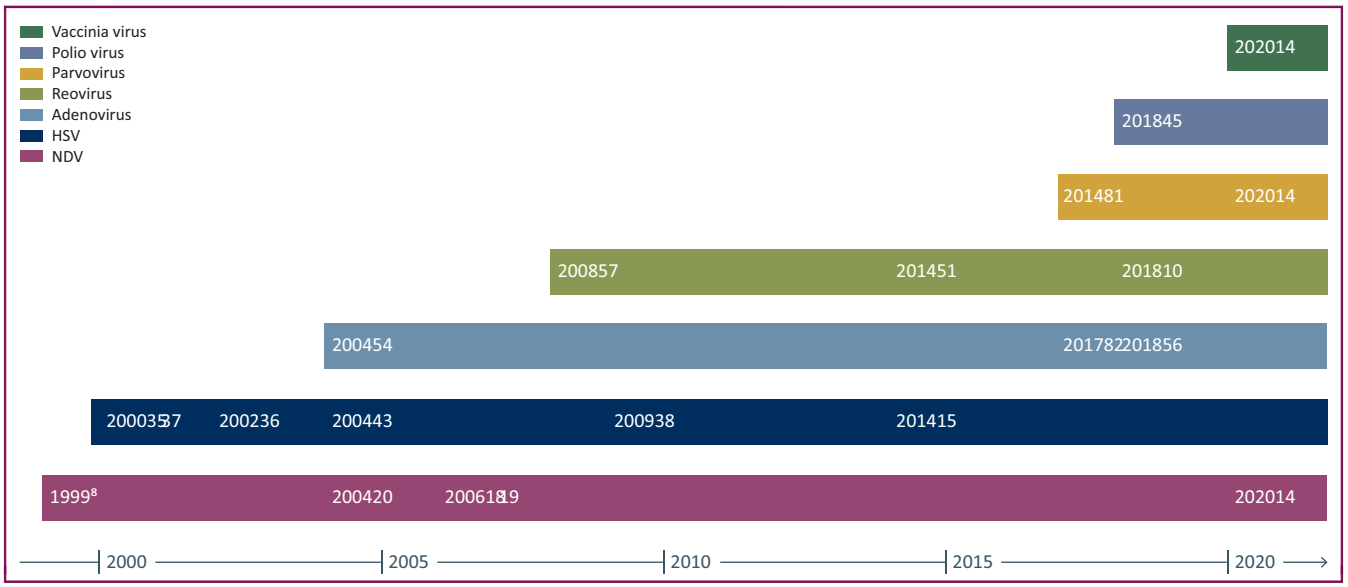

Figure 1. Clinical investigations of oncolytic viruses in gliomas by date.
HSV, herpes simplex virus; NDV, Newcastle disease virus.

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Table 2. Newcastle disease virus trials in glioma

Author and year	Patients (*n*)	Tumor type	Virus type	Virus features	Virus name	Trial design	Administration	Dose(s)	Previous treatment	Survival/outcome	Adverse events
Csatary and Bakács 1999[8]	3	Recurrent GBM	NDV	Passaged and purified from the NDV Hertfordshire strain	MTH-68/H	Case series	I.V.	10^7 up to four times per day for several weeks	S: 3 RT: 3 CT: 3	3 Years 24 Months 22 Months 'All live at publication date'	None reported
Csatary et al. 2004[20]	14	High-grade glioma	NDV	Passaged and purified from the NDV Hertfordshire strain	MTH-68/H	Case series	I.V.	2×10^7–8×10^7 daily	S: 4 RT: 4 CT: 4 'Data provided only for four patients'	Deaths: 5 from tumor progression, 2 from unrelated cause Alive: seven patients alive with four patients alive at 5-9 years	None reported
Freeman et al. 2006[18]	14	Recurrent GBM	NDV	Attenuated lentogenic strain	NDV-HUJ	Phase I/II single-center, open label. two parts 1. Six-step dose escalation. Three patients completed part I dosing. 2. Constant dosing three patients completed part II dosing.	I.V.	Part 1: 0.1, 0.32, 0.93, 5.9, 11, 55 BIU Part 2: 11 BIU constant	S: 10 Biopsy: 4 RT: 14 CT: 12	Survival ranged from 3 to 66 weeks. Time to radiological progression 2-37 weeks. One patient achieved a complete response.	Adverse events are unrelated to virotherapy. 1. Influenza-like illness 2. Injection site adverse event 3. Infusion reactions Adverse events were reduced with priming dosing and slow infusion rates
Wagner et al. 2006[19]	1	Anaplastic astrocytoma	NDV	Passaged and purified from the NDV Hertfordshire strain	MTH-68/H	Case report	I.V. and or inhalational	4×10^8 pfu 1500 mg valproate	S: 1 RT: 1 CT: 1	15 Months	None attributed to virotherapy
Gesundheit et al. 2020[14]	4	GBM	Combination therapy: NDV wild-type Parvovirus wild-type Vaccinia virus wild-type	Unmodified viruses	NDV, parvovirus, vaccinia virus	Case series	I.A. port at the carotid artery and I.V.	Viruses injected into patients at intervals of 2-3 weeks, administered by sequential 10 ml injections via the same catheter.	S: 4 RT: 4 CT: 4	14.5 Years, alive 6 Years, dead 8 Years, alive 4 Years, alive	Complications related to i.a. port.

AA, anaplastic astrocytoma; CT, chemotherapy; GBM, glioblastoma multiforme; I.A., intra-arterial; I.V., intravenous; NDV, Newcastle disease virus; OA, oligoastrocytoma; OS, overall survival; RT, radiation therapy; S, surgery; WT, wild-type.

accumulation of apoptotic tumor cell nuclei in a histological analysis after 5 months of continuous NDV treatment. They also first demonstrated virus replication within the tumor by the presence of NDV-like particles in the neoplastic cells. In all three studies with MTH-68/H, no adverse events due to virus administration were reported. It is unclear why additional trials were not conducted, despite these encouraging results.

NDV-HJU is also under investigation.[18] In 2006, Freeman et al.[18] reported a phase I/II dose escalation study. I.V. administration of NDV-HUJ was used in 14 patients aged 11-58 years with recurrent GBM refractory to surgery, radiation therapy, and chemotherapy. In this case series, one patient achieved a complete response with adverse events limited to grade 1/2 constitutional fevers.[18] Given that the i.v. administration was well tolerated with encouraging results, NDV-JHU warrants continued investigation for GBM.

Currently, there are no active clinical trials using NDV though there has been promising basic science research demonstrating its utility in both *in vivo* and *in vitro* models.[26-28]

HSV. HSVs are perhaps the most widely characterized OVs with six completed clinical reports in gliomas to date (Table 3). HSV viruses remain leading candidates in glioma treatment given the recent success and FDA approval of T-Vec in 2015 for melanoma. As is the case with NDV, the antitumor effect of oncolytic HSV is twofold: direct cytolysis followed by recruitment of an immune response. As mentioned, the genetic alterations allow conditional replication of oncolytic HSVs. This allows preferential viral replication in tumor cells and subsequent lysis further propagating local spread of the virus. Viral entry relies on one of three classes of membrane receptors.[29,30] Once tumor cells are lysed, there is a release of tumor-associated antigens which leads to induction of local and systemic antitumor immunity.[31] The success of viral propagation depends on a delicate balance between the hosts antiviral response versus its antitumor response. Oncolytic HSV administered in the brain induces immediate recruitment and activation of natural killer cells, macrophages, and microglia, which account for viral clearance and blunting of the antitumor efficacy of oncolytic HSV.[32] There is now evidence that antiviral responses also contribute to antitumor efficacy despite slowing viral replication as summarized in Figure 2.[33] As oncolytic HSV triggers an innate immune response, adaptive immune responses have also been characterized.[32] The immunogenic death of infected cells releases pathogen-associated molecular patterns and damage-associated molecular patterns that could facilitate dendritic cell presentation to T cells.[32] Efficacy of cellular immunity is often limited due to the immunologically suppressive microenvironment of grade III and IV gliomas. Numerous studies are underway addressing these challenges.

Martuza et al.[34] in 1991 provided the first report of a recombinant HSV specifically targeting the U87 human GBM cell line and demonstrated attenuating neurovirulence in non-dividing cells. Currently, there are two recombinant HSVs that have undergone clinical investigation, as summarized in Table 3: HSV1716 and HSVG207.[15,35-38]

HSV1716 is a selectively replication-competent mutant HSV that lacks both copies of the RL1 gene, which encodes the protein ICP34.5 and is a factor for neurovirulence.[35,39] It is avirulent in normal brains, having been demonstrated in animal models.[40,41] It fails to replicate in normal tissue but demonstrates lytic replication in human GBM cells *in vitro*.[42] As a result, Rampling et al.[35] reported a case series in 2000 with nine patients (eight with GBM and one with AA) treated with intratumoral (i.t.) HSV1716 injection. Four of the nine patients were alive at 14-24 months after treatment without significant adverse events. While OS was only slightly longer than standard of care, the authors concluded that HSV1716 is a safe and feasible option for continued study. A follow-up study was then carried out in 2002 with 12 GBM patients who underwent viral injection of HSV1716 followed by surgery.[36] This study showed the presence of HSV DNA by PCR at the inoculation sites in 10 patients and at distal tumor sites in 4. Again, they showed HSV1716 could be administered safely and that it replicates in GBM. A third trial was then completed in 2004 with 12 patients (10 GBM, 1 AA, 1 oligoastrocytoma).[43] This trial, while designed for safety, involved upfront surgery followed by viral injection into the resection cavity. The trial confirmed a lack of neurovirulence of HSV1716 and reported three patients surviving 15 months, 18 months, and 22 months after viral injection. While these authors hypothesized that surgery followed by injection is the most clinically efficacious treatment plan, trials designed for efficacy have not yet been carried out.

The most recent published data regarding HSV1716 and gliomas have been conducted in animal models. A phase I clinical trial was initiated in 2014 to study HSV1716 injected into or near the surgery resection cavity in pediatric patients with recurrent childhood gliomas. Two patients were enrolled but the results have not been published (NCT02031965).

HSVG207 is another OV under clinical investigation.[15,37,38] A phase I dose escalation study was carried out in 2000 with three groups at seven different doses.[37] Doses up to 3×10^9 pfu were injected at five inoculation sites. No adverse events were attributed to HSVG207, with mean survival from diagnosis reported to be 15.9 months for GBM and 40.5 months for AA. This trial established that intracerebral inoculation with G207 was safe; however, there was no demonstrated *in vivo* replication and the maximally tolerated dose was not achieved. Unfortunately, OS remained consistent with historical controls of 12-16 months. Fifteen years later, the group reported a patient's prolonged survival from the 2000 study: a 52-year-old woman experienced a 7.5-year survival with a 6-year 'disease-free' interval.[44] However, after treatment with HSVG207, the patient also underwent multiple additional treatments of surgery, radiation, and chemotherapy thus clouding the interpretation of the efficacy of the HSVG207. A follow-up phase Ib trial in 2009 was conducted using

Table 3. HSV trials in glioma

Author and year	Patients (*n*)	Tumor type	Virus type	Virus features	Virus name	Trial design	Administration	Dose(s)	Previous treatment	Survival/outcome	Adverse events
Rampling et al. 2000[35]	9	GBM 8 AA 1	HSV	Lacks RL1 gene that encodes the protein ICP34.5	HSV1716	Case series, single-center	Stereotactic I.T.	10^3 10^4 10^5 pfu	S: 9 RT: 9 CT: 7	Five patients died at 3, 6, 8, 9 months Stable at 14, 17, 19, 24 months, with one patient surviving 7.5 years[44]	None ascribed to HSV1716
Markert et al. 2000[37]	21	GBM 16 AA 5	HSV	Deletions of g134.5 loci and a lacZ insertion disabling UL39 of ribonucleotide reductase	G207	Phase I trial, five institutions, dose escalation in three groups	I.T.	10^6–3×10^9 pfu injected over 2 min in 5 operative locations	S: 17 Biopsy: 4 RT: 21 CT: 10	4 Survived 12.8 months AA 28 months	None ascribed to G207
Papanastassiou 2002[36]	12	Recurrent GBM 6 Secondary GBM 5 Newly diagnosed GBM	HSV	Lacks RL1 gene that encodes the protein ICP34.5	HSV1716	Viral injection followed by surgery 4-9 days later.	I.T.	10^5 pfu in 1 ml HSV1716, injected in nine aliquots.	S: 11 RT: 10 CT: 3	Deaths at: 1, 2, 4, 6, 9, 10 months Alive: 15, 16, 22 months	None ascribed to HSV1716
Harrow 2004[43]	12	GBM 10 AA 1 OA 1	HSV	Lacks RL1 gene that encodes the protein ICP34.5	HSV1716	Phase I study, surgery then, viral injection into resection cavity	I.T. cavity	10^5 pfu in 1 ml injected in 8-10 brain regions after surgical resection	S: 5 RT: 6 CT: 1	Alive: 15, 18, 22 months after injection Died: 3, 6, 8, 9, 11, 11, 11.5, 14, 15 months	None ascribed to HSV1716
Markert et al. 2009[38]	6	GBM 6	HSV	Deletions of g134.5 loci and a lacZ insertion disabling UL39 of ribonucleotide reductase	G207	Phase Ib, intratumoral injection then 2-5 days later surgery with injection into resection cavity	I.T.	Two doses of G207 totaling 1.15×10^9	S: 6 RT: 6 CT: 5	Median TTP: 3 months Median OS: 23 months after initial tumor diagnosis Median survival from G207 therapy 6.6 months	None ascribed to G207
Markert et al. 2014[15]	9	GBM 7 AA 2	HSV	Deletions of g134.5 loci and a lacZ insertion disabling UL39 of ribonucleotide reductase	G207	Phase I, single-center, dose escalation, combined with 5 gray radiotherapy	I.T.	One dose injected into multiple regions of tumor margin, $1.0 \times 10^9 + 5$ gray rads 24 h after injection	S: 9 RT: 9 CT: 9	Median OS from virus inoculation to death: 7.5 months Median progression-free survival: 2.5 months	Seizures and pyrexia possibly attributed to virotherapy

AA, anaplastic astrocytoma; CT, chemotherapy; GBM, glioblastoma multiforme; HSV, herpes simplex virus; I.T., intratumoral; OA, oligoastrocytoma; OS, overall survival; RT, radiation therapy; S, surgery; TTP, time to progression.

HSVG207 in patients with recurrent GBM.[38] The trial was designed for i.t. inoculation followed by surgical resection and then re-injection into the resection cavity. Again, there were no adverse events ascribed to HSVG207 and median OS from diagnosis was 23 months, median OS from HSVG207 treatment was 6.6 months, and median time to progression was 3 months. This trial took the development of HSVG207 another step forward demonstrating that multiple doses of HSV can be administered without evidence of encephalitis. The most recent trial with HSVG207 was reported in 2014 and combined a single dose of HSVG207 OV therapy with radiation.[15] This phase I dose escalation study enrolled nine patients (seven GBM and two AA) to be treated with a single dose of HSVG207 combined with 5 Gy radiation 24 h after injection. Median survival after inoculation was 7.5 months and progression-free survival (PFS) was 2.5 months. The authors also highlight that the 5-Gy dose of radiation is known to be a subtherapeutic dose of radiation in malignant gliomas and that a treatment effect is therefore unlikely unless there was a synergistic effect between HSVG207 and radiation, which is not yet identified. In sum, this was the third trial for HSVG207 that showed safety at the doses tested and that there were variable responses with some living longer than the historical survival times.

Poliovirus. A recombinant poliovirus-rhinovirus chimera, PVSRIPO, completed a phase I clinical trial where it was granted Breakthrough Therapy Designation by the FDA on 10 May 2016 (Table 4).[12,45] PVSRIPO is a replication competent, live attenuated virus developed from the type 1 Sabin live attenuated poliovirus vaccine with the poliovirus internal ribosomal entry site (IRES) substituted with that of rhinovirus.[46] The safety of PVSRIPO relies on the substitution of the IRES for that of rhinovirus. In turn, this leads to attenuation of neurovirulence and conditional replication in non-neuronal cells. PVSRIPO binds to CD55 (nectin-like molecule-5), which is up-regulated in many cancer types.[46-50]

PVSRIPO works via direct oncolysis followed by a triggered immune response, similar to the viruses described above. PVSRIPO elicits a mix of early innate antiviral activation, proinflammatory stimulation, and immune cell invasion that may generate long lasting antitumor immunity.[46] This chimera elicits a strong neutrophil response—neutrophils are suspected to participate in direct tumor cell killing with cytotoxic proteins, reactive oxygen species, and TNF. Additionally, the neutrophil response is thought to mediate the recruitment of the adaptive antitumor response.[46] This recruitment may prove especially significant in promoting immune cell invasion into notoriously immunologically 'cold' tumors, thereby providing a durable immune response.

The single trial with PVSRIPO was completed in 2018 with 61 recurrent GBM patients.[11] Patients received an i.t. injection with convection enhanced delivery (CED) and showed a modest improvement in OS (12.5 months compared with 11.3).[45] CED relies on sustained, continuous

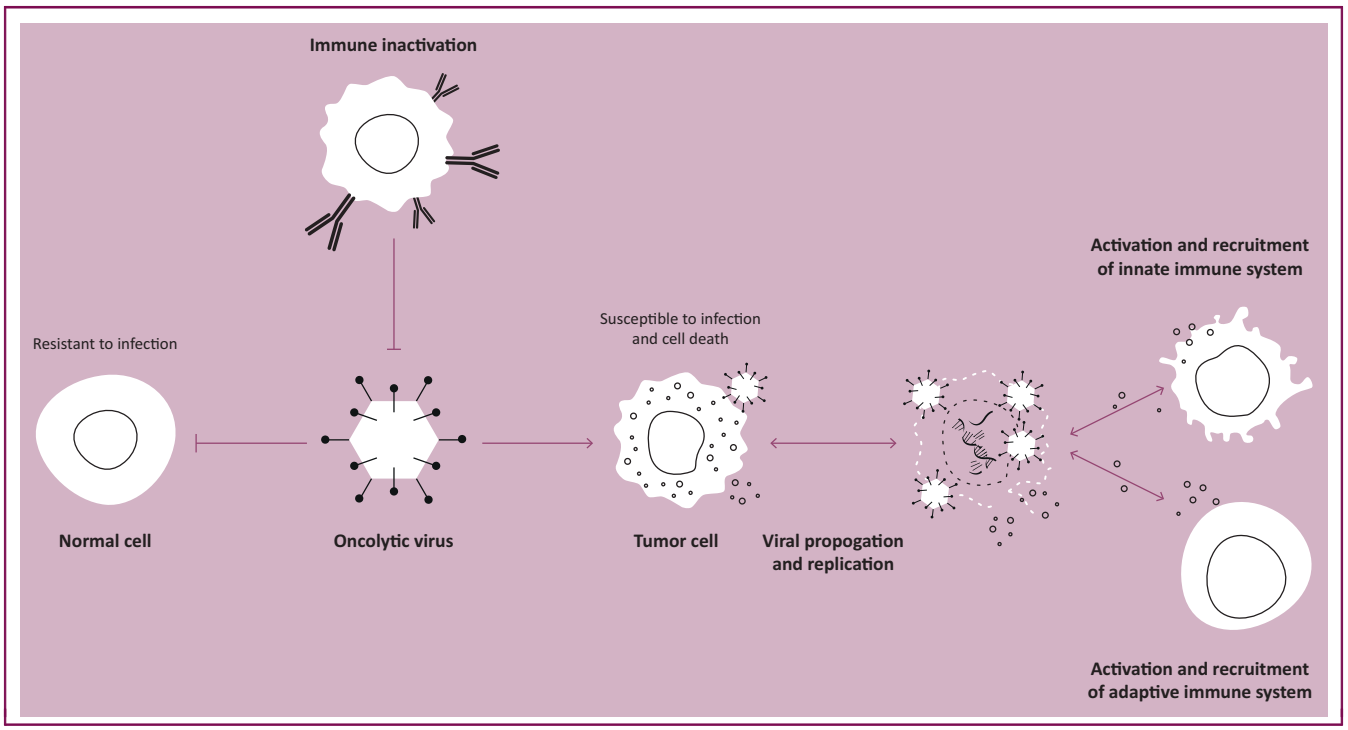

Figure 2. Mechanisms of administered oncolytic viruses.
Normal cells are resistant to infection while conditional replication is limited to tumor cells due to specific tropism. Once infected, tumor cells are susceptible to cell death, which leads to propagation and viral replication. Subsequently, both the innate and adaptive immune responses are recruited to the tumor. While it is thought the main antitumor effect is derived from the immune response, it also has the potential, however, to inactivate the circulating virus and in fact blunt viral replication and propagation.

Table 4. Poliovirus trials in glioma

Author and year	Patients	Tumor type	Virus type	Virus features	Virus name	Trial design	Administration	Dose(s)	Previous treatment	Survival/outcome	Adverse events
Desjardins et al. 2018[45]	61	Recurrent malignant glioma	Poliovirus	Poliovirus chimera with the IRES substituted with that of rhinovirus	PVSRIPO	Phase I single-center dose escalation	Intratumoral CED	10^7-10^{10} CED − 6.5 h, at a rate of 500 µl/h	S: 61 RT: 61 CT: 61 BEV: 61	Median OS 12.5 months. Survival rate at 36 months was 21%, with patients remaining alive >70, >69, and >57 months.	Two deaths due to tumor progression and intracranial hemorrhage after removal of catheter. 69% Had adverse events of grade 1 or 2. 19% Had adverse events of grade 3 or higher.

BEV, bevacizumab; CED, convection enhanced delivery; CT, chemotherapy; IRES, internal ribosomal entry site; OS, overall survival; RT, radiation therapy; S, surgery.

low-pressure infusion allowing greater distribution over a larger area of the brain.[51] However, a statistically significant improvement in long-term survival was observed with 21% of patients surviving at both 24 and 36 months compared with historical controls of 14% at 24 months and 4% at 36 months.[4,11]

Adenovirus. The well-understood nature of adenovirus biology allows for genetic manipulation of conditionally replicative adenovirus (Table 5).[52] The first recombinant adenovirus was Onyx-015.[52] Attenuation and conditional replication of Onyx-015 was achieved with a deletion at the E1B locus, thereby blocking expression of the E1B-55kD protein.[52,53] This deletion prevents Onyx-015 from replicating in normal cells.[52] Onyx-015 was investigated in several clinical trials for a variety of cancers and for malignant glioma (grade III and IV) in a 2004 study.[54] This trial enrolled 24 patients with recurrent malignant glioma who received i.t. injection in the surrounding surgical resection cavity. While lymphocytes and plasma cells were identified within the tumors on histological analysis, no definite antitumor response was demonstrated. Investigation into Onyx-015 was ultimately discontinued after an unsuccessful phase III trial in patients with head and neck cancers.[52,55]

Among the second generation of OVs is DNX-2401, an oncolytic adenovirus that has tumor selectivity from a 24-base pair deletion in the E1A gene.[56] DNX-2401 is unable to replicate in normal cells that maintain a functional retinoblastoma pathway, but can replicate in tumor cells, as nearly all gliomas contain altered retinoblastoma pathways.[13,52,56] The phase I trial with DNX-2401 was reported by Lang et al.[56] in 2018 and enrolled 37 patients with malignant glioma. Twenty-five patients in Group A were assigned to one of eight dose escalation protocols (1×10^7—3×10^{10}) that were administered in a single i.t. dose. Patients in Group B were assigned one of four dose escalation schedules (1×10^7—3×10^8) administered through an i.t. catheter, underwent *en bloc* tumor resection 14 days later, and then received a second injection of DNX-2401 into the resection cavity intraoperatively. Analysis of the resected tumors showed evidence of virus-induced necrosis and active viral replication, thus providing evidence of viral lysis and propagation. Group A had a median OS of 9.5 months with five patients surviving >3 years. In Group B, the median OS was 13 months with two patients surviving >2 years. Analysis of samples from Group B found an increased population of CD4+ T cells suggesting a treatment-induced immunological response. Given the subgroups of strong responders in this study as well as evidence of viral replication and immunological response, DNX-2401 was granted Fast Track Orphan Drug Designation from the FDA.[13]

Reovirus. Reoviruses have been investigated in both wild-type and recombinant forms (Table 6). Forsyth et al.[57] in 2008 reported the first phase I clinical study with wild-type reovirus that enrolled 12 patients with malignant glioma. In this trial, reovirus was administered i.t. in a dose escalation protocol. Reovirus inoculation was well tolerated with no

Table 5. Adenovirus trials in glioma

Author and year	Patients (*n*)	Tumor type	Virus type	Virus features	Virus name	Trial design	Administration	Dose(s)	Previous treatment	Survival/outcome	Adverse events
Chiocca et al. 2004[54]	24	Recurrent GBM 17 Recurrent AA 5 OA 2	Adenovirus	E1B-55K deletion and substitution	ONYX- 015	Phase I, six-center, dose escalation with four cohorts of six patients, i.t. injection then surgery	I.T.	10^7-10^{10} 10 Injections 100 ul per injection intraoperatively after glioma resection	S: 24 RT: 24 CT: 24	Median TTP: 46 days. 20 Deaths from progression. 1 Unrelated death. 3 Alive—AA and OA. Median survival time 6.2 months. Median survival time for GBM 4.9 months. Median survival time for AO 11.3 months.	Neuropathy, diarrhea, confusion. None attributed to virotherapy
Lang et al. 2017[81]	27	Recurrent GBM	Adenovirus	24 Base pair deletion in the E1A gene	Delta 24 RGD DNX 2401	Phase Ib, i.t. DNX-2401 followed by IFN or DNX-2401 alone	I.T.	A single i.t. injection of 3e10 VP DNX-2401 IFN subcutaneous at 5 receive 50 $\mu g/m^2$	S: 27 RT: 27 CT: 27	Three patients remain alive at 19, 21, and 22 months. IFN did not provide additional benefit.	None attributed to virotherapy
Lang et al. 2018[56]	37	GBM 89% AA 5% Gliosarcoma 5%	Adenovirus	24 Base pair deletion in the E1A gene	Delta 24 RGD DNX 2401	Phase 1, single-center, two-arm study. Arm 1: single i.t. injection. Arm 2: injection followed by surgical resection	I.T.	8 Dose levels Arm 1: up to 3 $\times$ 10^{10} VP Arm 2: up to 3 $\times$ 10^8 VP	S: 25 RT: 37 CT: 36	Median OS: 13.0 months across all cohorts. Arm 1: median OS 9.5 months regardless of dose, with five patients alive at 3 years. Arm 2: 17% survived for 2 years	None attributed to virotherapy

AA, anaplastic astrocytoma; CT, chemotherapy; GBM, glioblastoma multiforme; IFN, interferon; i.t./I.T., intratumoral; OA, oligoastrocytoma; OS, overall survival; RT, radiation therapy; TTP, time to progression; S, surgery; VP, viral particles.

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Table 6. Reovirus trials in glioma

Author and year	Patients (*n*)	Tumor type	Virus type	Virus features	Virus name	Trial design	Administration	Dose(s)	Previous treatment	Survival/outcome	Adverse events
Forsyth et al. 2008[57]	12	GBM 9 AA 2 OA 1	Reovirus	Wild-type	Reovirus	Phase I, three groups of three	I.T.	10^7 — three patients 10^8 — six patients 10^9 — three patients 72-h infusion	S: 12 RT: 12 CT: 10	Median OS 21 weeks Median TTP 4.3 weeks One patient alive at 54 months with GBM	None attributed to virotherapy.
Kicielinski et al. 2014[51]	15	GBM 12 AA 3	Reovirus	Wild-type reovirus variant	Reolysin	Phase I, three-center, dose escalation	I.T. CED	10^8 -10^{10} pfu	S: 15 RT: 15 CT: 15	Median OS: 140 days Median TTP: 61 days	Convulsions in three patients
Samson et al. 2018[10]	9	HGG 6 Melanoma metastasis 2 CRC metastasis 1	Reovirus	Wild-type reovirus variant	Reolysin	Phase Ib window of opportunity trial.	I.V.	Single, 1-h i.v. infusion of 1×10^{10} ahead of planned surgical resection surgery was undertaken 3—17 days after reovirus infusion.	S: 6 RT: 6 CT: 6 BEV: 1 'Melanoma and CRC patients had no prior brain metastasis therapy'	Median OS: 469 days HGG median OS: 450 days Metastasis median OS: 532 days	Lymphopenia grade 1-2 in all nine patients. Grade 3-4 adverse events in six patients with influenza-like symptoms

AA, anaplastic astrocytoma; BEV, bevacizumab; CED, convection enhanced delivery; CRC, colorectal cancer; CT, chemotherapy; GBM, glioblastoma multiforme; HGG, high-grade glioma; I.T., intratumoral; i.v./I.V., intravenous; OA, oligoastrocytoma; OS, overall survival; RT, radiation therapy; S, surgery; TTP, time to progression.

significant adverse events. Three patients survived >1 year; one patient survived 54 months; the median OS was 21 weeks. Intratumoral viral replication and viral cell killing, however, were not assessed.

Kicielinski et al.[51] in 2014 designed a trial to reach higher doses and better intratumoral distribution of reovirus with Reolysin, another wild-type reovirus. To achieve this, the group used CED, marking the first use of CED for OV therapy. Median survival was 140 days with one patient surviving nearly 2 years and another nearly 3 years. No significant adverse events were attributed to Reolysin. Like the previous study, histological analysis of post-treatment samples was not carried out. This early success led to the FDA granting Reolysin Orphan Drug status in 2015.

Samson et al.[10] in 2018 continued investigation with Reolysin in a phase 1b window of opportunity trial. Nine patients with high-grade gliomas and brain metastases were enrolled and treated with a single, 1-h i.v. infusion before brain tumor surgery 3-17 days later. Median OS was 469 days with common adverse events of lymphopenia and influenza-like symptoms. IFN-γ from patient sera significantly increased after treatment compared with samples taken before, indicating systemic inflammatory response to treatment. Additionally, evidence of Reolysin was found in all tumors analyzed after surgical resection, with RNA found diffusely throughout the tumor and viral protein more localized. This indicates reovirus translation being localized to a select few regions of the tumor. Additionally, tumors with higher proliferative rates were more susceptible to reovirus infection. These data support the promise of i.v. administration of oncolytic viral agents as an effective means of delivering OV to brain tumors. The mechanism of how reovirus enters through the blood-brain barrier (BBB) has yet to be elucidated.

Parvovirus. Clinical investigation for cancer treatment with parvovirus, specifically H-1PV, began as early as 1965 and established safety in humans. H-1PV continued to undergo preclinical investigation in both pancreatic and glioma models until a trial was launched for GBM in 2011—the first clinical trial in Germany to use OVs (Table 7).[58,59]

The natural host of H-1PV is the rat and therefore, is nonpathogenic to humans. It was found to selectively replicate and kill human cancer cells through numerous mechanisms, as well as triggering an immunogenic anti-tumor response.[58] A distinguishing feature of H-1PV is its small size: with a diameter of 25 nm it is equal to the size of a ribosome.[58] This unique feature offers clinical significance as it allows H-1PV to cross the BBB to reach tumor cells and offers the potential of i.v. administration.[58,59]

The 2011 GBM trial design included 18 patients divided into two arms treated with escalating doses of H-1PV administered either i.t. or i.v.[58,59] The trial met its primary endpoints of demonstrating safety and tolerability. They also found wide distribution of the virus within the tumor microenvironment, viral RNA in tumor cells in a post-resection analysis, a triggered inflammatory response,

Table 7. Parvovirus trials in glioma

Author and year	Patients (*n*)	Tumor type	Virus type	Virus features	Virus name	Trial design	Administration	Dose(s)	Previous treatment	Survival/outcome	Adverse events
Geletneky et al. 2017[59]	18	Recurrent GBM	Parvovirus	Wild-type H-1PV strain	ParvOryx	Phase I/IIa open non-controlled two-arm, three group intragroup dose escalation single-center study. Arm 1: i.t. injection, followed by surgery, and re-injection into cavity. Arm 2: 5 i.v. virus infusions, followed by resection, and re-injection into cavity.	Two arms i.t. and one arm i.v.	Arm 1 i.t.: 5×10^9 pfu Arm 2 i.v.: 1×10^9 pfu	S: 18 RT: 18 CT: 18	Overall median PFS: 111 days Overall median OS: 464 days Arm 1: median OS: 411 days Arms 2: median OS: 208 days	None attributed to virotherapy
Gesundheit et al. 2020[14]	4	GBM	Combination therapy: NDV wild-type Parvovirus wild-type Vaccinia virus wild-type	Unmodified viruses	NDV, parvovirus, vaccinia virus	Case series	I.A. port at the carotid artery and i.v.	Viruses injected into patients at intervals of 2-3 weeks, administered by sequential 10 ml injections via the same catheter.	S: 4 RT: 4 CT: 4	14.5 Years, alive 6 Years, dead 8 Years, alive 4 Years, alive	Complications related to i.a. port

CT, chemotherapy; GBM, glioblastoma; i.a./I.A., intra-arterial; i.t., intratumoral; i.v., intravenous; NDV, Newcastle disease virus; OS, overall survival; PFS, progression-free survival; RT, radiation therapy; S, surgery.

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dose-dependent antibody formation, and a specific T cell response.[58,59] OS and PFS were improved; however, all patients ultimately died. While improvements in OS (464 days) and PFS (15.9 weeks) were found combined for both groups, a randomized, double blinded study is the obvious next step.

Combined viral therapy. As multimodality treatment is expanding in oncology, so too are treatments with combinations of OVs. In a recent study from May 2020, Gesundheit et al.[14] used mixtures of OVs to treat four patients with GBM. The group sought to evaluate the synergy of intra-arterial (i.a.) NDV, wild-type parvovirus, and wild-type vaccinia. Delivery of OV was both i.a. and i.v.[14] Survival to date from diagnosis for the four patients ranged from 4 to 14.5 years, with three remaining alive at the date of publication. The second treated patient died 6 years after starting OV therapy and died 1 year after electing to stop OV treatment. This unique approach provided both clinical and radiologic responses with long-term survivors.

Despite the early success of this case series, phase I trials and randomized, controlled trials are needed to further develop and standardize clinical protocols. Future innovations in OV mixtures include further engineering these wild-type viruses for greater specificity and immune activation, as well as combining OV mixtures with other immunotherapies. Dendritic cell therapies are thought to offer particular synergy with OVs and warrant further clinical investigation.[14,60,61]

Administration and dosing. Despite the limited number of trials for OVs in gliomas, numerous routes of administration and dosing protocols have been used in clinical trials to date, as summarized in Figure 3. Route of administration and sequence of treatment continue to be investigated, including i.v., i.a., i.t., i.t. with CED, and even inhalational.

Direct i.t. inoculation has demonstrated the most success given the FDA approval of T-Vec as well as the FDA Fast Track Designation for both PVSRIPO and DNX-2401. I.T. injection has been used in most glioma trials and leads to favorable pharmacokinetics, maximizes drug concentration at the site of the lesion, and can lead to robust immunological response.[9,46] I.T. administration overcomes several hurdles of systemic therapy like BBB penetration, accumulating OV at the cancer site from a remote access site, accumulating sufficient dose to have a clinical effect, and protecting the integrity of the OV from the immune system.[9] By contrast, due to the expense and complexity of neurosurgical procedures, repeat i.t. dosing is often not feasible.[29] Additionally, deep-seated tumors, or tumors in eloquent areas of the brain, further limit the applicability of

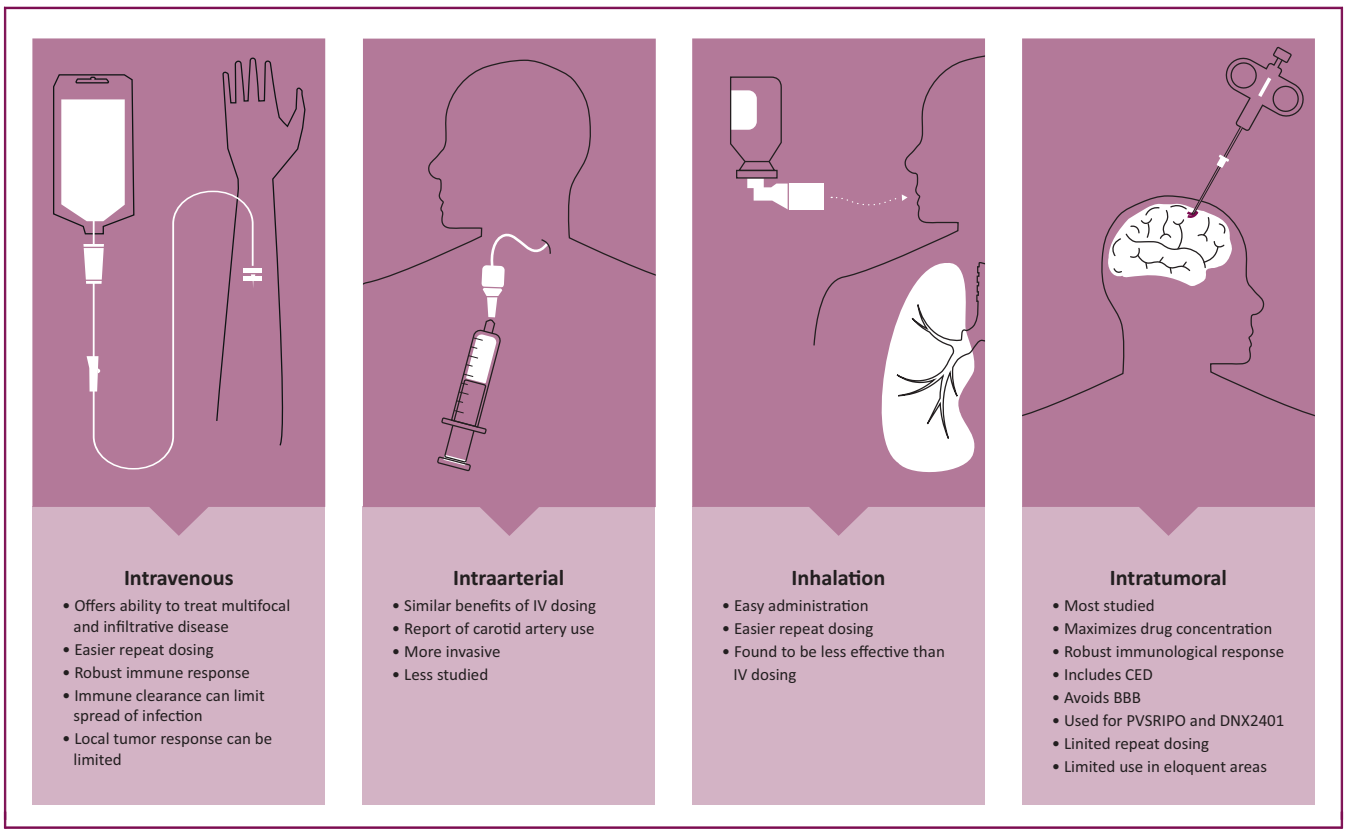

Figure 3. Routes of administration of oncolytic viruses. Throughout the development of oncolytic viruses, many methods of administration have been attempted and studied including intravenous (i.v.),[8,10,14,18-20,59] intra-arterial (i.a.),[14] inhalation,[19] and intratumoral (i.t.),[9,15,36-38,43,45,51,54,56,57,59,81] which also includes convection enhanced delivery (CED). While there are benefits and shortcomings to each method, i.t. and i.v. administration have been the most studied given the efficacy of local therapy and ease of repeat dosing.
BBB, blood-brain barrier.

i.t. dosing.[29] I.T. injections may also be suboptimal for multifocal and/or infiltrative diseases given the local nature of the injection. CED has sought to improve viral distribution but has yet to fulfill its theoretical and preclinical potential. While CED provides positive pressure, backflow can limit the distribution to the tumor edge and infiltrating tumor cells, which is the area most difficult to resect and achieve tumor control.[62] It is these distant cells, however, that are the likely source of recurrence and most desperately need tumor control which CED appears to provide.

Systemic therapy, including both i.v. and i.a., has shown some efficacy. I.V. injection may enhance the generation of the antitumor immune response as well as offer the ability to treat multifocal and infiltrative pathology.[9,10] Systemic therapy is the likely direction for treatment with OVs as it is the standard for anticancer agents and therefore is available for most cancer patients. Intravascular therapy, however, has several challenges to overcome. Systemic toxicity becomes an issue. Immune clearance dramatically impedes the efficacy of OVs with neutralizing antibodies greatly impeding the dose that can reach the tumor site. BBB penetration also remains a longstanding obstacle. Nevertheless, intravascular OV therapy continues to be a promising avenue of investigation given the potential to enhance OV spread throughout the tumor, including the periphery and distant tumor cells.[62] To overcome these challenges associated with intravascular OV delivery, methods of circumventing the BBB and neutralizing antibodies have been recently explored: mannitol infusions, focused ultrasound, and microbubble-mediated drug delivery systems are under active investigation.[62-64] Further modifying OVs either by genetic or chemical means is an active area of further investigation.[9] A recent report cites production of a recombinant measles virus with modifications that maintain oncolytic activity while evading anti-measles antibodies.[65,66] This 'stealth virus', while originally generated for non-CNS tumors, may offer benefits for brain tumors as it maintains circulating virus. Combination therapy with both i.t. and systemic therapy may offer a synergistic effect, including both a local and systemic immune response.[67] I.T. therapy ensures direct tumor cell infection and, if further optimized, could be combined with intravascular therapy for distant tumor control.

Another form of combination therapy is OVs and ICIs. Blockade of immune checkpoints has demonstrated efficacy in non-CNS tumors but has only been sparsely investigated in brain tumors. Initial studies with ICIs alone in gliomas have demonstrated little success. A 2020 phase III trial compared programmed cell death protein 1 (PD-1) inhibitor nivolumab with vascular endothelial growth factor-A inhibitor bevacizumab in patients with GBM, but failed to show an improvement in OS.[68] A 2019 phase II trial in patients with GBM evaluated nivolumab administered both before and after surgery, but no clinical benefit was observed.[69] In a 2019 study, Cloughesy et al.[70] did report a survival benefit with neoadjuvant pembrolizumab of 417 days compared with 228.5 days in the control adjuvant cohort. Active and ongoing trials with ICIs are summarized by Mende et al.[71] Less than expected success with ICIs is thought to be due to the lack of T cells within the glioma tumor microenvironment.[45,71] Combined OV and ICI has been proposed to address the shortcomings of ICI therapy alone, as OV therapy is recognized to alter the tumor microenvironment and have synergistic effects with ICIs.[10,72,73] Preclinical studies have demonstrated efficacy in animal models where an experimental oncolytic HSV (G47Δ-mIL12) was combined with anti-PD-1 and anti-cytotoxic T-lymphocyte-associated protein 4 checkpoint inhibitors that cured existing tumor burden and generated immunological memory where animals were resistant to rechallenge.[74] This treatment paradigm has also undergone clinical investigation in unresectable melanoma with T-Vec and ipilimumab compared with ipilimumab alone, which found an increased rate of objective response from 18% to 39% when using combined therapies.[75] Subsequent trials are now underway combining T-Vec with other forms of ICI. With the precedent set for combined OV and ICI therapy in both preclinical glioma models and other cancers, further investigation is on the horizon.

Limitations of completed trials, future directions, and conclusions. Despite the early success of OV trials, several limitations must be recognized. The first limitation with many of these studies is the small number of patients. While many of these trials were designed first for safety, a greater number of patients must be included to evaluate efficacy and potential long-term consequences of OVs in the brain. Furthermore, dose-limiting toxicity was not determined for many viruses. Many of these trials, particularly the earlier ones, also failed to describe the genetic landscape of the tumors. Without controlling for these prognostic markers, interpretation of survival data is limited. As the clinical studies reviewed here lacked control groups, their survival can only be interpreted in context of historical controls. Prospective randomized trials are needed for proper evaluation of OV efficacy. Analysis of patients with favorable and unfavorable prognostic markers must be carried out to determine OV efficacy in both subgroups. There is not, however, a generally accepted molecular biomarker used to predict response, though the following are associated with treatment response: tumor receptor expression, tumor mutation burden, and alterations to several molecular pathways including IFN expression, autophagy, and ubiquitination.[3,71,76,77] While only proposed, these data points may offer an insight into virus selection and response to treatment. Concerns remain about dosing protocols, genetic stability, viral integrity after replication, off-target replication, and immune-mediated clearance of OVs.

Nevertheless, the future of OVs in the treatment of gliomas remains promising, perhaps most sustained by evidence of long-term responders. Chiocca et al.[3] in 2019 reported an increased rate of durable responders in patients with GBM treated with OV compared with patients treated with non-virotherapy. A pooled analysis of 2472 patients suggests that the overall proportion of 24-month

Table 8. Ongoing, active, and recruiting human clinical trials of oncolytic virus for glioma tumors

Virus	Virus name	Reference	Phase	Patients	Administration	Primary outcome measure
HSV	HSV G207	NCT04482933	Phase II	30	I.T.	OS at 1 and 2 years
	HSV G207 rQNestin34.5v.2	NCT03911388	Phase I	15	I.T.	Tolerability
	C134	NCT03152318	Phase I	108	I.T.	Maximum tolerated dose
	M032	NCT03657576	Phase I	24	I.T.	Safety and tolerability through 12 months
		NCT02062827	Phase I	36	I.T.	Maximum tolerated dose through 12 months
Adenovirus	DNX-2401	NCT02798406	Phase II	49	I.T.	Objective response rate at 3.5 years
	DNX-2401	NCT03896568	Phase I	36	I.A.	Maximum tolerated dose and incidence of adverse events
	DNX-2401	NCT03178032	Phase I	12	I.A.	Safety, tolerability, and toxicity
	DNX-2401	NCT03896568	Phase I	36	I.A.	Maximum tolerated dose and incidence of adverse Events
	CRAd-Survivin-pk7	NCT03072134	Phase I	13	I.T.	Maximum dose and tumor response at 2 years
	DNX-2440	NCT03714334	Phase I	24	I.T.	Adverse events at 8 weeks
Reovirus	Wild-type reovirus	NCT02444546	Phase I	6	I.V.	Maximum tolerated dose
Poliovirus	PVSRIPO	NCT02986178	Phase II	122	I.T.	Objective radiographic response at 36 months
	PVSRIPO	NCT04479241	Phase II	30	I.T.	Objective response rate at 24 months
	PVSRIPO	NCT03043391	Phase Ib	12	I.T.	Toxicity
Vaccinia virus	TG6002	NCT03294486	Phase I/II	78	I.V.	Safety, tolerability, and dose-limited toxicity

I.T., intratumoral; I.A., intra-arterial; I.V., intravenous; OS, overall survival.

survival is 12% and 36-month survival is 6%, while patients treated with virotherapy demonstrate 24- and 36-month survival of 15% and 9%, respectively.[3] They highlight a 'tail' of durable responders that reveals the potential for certain GBM patients to respond very favorably to OV therapy.

Table 8 summarizes all active and recruiting human trials with OVs in glioma. These trials include continuations of previously mentioned trials as well as new phase 1 trials involving pediatric patients, novel human trials, and new combinations of therapies for gliomas. PVSRIPO has undergone further preclinical immunological studies as we await the start of several clinical trials both in glioma and in non-CNS cancers.[78-80] Further study in gliomas will continue with a phase II trial with CED (NCT02986178), a phase I trial combining PVSRIPO-CED with pembrolizumab (NCT04479241), and a phase Ib trial in pediatric glioma also with CED (NCT03043391). DNX-2401 likewise is awaiting the start of several clinical trials including a phase II combined treatment with pembrolizumab (NCT02798406), a phase I trial with i.a. administration combined with surgery (NCT03896568), and a phase I i.t. injection in pediatric glioma (NCT03178032).

Imminent OV trials include rQNestin, M032, C134, CRAd-survivin-pk7, and TG6002 (Table 8). These will all be administered intratumorally in an effort to establish maximum tolerated dose and tumor response. While the field eagerly awaits the results of these trials, none of them, however, are controlled studies, a shortcoming in evaluating efficacy. We look forward to the results of these trials and future introduction of controlled studies that will undoubtedly shape the future landscape of OVs in glioma therapy.

CONCLUSION

This review presents the most up-to-date account of OV trials for high-grade gliomas. The focus is on OVs as replication-competent viruses that work by inducing tumor cell lysis and activating the immune system. Although these remain early-stage trials with none entering phase III, results are promising and inclusion in standard of care management seems within reach.

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