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REVIEW

Oncolytic Virotherapy for the Treatment of Malignant Glioma

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Abstract Malignant glioma is the most common primary brain tumor and carries a grim prognosis, with a median survival of just over 14 months. Given the poor outcomes with standard-of-care treatments, novel treatment strategies are needed. The concept of virotherapy for the treatment of malignant tumors dates back more than a century and can be divided into replication-competent oncolytic viruses and replication-deficient viral vectors. Oncolytic viruses are designed to selectively target, infect, and replicate in tumor cells, while sparing surrounding normal brain. A host of oncolytic viruses has been evaluated in early phase human trials with promising safety results, but none has progressed to phase III trials. Despite the 25 years that has passed since the initial publication of genetically engineered oncolytic viruses for the treatment of glioma, much remains to be learned about the use of this therapy, including its mechanism of action, optimal treatment paradigm, appropriate targets, and integration with adjuvant agents. Oncolytic viral therapy for glioma remains promising and will undoubtedly impact the future of patient care.

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Introduction

Despite a continuous decline in cancer death rates over the last 20 years, cancer remains the second leading cause of death in the USA [1]. New cases affecting the nervous system occur in nearly 24,000 people in the US alone, and result in 16,000 deaths [1]. Of these, malignant glioma (World Health Organization grade III and IV) is the most common primary brain tumor and is associated with a dismal prognosis [2]. Glioblastoma multiforme (GBM; World Health Organization grade IV glioma) is the most common malignant glioma and carries a median survival of only 14.6 months when treated with current standard of care, consisting of maximal safe surgical resection followed by radiotherapy with concomitant and adjuvant temozolomide [3]. The infiltrative nature, relentless growth of these tumors, and resistance to therapy over time are hallmarks of their malignant behavior [4]. Given the poor outcomes with current standard-of-care treatments of these highly malignant tumors, novel treatment strategies are desperately needed.

Features of malignant gliomas that contribute to their poor prognosis include: 1) their relative resistance to traditional radiation and chemotherapies; 2) physiologic isolation of the tumor due to the blood–brain barrier; 3) the infiltrative nature of these tumors; 4) the relative immune privileged status of the brain; and 5) identification of cancer stem cells with the ability for self-renewal and resistance to conventional therapy [4–6]. An evolving understanding of these tumors has led to treatment strategies targeting the basic elements of these malignant cells and their microenvironments with hopes of complementing the existing standard of care [4, 7].

The concept of virotherapy for the treatment of malignant tumors dates back more than a century, when De Pace, in a case report from 1912, described a case of cervical cancer that regressed after receiving Pasteur's attenuated rabies vaccine following a dog bite [8]. Subsequently, 30 patients with melanomatosis were treated with the rabies vaccines, and 8 demonstrated regressive changes [9, 10]. Infection with naturally occurring viruses, such as measles, has also demonstrated cancer regression in cases of Burkitt's lymphoma and Hodgkin's disease [11, 12]. However, concern of serious adverse events and the rise of chemotherapy halted early progress [7].

The last 3 decades has seen a resurgence of interest in genetically engineered viruses for the treatment of malignant glioma. Advancements in the field of molecular virology and genetics have allowed for the rational modification of viruses to combat cancer. Viral therapy can be divided into 2 groups: 1) replication-competent oncolytic viruses (OVs); and 2) replication-deficient viral vectors used as a delivery mechanism for therapeutic genes [7]. To date, virotherapy for malignant glioma has proven safe. Furthermore, the recent success of the talimogene laherparepvec, an oncolytic herpes simplex virus armed with granulocyte macrophage colony-stimulating factor (GM-CSF), in a phase III clinical trial followed by its Food and Drug Administration (FDA) approval for metastatic melanoma highlights the potential for virotherapy success in the treatment of neoplastic diseases [13].

This review will cover oncolytic virotherapy for the treatment of malignant glioma with a focus on viral agents that have completed or are currently being evaluated in a clinical trial.

This review will focus on replication-competent viruses that are designed to selectively target, infect, and replicate in tumor cells, while sparing the surrounding normal brain parenchyma (Tables 1 and 2). Their ability to replicate serves as an advantage for overcoming the low transduction efficiency and vector loss that can hamper non-replicating viruses [7]. Influential work on nonreplicating viruses that exert their antitumor effect through gene-mediated cytotoxic immunotherapy, such as AdV-tk (aglatimagene besadenovec) + valaciclovir, as proposed by Wheeler et al. [14], remains an exciting therapeutic opportunity but falls outside the scope of the current review.

Oncolytic Viruses

Unique to OVs is the ability of the virus to infect specifically a tumor cell and induce tumor lysis through the release of viral progeny, which can subsequently infect nearby tumor cells. In 1991, the pioneering work of Martuza et al. [15] described a herpes simplex virus type 1 (HSV-1) thymidine-kinase (tk) deleted mutant, *dl*sptk, which was replication-attenuated in quiescent cells such as neurons [15]. The thymidine kinase (tk) gene deletion required the virus to rely on actively dividing cells to supply thymidine kinase for DNA replication. The *dl*sptk virus displayed an encouraging therapeutic profile in the treatment of malignant glioma in animal studies. However, the tk gene deletion rendered *dl*sptk resistant to antiviral agents that target the viral tk, such as aciclovir and ganciclovir. Lack of susceptibility to the viral tk-targeted drugs prohibited advancement of this OV into clinical trials [2]. Despite this limitation, this early work led to the development of a new generation of genetically engineered OVs for the treatment of malignant glioma. Nearly a decade after the introduction of the first HSV-1 tk-deleted mutant, the results of two phase I trials using genetically engineered HSV-1 for the treatment of malignant glioma were published [16, 17].

HSV-1

HSV-1 is a large double-stranded DNA and a common human pathogen, capable of establishing lifelong infection through latency [18]. It is a neurotropic virus and is perhaps the best studied of the OVs. The genes involved in oncolysis are distinct from the genes for neurovirulence, allowing for genetic manipulation that permits conditional replication and oncolysis of tumor cells [2]. An additional safety feature of HSV-1 is its sensitivity to aciclovir and ganciclovir when its tk gene is intact; this improves the safety profile of the virus when used in clinical trials.[2]

Since the examination of *dl*sptk in 1991 [15], a host of HSV-1 mutants has been engineered to reduce neurotoxicity while retaining the ability to infect and lyse actively dividing tumor cells. These attenuating mutations involve deletion of both copies of $\gamma_1$34.5 or disruption of U_L39 utilizing a *lacZ* insertion. The $\gamma_1$34.5 gene has been recognized as essential for neurovirulence, as it produces infected cell protein (ICP) 34.5, which activates a phosphatase which then dephosphorylates eukaryotic initiation factor (eIF2α), restoring protein synthesis in the infected cell [2, 19]. Deletion of this gene also removes part of the latency-activated transcripts, preventing the virus from establishing latency following initial infection [2, 20]. U_L39 encodes the large subunit of ribonucleotide reductase (ICP 6), which is essential for DNA replication in postmitotic cells such as the neuron [2]. However, in actively dividing cells the U_L39 mutation is complemented *in trans* by the cellular version of the enzyme [21], allowing for continued viral replication [2]. This ribonucleotide reductase mutation also provides an additional level of safety by not only limiting replication to actively dividing cells, but also increasing the sensitivity of the virus to aciclovir [22].

Table 1 Completed oncolytic virotherapy trials

Study phase	Year of publication	Disease	Experimental therapy	Delivery and dosing	Results	Survival
Case report	1999	Recurrent GBM	MTH-68/h: live attenuated NDV	i.v. administration initiated at 1 vial ($10^{7.4}$ electroimmunodiffusion, 50 per vial) daily, increased stepwise to 4 vials daily	No significant toxicity; neurologic improvement; progressive tumor shrinkage	Alive at 3 years
Case series (includes the case report patient)	2004	Progressive high-grade glioma	MTH-68/h: live attenuated NDV	i.v. administration with variable dosing	No adverse effects; neurologic improvement; tumor shrinkage	All patients alive at 5–9 years
Case report	2006	Recurrent anaplastic astrocytoma	MTH-68/h: live attenuated NDV + valproic acid	Daily alternating i.v. and inhalational of 4×10^8 pfu with 1500 mg of valproic acid daily	Partial tumor response; oncolytic activity a result of viral replication	16 months
I	2000	Recurrent malignant glioma	HSV171:ICP34.5-deleted HSV	Intratumoral inoculation up to 1×10^5 pfu	No adverse clinical symptoms, encephalitis, or reactivation of latent virus	4/9 patients alive 14–24 months postinoculation
I	2002	Malignant glioma	HSV1716: ICP34.5-deleted HSV	Intratumoral inoculation up to 1×10^5 pfu	No toxicity; evidence of viral replication in tumor	Median > 7 months postinoculation
I	2004	High-grade glioma	HSV1716: ICP34.5-deleted HSV	Resection bed inoculation up to 1×10^5 pfu	No toxicity; encouraging imaging data	3/12 patients alive 15–22 months postinoculation
I	2000	Recurrent malignant glioma	G207: ICP6-inactivated and ICP34.5-deleted HSV	Intatumoral inoculation up to 3×10^9 pfu	No viral-related toxicity; evidence of antitumor activity	4/21 alive 7–19 months following inoculation; of deceased, mean of 6.2 months postinoculation
Ib	2009	Recurrent GBM	G207: ICP6-inactivated and ICP34.5-deleted HSV	Intratumoral inoculation of 1.5×10^8 pfu followed by 1×10^9 pfu into the resection bed	No encephalitis; evidence of antitumor activity and viral replication	Median 23 months from diagnosis and 6.6 months postinoculation
I	2014	Malignant glioma	G207: ICP6-inactivated and ICP34.5-deleted HSV + radiation	Intratumoral inoculation of 1×10^9 pfu followed by single 5-Gy radiation dose	Treatment well tolerated; 3 instances of marked radiographic response	Median 7.5 months postinoculation
I	2004	Recurrent malignant glioma	ONYX-015: E1B and E3-deleted adenovirus	Resection bed inoculation up to 1×10^{10} pfu	No serious virus-related adverse events; MTD was not reached	Median 6.2 months postinoculation
I/II	2006	Recurrent GBM	NDV-HJU: lentogenic NDV	1) i.v. intrapatient dose escalation to 11 BIU + 3 cycles 55 BIU 2) 3 cycles i.v. dosing at 11 BIU + 2 doses of 11 BIU weekly	Minimal toxicity; MTD not achieved; 1 patient with complete response	Median 7.3 postviral therapy
I	2008	Recurrent malignant glioma	Reolysin: reovirus	Intratumoral inoculation up to 1×10^9 $TCID_{50}$	No serious adverse events related to treatment; MTD was not reached	Median 4.8 postinoculation
I	2014		Reolysin: reovirus			Median 4.5 postinfusion

Table 1 (continued)

Study phase	Year of publication	Disease	Experimental therapy	Delivery and dosing	Results	Survival
		Recurrent malignant glioma		Intratumoral infusion up to 1×10^{10} $TCID_{50}$	DLT not identified; MTD not reached; evidence of anti-glioma activity	
I	Not published	Recurrent malignant glioma	DNX2401: E1A-deleted adenovirus targeted to integrins with RGD peptide			
I/II	Not published	Recurrent GBM	Delta-24-RGD: E1A-deleted adenovirus targeted to integrins with RGD peptide			
I/IIa	Not published	Progressive primary or recurrent GBM	ParvOryx: H-1 parvovirus			

GBM = glioblastoma multiforme; NDV = Newcastle disease virus; i.v. = intravenous; pfu = plaque-forming units; HSV = herpes simplex virus; ICP = infected cell protein; MTD = maximally tolerated dose; BIU = billion infectious units; $TCID_{50}$ = tissue culture infectious dose 50; DLT = dose-limiting toxicity; RGD = arginine–glycine–aspartic acid

G207

G207 is an HSV-1 mutant with deletions of both copies of $\gamma_1$34.5 gene and a *lacZ* insertion into the U_L39 gene, rendering the virus sensitive to aciclovir, favoring conditional replication in actively dividing cells. Building on the encouraging results of murine and nonhuman primate studies [23–25], 3 early-phase trials have been completed using G207 alone or in combination with radiation [16, 26, 27]. The first of these phase I trials enrolled 21 patients with evidence of recurrent or progressive malignant glioma despite standard therapy (surgery and/or biopsy followed by radiation) [16]. Patients received a dose of 10^6 plaque-forming units (pfu) inoculated at a single tumor site up to 3×10^9 pfu at 5 sites, the maximally planned dose. No toxicity or serious adverse events could be attributed to the experimental therapy and a maximum tolerated dose was not established. Decreased enhancement volume was present in 8 patients on 1-month post-inoculation scans.

Building on the results of the initial trial, a second phase Ib trial was performed with the aims of: 1) determining the safety of direct inoculation into the surrounding brain; 2) determining the safety of 2 inoculations within 1 week; 3) identifying evidence of HSV replication; and 4) determining the degree of early immune response [26]. Six patients with recurrent malignant glioma were included. Patients were treated with a total of 2 doses of G207 totaling 1.15×10^9 pfu. The initial dose (13% of total dose) was injected via a stereotactically placed catheter within the enhancing portion of the tumor. Two or 5 days later, tumor was resected *en bloc*, and the remainder of the G207 dose was injected into the surrounding resection cavity. Again, radiographic and neuropathologic evidence of antitumor activity was identified in the absence of HSV encephalitis. Furthermore, evidence of viral replication was identified. This affirmed that G207 was safe for multidose delivery, including inoculation of the resection cavity.

Another phase I trial of G207 was designed to exploit the improved HSV antitumor activity identified in preclinical studies when the virus was followed by radiation [27–29]. Nine patients with progressive, recurrent malignant glioma were enrolled. Patients received 1 dose of G207 stereotactically inoculated into the enhancing tumor margin and were then treated with 5 Gy of radiation the following day. Median survival from inoculation was 7.5 months with 6 of 9 patients demonstrating stable disease or partial response for at least 1 time point. The authors concluded that G207 inoculation followed by radiation was safe and demonstrated the potential for clinical response.

Based on promising preclinical results in pediatric tumors [30, 31], a phase I trial of G207 alone or with a single radiation dose for children with recurrent supratentorial brain tumors is currently enrolling at the University of Alabama Birmingham (NCT02457845). The primary outcome will be the safety and tolerability of the experimental therapy, with a secondary aim of assessing the potential efficacy of and biological and immune repsonse to G207.

Table 2 Ongoing oncolytic virotherapy trials

Study phase	Disease	Experimental therapy	Design	Identifier
I	Recurrent supratentorial GBM	PVS-RIPO: live attenuated poliovirus vaccine with human rhinovirus type 2 IRES	Dose escalation of intratumoral convection-enhanced delivery	NCT01491893
I	Recurrent GBM	MV-CEA: measles Edmonston vaccine strain expressing CEA	Group 1: dose escalation of resection cavity administration Group 2: dose escalation of intratumoral and resection bed administration	NCT00390299
I	Children with relapsed or refractory brain tumors	Reolysin: reovirus + GM-CSF	Dose escalation of i.v. virus + GM-CSF	NCT02444546
I	Recurrent or progressive GBM, anaplastic astrocytoma, or gliosarcoma	M032: ICP34.5 deleted HSV expressing IL-12	Dose escalation of intratumoral infusion	NCT02062827
I	Refractory or recurrent childhood HGG	HSV1716: ICP34.5-deleted HSV	Dose escalation of intratumoral and resection bed administration	NCT02031965
I	Recurrent supratentorial brain tumors in children	G207: ICP6-inactivated and ICP34.5-deleted HSV + radiation	Dose escalation of intratumoral infusion of virus + 5 Gy radiation	NCT02457845
I/II	Recurrent or progressive GBM	G47Δ: ICP6 inactivated, ICP34.5 deleted, and ICP47-deleted HSV	Dose escalation of intratumoral administration	JPRN-UMIN000002661
I	First GBM recurrence	DNX2401: E1A-deleted adenovirus targeted to integrins with RGD peptide + temozolomide	Dose escalation of intratumoral or resection bed administration with short-course temozolomide	NCT01956734
Ib	Recurrent GBM or gliosarcoma	DNX-2401: E1A-deleted adenovirus targeted to integrins with RGD peptide + IFN-γ	RCT; group 1: intratumoral injection of virus Group 2: intratumoral injection of virus + IFN-γ	NCT02197169

GBM = glioblastoma multiforme; IRES = internal ribosomal entry site; CEA = carcinoembryonic antigen; GM-CSF = granulocyte-macrophage colony-stimulating factor; i.v. = intravenous; ICP = infected cell protein; HSV = herpes simplex virus; IL = interleukin; HGG = high-grade glioma; RGD = arginine–glycine–aspartic acid; IFN = interferon; RCT = randomized controlled trial

HSV1716

Coincident with the G207 trials in the USA, HSV1716 has been evaluated by a group in the UK. HSV1716 is a $\gamma_1$34.5 null mutant with an intact U_L39 gene that replicates selectively in actively dividing cells. The initial phase I trial treated 9 patients with recurrent malignant glioma with intratumoral inoculation of up to 10^5 pfu without adverse clinical sequelae [17]. Four of the 9 patients treated were alive 14–24 months after treatment,

leading the authors to conclude the feasibility of replication-competent HSV in human glioma therapy.

A second phase I study was conducted to establish a "proof of principle" by demonstrating viral replication following intratumoral inoculation [32]. Twelve patients with biopsy-proven malignant glioma were treated with intratumoral injections of 10^5 pfu of HSV1716. Four to 9 days later, tumors were resected and assayed for viral replication, with findings confirming viral replication in the absence of toxicity in both HSV seropositive and HSV seronegative patients.

A third phase I trial, intended to evaluate the safety of HSV1716 into the resection cavity, was performed by the same UK group [33]. Twelve patients with newly diagnosed high-grade glioma underwent surgical resection followed by inoculation of the tumor bed with HSV1716. In addition to a lack of toxicity, they reported long-term survival (15–22 months) of 3 treated patients. Clinical trials powered to demonstrate efficacy have not yet been completed.

M032

Genetically engineered, neuroattenuated HSV expressing *trans*-genes that code for cytokines such as interleukin (IL)-12 have been shown to provide a survival benefit in murine brain tumor models via oncolytic effects combined with immunologic effects mediated by T cells [34]. M032 is a second-generation oncolytic HSV that is rendered conditionally replicative by the deletion of both $\gamma_1$34.5 gene copies. Additionally, the virus serves as a gene therapy vector, armed with a bicistronic expression cassette encoding human IL-12. A phase I clinical trial is currently enrolling at the University of Alabama Birmingham (NCT02062827). The trial seeks to determine the maximum tolerated dose of M032 in patients with recurrent or progressive GBM, anaplastic astrocytoma, and gliosarcoma.

G47Δ

This multimutated replication competent oncolytic HSV is built from G207 by the deletion of the α47 gene [35, 36]. Owing to overlapping transcripts encoding ICP47 and US11, the deletion has dual functions: 1) enhanced major histocompatibility complex class I presentation because ICP47 inhibits the transporter associated with antigen presentation; and 2) enhanced viral replication due to the late *US11* gene being under control of the immediate-early *α47* promoter [36]. Taken together, these results suggest that G47Δ may have enhanced antitumor activity; the results of a phase I/II Japanese study in recurrent and progressive GBM are pending (JPRN-UMIN000002661).

Conditionally Replicating Adenovirus

Adenovirus is a double-stranded, nonenveloped DNA virus that is a common human pathogen, usually causing mild upper respiratory symptoms [18]. Human adenovirus serotype 5 is one of the most studied OVs and has served as a backbone for multiple genetically engineered viral agents. Gene deletions involving E1A and E1B have been used to generate conditionally replicating adenoviruses. Adenovirus-Δ24 conditionally replicates in tumors with a deregulated retinoblastoma pathway due to a 24-base pair deletion in the E1A gene, which renders the protein unable to bind cellular retinoblastoma [37, 38]. ONYX-015, as a result of E1B deletion, was conceived as an OV that would conditionally replicate in tumor cells with a dysfunctional p53 pathway [39, 40]. However, later studies of ONYX-015 demonstrated that its tumor selected replication is instead determined by efficient export of late viral RNA in tumor cells in the absence of E1B-55K [40]. Consequently, loss of E1B-55K-mediated late viral RNA export, and not p53 degradation, is the key feature of tumor-selective replication [40].

ONYX-015

In a phase I dose escalation trial of ONYX-015, an E1B-attenuated adenovirus, for the treatment of malignant glioma [41], 24 patients received peritumoral injection of ONYX-015 following resection of a recurrent malignant glioma. No patient experienced an adverse event related to the experimental therapy, and the maximum tolerated dose was not reached

Of interest, H101, an E1B gene-deleted adenovirus very similar to ONYX-015, was approved in China in 2005 for the treatment of head and neck or esophageal squamous cell carcinoma [42, 43]; however, there have been no human clinical trials of this virus conducted in gliomas.

DNX2401/DELTA-24-RGD

In an effort to overcome untargeted viral replication and the relative paucity of coxsackie–adenovirus receptors (CARs) on the surface of glioma cells, a RGD-4C peptide motif was inserted into the adenoviral fiber of adenovirus-Δ24 [44]. This modification allows the virus to bind to integrins in a CAR-independent manner, increasing infectivity of glioma cells with low CAR expression [44]. This virus was evaluated in a clinical trial of recurrent malignant glioma. Although the phase I results of DNX2401 (formerly known as DELTA-24-RGD) have not been published, Lang et al. presented their findings at the Society for Neuro-Oncology Meeting in 2004. No toxicity was reported with a maximum tolerated dose of 3×10^{10} virus particles. Histologic analysis of post-treatment surgical specimens identified evidence of infection, replication, and tumor lysis; additionally, complete responses were seen in 3 (12%) patients.

A Dutch group has completed a phase I/II trial of DELTA-24-RGD administered by convection-enhanced delivery for the treatment of recurrent GBM (NCT01582516); the results are expected to elucidate whether this delivery approach will prove effective for DNX 2401.

Ongoing trials include a phase I trial of DNX2401 in combination with a short course of temozolomide for the treatment of GBM at first recurrence (NCT01956734) and a multicenter phase Ib trial that will randomize patients between DNX-2401 alone or DNX-2401 + interferon-γ for recurrent GBM or gliosarcoma (NCT02197169). These trials will evaluate the apparent synergy between oncolytic adenovirus and temozolomide in the treatment of glioma [45], and the anti-tumor effects of interferon-γ [46] when combined with OVs, respectively.

Reovirus

Reoviruses are nonenveloped, double-stranded RNA viruses generally associated with mild or subclinical symptoms in humans [18]. The unique double-stranded RNA genome of the virus, through interaction with the protein kinase R pathway, renders the virus naturally oncoselective for tumors with upregulated Ras pathways [47]. Under normal conditions, the double-stranded RNA of the virus activates protein kinase R, thus halting protein synthesis and promoting apoptosis. In many tumor cells the activation of this pathway is inhibited by upregulated Ras signaling via epidermal growth factor receptor mutations [48]. While activated Ras signaling appears important to reovirus replication, the mechanism of oncolysis remains unclear and is the source of ongoing research [49].

Reolysin™

Following the encouraging results of reovirus inoculation into immunocompetent nonhuman primates [50], a phase I dose escalation trial was performed to determine the safety of a single intratumoral injection of genetically unmodified reovirus [51]. Twelve patients with histologically confirmed recurrent malignant glioma were enrolled. Median survival was 21 weeks and the maximum tolerated dose was not reached. A follow-up multi-institution phase I study confirmed the safety of utilizing infusion over a 72-h period [52]. This is the first report of this delivery method for an OV in a clinical trial in North America. Despite 1 grade 3 adverse event, dose-limiting toxicities were not identified and a maximum tolerated dose was not reached.

A Mayo Clinic group is currently enrolling patients in a phase I study of reovirus in combination with GM-CSF in pediatric patients with relapsed or refractory brain tumors (NCT02444546). The goal of adding GM-CSF is to enhance the antitumor immune response and it has been used in other immunotherapy and viral trials [53].

Paramyxovirus

Paramyxoviridae is a family of negative-stranded RNA viruses that include measles and Newcastle disease virus (NDV). The measles virus is a highly contagious human pathogen causing a characteristic erythematous maculopapular rash and in very rare cases subacute sclerosing panencephalitis. In contrast, NDV is primarily an avian pathogen, causing only mild symptoms in humans [54].

NDV

The ability of NDV to replicate in and lyse tumor cells was demonstrated as early as 1955 when Flanagan et al. [55] introduced NDV into Ehrlich ascites cells. This led to early studies in animal tumor models, with encouraging results in both human neuroblastoma [56] and fibrosarcoma [57] following direct administration of the virus. Two strains of NDV have been evaluated in early-phase studies for glioma: MTH-68/H and NDV-HJU.

Csatary et al. [58] detailed a case report of a 14-year-old boy with a recurrent, progressive GBM treated with intravenous NDV vaccine (MTH-68/h, a live attenuated oncolytic strain) during a 2-year period, resulting in marked tumor shrinkage [58]. This group followed their initial publication with a series of 4 patients (3 children and 1 adult) with treatment refractory high-grade gliomas, resulting in survival rates of 5 to 9 years [59]. At the time of publication, all patients were alive and receiving MTH-68/h as their sole form of oncotherapy. The first histologic evidence of MTH-68/h-induced apoptosis of human glioma cells *in vivo* was provided in a case report of a young boy with a refractory anaplastic astrocytoma treated with MTH-68/h plus valproic acid [60]. This led the authors to conclude that combination therapy with OV and valproic acid may result in synergistic antineoplastic effects.

The lentogenic NDV, known as NDV-HJU, was evaluated in a phase I/II trial for treatment of recurrent GBM and represented the first study of systemically administered lentogenic NDV in patients with GBM patients [61]. Lentogenic (avirulent) strains of NDV cause mild or asymptomatic illness in birds, whereas MTH-68/h is a mesogenic (moderately pathogenic) strain [2]. A total of 14 patients were enrolled and 11 treated. Toxicity was minimal and 1 patient achieved a complete response.

Measles

In contrast to wild-type measles virus, the Edmonston vaccine strain exhibits oncoselectivity at the level of cell surface binding. The H protein mutation of the Edmonston vaccine strain causes the virus to display a high affinity for cellular CD46 receptors [62], which are commonly overexpressed on tumors [63]. In response to the difficulty of tracking viral gene expression *in vivo*, human carcinoembryonic antigen (CEA) was inserted as an easily detectable serum maker of gene expression [64]. The resulting OV, MV-CEA, demonstrated potent antitumor activity in subcutaneous and orthotopic U87 animal models [65], and proved safe in a phase I/II trial in nonhuman primates [66]. A phase I trial of MV-CEA for the treatment of recurrent GBM is currently enrolling (NCT00390299). This trial consists of 2 arms: 1) viral administration to the resection cavity; 2) intratumoral catheter administration of the virus followed by resection with virus administered to the tumor bed.

Poliovirus

The poliovirus (PV) is a single-stranded, positive-sense RNA human enterovirus and the causative agent of poliomyelitis [18]. However, the neurovirulence of the poliovirus is attenuated by mutations in the internal ribosomal entry site (IRES) [67]. Transposition of the human rhinovirus type 2 I.E. element into the poliovirus type 1 (Mahoney) results in PV-1–RIPO [67], which proved safe in animal glioma models [68]. This PV recombinant retained its natural affinity for the poliovirus receptor CD155, a receptor commonly overexpressed in high-grade glioma [69].

PVS–RIPO

In an effort to further attenuate RIPO, PVS–RIPO was derived from the live attenuated SABIN poliovirus vaccine [70]. This virus combines the neuroattenuation of the human rhinovirus type 2 I.E. with attenuating mutations affecting the RNA-dependent RNA polymerase [70]. Subsequently PVS-RIPO was shown to eliminate glioma cells *in vivo* without adapting to a pathogenic phenotype, thus supporting its use in clinical trials [71]. While the phase I dose escalation trial for convection-enhanced delivery of PVS-RIPO in recurrent GBM is currently enrolling (NCT01491893), preliminary results are promising, with 3 of 7 patients demonstrating a complete response [72].

Parvovirus

Parvoviruses are small, nonenveloped, single-stranded DNA viruses and include parvovirus B19, a well-known human pathogen and the causative agent of fifth disease [18]. The parvoviridae family includes adenoassociated viruses (genus *Dependoparvovirus*; usually requiring a helper virus for replication) and autonomous parvoviruses (genus *Parvovirus*; capable of independent replication in permissive cells) [73]. Autonomous parvoviruses demonstrate oncoselectivity owing to more efficient viral replication in malignant cells rather than superior virus uptake [74, 75]. Their oncosupressive effect appears to result from 2 interrelated actions: direct oncolytic effects and indirect immune reaction [74, 75]. These oncosuppressive effects were confirmed in human glioma cell lines, paving the way for early-phase clinical trials [76, 77].

ParvOryx

The rat is the natural host for parvovirus H-1 (H-1PV), and, in general, this wild-type strain is considered nonpathogenic but able to replicate in human cells [73, 78]. This wild-type strain was tested in orthotopic glioma models demonstrating evidence of tumor regression and improved survival [77]. The first trial of a parvovirus in humans was a phase I/IIa of ParvOryx (parvovirus H-1) for progressive primary or recurrent GBM (NCT01301430) [78]. This completed, dose-escalation study consisted of 2 groups: group 1 received H1-PV by intratumoral injection followed by administration into the tumor cavity; group 2 patients were treated with intravenous H1-PV followed by administration into the tumor cavity [78]. Results from this study are awaited.

Future Directions

Our understanding of the use of OVs as potential therapeutic agents for treating glioma and other tumors of the nervous system continues to expand rapidly. Since the first paper examining the possibility of using OVs for treating glioma was published 25 years ago, the number of viruses under examination and the groups studying this approach have exploded. The findings that have emerged over time have demonstrated that this approach is promising, but also have led to important new discoveries, which are shaping the direction of current and future research.

The first significant finding is that oncolytic viral therapy for glioma has, for the most part, been well tolerated with relatively few serious adverse events. In fact, there is often mention of a “maximal affordable dose” (tongue-in-cheek) instead of a maximum tolerated dose. When toxicities do occur, they often are related to the underlying disease and include events such as headache, seizure, fever, or progressive neurologic deficit. Presumed viral-related toxicities tend to be related to the inflammatory response produced as a result of viral infection and can produce many of the same symptoms. Serious adverse events definitively attributed to viral therapy

(i.e., encephalitis, death) have not been reported. Additionally, in studies where virus has been administrated into the tumor cavity surrounding the tumor, there does not appear to be an increased risk of toxicity to the patient, suggesting that for the majority of viruses tested, direct toxicity of the nervous system from viral replication is not a frequent event.

However, perhaps the most important finding over the course of these studies is the critical nature of the immune response in generating the antitumor effect. In some circles, rather than oncolytic viral therapy, treatment is referred to as oncolytic immunotherapy. It seems clear at this juncture that viral replication and local infection of the tumor is producing an inflammatory response, despite the often heavily immunosuppressed nature of these patients. Malignant gliomas produce a highly immunosuppressive local tumor microenvironment, with high levels of transforming growth factor-β, IL-10, and prostaglandin E2. There is significant numbers of immunosuppressive T regulatory cells and M2 phenotype macrophages present in association with these tumors. Additionally, patients have tended to be treated at time of recurrence, at which point they have received glucocorticoid, radiation, and chemotherapy, all of which combine to suppress the immune response. Despite these factors, multiple studies have shown the immune response that occurs after viral therapy and suggest that induction of a T_H1 cell response can be produced by treatment with a variety of OVs. Both clinical and preclinical studies to date have shown increases in inflammatory cells, including T cells, natural killer cells and macrophages, and T_H1 cell-related cytokines, after successful treatment with OVs, and the efficacy of OV is often decreased or lost in immunosuppressed mouse models, further implicating the immune system in the antitumor response. It seems promising therefore that combination treatment with other immunotherapies might be augmented by usage with OV. One question for the field to address is whether the type of virus administered is really as important as the immune response generated. Nonetheless, there does appear to be a difference in the antitumor response that is produced by different viruses, and it will be important to learn whether these differences and responses are more related to tumor genotype, viral genotype, or the antitumor immune response generated by the viral treatment.

Hand in hand with the inflammatory response induced by the viruses is the appearance of pseudoprogression, a state in which imaging findings are suggestive of tumor progression when, in fact, the patient is simply undergoing an antitumor immune response produced by the viral therapy With the development of the iRANO criteria, a standardized protocol for determining whether such progression represents actual tumor progression or simply inflammatory response to treatment has been initially outlined. However, the community as a whole will be interested in developing new and more sophisticated approaches to outlining which patients actually developed an antitumor immune response and which patients have simply sustained tumor progression earlier in the post-treatment time course. A third development in the oncolytic viral therapy field has been the utilization of OVs that express foreign genes to augment the antitumor immune response. This was initially described by Parker et al. [34] utilizing a virus that expresses IL-12. A humanized version of this virus is now in clinical trials (M032; described above); however, the only OV FDA approved today for human use is Imlygic©, formerly known as talimogene laherparepvec, which is being marketed by Amgen for use in metastatic melanoma. This OV expresses GM-CSF (described above). It will be interesting to see how further engineering of OVs in the future may lead to improved responses as the immune system is even better harnessed to augment the antitumor response.

In addition to provoking an increased immune response against the tumor by utilizing foreign gene expression from within the virus, the utilization of adjuvant agents to augment the immune response is being studied as well. Specifically, with the advent of checkpoint inhibition and its success in anticancer treatment, particularly in melanoma and other immunogenic cancers, the interest in this approach is high. In particular, with early results suggesting that checkpoint inhibition alone may not increase survival in patients with recurrent malignant glioma, the possibility of increasing the intratumoral immune response by utilization of oncolytic viral therapy followed by checkpoint inhibition appears to hold increased promise. Preclinical studies have already suggested this approach to be valid. A clinical trial utilizing Imlygic in melanoma in conjunction with checkpoint inhibitors is also underway (NCT02263508) [79].

Finally, the question of the importance of the viral replication and host cell lysis remains unanswered. Other approaches not discussed in this review include the use of nonreplicating viruses for the induction of immune response, as well as the utilization of nonlytic viruses such as Toca-511, a retrovirus that expresses cytosine deaminase but does not actually lyse the host cell. Instead, a long acting form of 5-fluoroscytosine is administered and tumor death occurs, both as a result of the use of the prodrug suicide gene expression, as well as the associated antitumor response. These various approaches will also add to our understanding of the mechanism of action of oncolytic viral therapy in general as they are further dissected.

Administration of Oncolytic Viral Therapy

The mechanism of administration of oncolytic viral therapy remains nonstandardized. Current approaches include simple injection *versus* attempts at convection-enhanced delivery. The mechanism of administration of oncolytic viral therapy remains nonstandardized. Current approaches include direct intratumoral injection, convection-enhanced delivery, and

systemic vascular delivery (intra-arterial and intravenous). Intratumoral or resection bed inoculation is the most studied and simplest method of introducing viral vectors into high-grade gliomas. It has the advantage of bypassing the blood–brain barrier and can introduce a high concentration of virus directly into the tumor. However, it is limited by its invasive nature and only delivers a single dose. The problem of virus reflux out of the injection tract (and out of the tumor) is significant. Total volume of delivery is limited as well, though this may be overcome in oncolytic virotherapy by viral replication. Convection-enhanced delivery, however, enables continuous delivery of virus into the region of interest enabling higher volumes of virus (and thus requiring lower titers!) and achieves a greater volume of distribution. Ongoing device development and various apparatuses in use are aimed at minimizing reflux and spill into the cerebrospinal fluid spaces. Much like intratumoral injection, convection-enhanced delivery is limited by the need for an invasive and at times complex surgical procedure. Advances in this area of bioengineering will have an important impact on the ability to deliver OVs. The development of novel image processing to delineate portions of the tumor that have not received viral treatment will also be important. Utilization of systemic approaches that minimize the need for neurosurgical administration of the viruses are also under consideration. While systemic methods could obviate the need for neurosurgical procedures, they will be subject to passage through the blood–brain barrier, potentially necessitating high doses of virus to reach therapeutic concentrations in tumor. The possibility of destruction of virus by the complement system and other aspects of the innate immune response can also be problematic. Finally, the possibility of utilizing cell-based therapy for delivery and/or augmentation of oncolysis remains to be explored.

Challenges Facing Oncolytic Virotherapy

The major hurdles for moving oncolytic viral therapy into the clinic have, in great part, been overcome. Specifically, initial viral therapy proposals were met with skepticism and concern by both reviewers and the FDA. As a result, extensive preclinical safety investigations were required involving the use of multiple species prior to *in vivo* evaluation in humans. As viral therapy has become more common and established a history of safety in humans, whatever the indication, some of these concerns (but not all) have been alleviated. Thus, the actual requirements from both reviewers and the FDA have somewhat softened over the years. Other hurdles for entering the clinic include the ability to make these viruses and improved molecular techniques have greatly assisted in the actual construction and purification of these viruses. The expense of manufacturing the virus in clinical grade remains a major hurdle; also, the small number of Good Manufacturing Practice facilities that are available to make such virus are sometimes backlogged. Again, as these agents become more and more accepted and routine, these obstacles are expected to become less problematic.

The future of oncolytic viral therapy is clearly bright. Speculation about the future of such therapy remains exactly that, speculation. However, it is clear the community in general views the immunotherapeutic aspect of viral therapy as critical to its success and perhaps the dominating force behind any therapeutic response. While opinions regarding the necessity for different viral approaches vary, it is clear that certain viruses replicate better and produce more robust immunologic responses than others. Thus, questions to be answered in the future remain: 1) can any virus be used, once modified appropriately, in a given cancer type, and even more so, within different subgroupings of that cancer as determined by mutational profiles; 2) might specific patient immune states result in a better response from a virus that is say more immunogenic (e.g., adenovirus), less immunogenic (e.g., measles), or moderately so (HSV); and 3) utilization of these agents with adjuvant interventions such as radiation therapy, chemotherapy/antiandrogenic therapy, and, most importantly, adjuvant immunotherapy, such as checkpoint inhibitors, is likely to be a major force in the evolution of viral therapy for human cancer.

Conclusion

While a quarter of a century has passed since the initial publication of the use of genetically engineered OVs for the treatment of glioma, much remains to be learned about optimal use of this therapy, including its mechanism of actions, optimal treatment paradigms, appropriate tumors for treatment, and integration with adjuvant agents. The future of oncolytic viral therapy for glioma remains promising and will undoubtedly impact the future of patient care in years to come.

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