Immunovirotherapy for the Treatment of Glioblastoma and Other Malignant Gliomas

Dagoberto Estevez-Ordonez, MD[a], Gustavo Chagoya, MD[a], Arsalaan Salehani, MD[a], Travis J. Atchley, MD[a], Nicholas M.B. Laskay, MD[a], Matthew S. Parr, MD[a], Galal A. Elsayed, MD[a], Anil K. Mahavadi, MD[a], Sage P. Rahm, MD[a], Gregory K. Friedman, MD[a,b], James M. Markert, MD, MPH[c,*]

KEYWORDS

• Glioblastoma • Immunovirotherapy • Oncolytic virus • Herpes simplex virus (HSV-1) • Brain tumors • Oncolytic virotherapy

KEY POINTS

- Immunovirotherapy has emerged as a promising targeted approach for treatment of GBM and other malignant gliomas.
- There are multiple viral prototypes for targeted oncolytic virotherapy and targeted drug delivery in various stages of clinical development with promising results.
- Herpes Simplex Virus type 1 offers numerous advantages as an oncolytic virus with several genetic enhancements currently being tested in clinical trials in adults and children.

INTRODUCTION

Glioblastoma multiforme (GBM) represents nearly half of all primary malignant brain tumors in adults, and malignant gliomas are a leading cause of cancer-related morbidity and mortality in children.[1–3] Outcomes for patients with GBM are poor, and effective treatment options are limited with individuals having a median survival of approximately 15 months.[2,4] The current treatment protocol focuses on maximal safe resection, radiotherapy, and concurrent tumor-treating fields/chemotherapy with temozolomide (TMZ) with only a modest effect on outcomes.[4–8] There are multiple factors that contribute to treatment resistance and recurrence of GBM. It is highly invasive, with glioma cells spreading widely within normal brain tissue at early stages.[9–11] GBMs contain tumorigenic glioma stem cells that contribute to tumor initiation, therapeutic resistance, and recurrence.[12] GBM also exhibits both intertumoral and intratumoral heterogeneity, which contributes to diagnostic complexity and limits the application of personalized, targeted therapies.[12]

There is a substantial need for novel therapeutic approaches that address several of these challenges. Immunovirotherapy has emerged as a targeted approach for treatment of GBM and other malignant gliomas with promising results.[5,13,14] Multiple viral vectors have been genetically altered and developed as oncolytic viruses and for targeted drug delivery. There are currently several ongoing clinical trials for the treatment of GBM with immunovirotherapy.[12,15,16] In this review, we

[a] Department of Neurosurgery, The University of Alabama at Birmingham, 1060 Faculty Office Tower 510 20th Street South, Birmingham, AL, USA; [b] Department of Pediatrics, Division of Pediatric Hematology-Oncology, The University of Alabama at Birmingham; [c] Department of Neurosurgery, Neurosurgery, Pediatrics, and Cell, Developmental and Integrative Biology, The University of Alabama at Birmingham, 1060 Faculty Office Tower 510 20th Street South, Birmingham, AL, USA
* Corresponding author.
E-mail address: jmarkert@uabmc.edu

Neurosurg Clin N Am 32 (2021) 265–281
https://doi.org/10.1016/j.nec.2020.12.008

discuss the recent advances and current state of viral vectors developed for the targeted treatment of GBM and malignant gliomas including their mechanism of action and clinical applications.

HUMAN ONCOLYTIC VIRUS MODELS

Adenovirus

Adenovirus (Adv) is a double-stranded nonenveloped DNA virus causing mild upper respiratory symptoms in humans that typically self-resolve. Within the realm of immunovirotherapy, recombinants of Adv that show conditional replication are some of the most studied oncolytic viruses.[16,17] The key to the multiple immunovirotherapy applications of the oncolytic Adv comes from its E1A gene, which is essential in its replication and is the first gene expressed on viral infection.[18] The Ki67 promoter for E1A expression can be upregulated in conjunction with arming the oncolytic Adv with interleukin (IL)-15 gene expression against GBM cells with resultant enhanced anti-GBM efficacy via activation of microglial cells.[18] Adenovirus can also be used to deliver suicide gene therapy.[19] These suicide genes have successfully induced apoptosis via conversion of the prodrug 5-FC into 5-fluorouracil in the presence of *Escherichia coli* cytosine deaminase (CD) and have encoded proteins that terminate protein synthesis within tumor cells.[19] Adenovirus, therefore, represents a multifaceted vector in the immunovirotherapy arsenal against GBM.

In 2018, Lang and colleagues[20] published landmark results from a Phase I, dose-escalation, biologic-end-point study investigating Delta-24-RGD oncolytic virus. Participants were separated into 2 groups, with group A receiving a single intratumoral injection of the virus into biopsy-confirmed recurrent tumor and group B undergoing intratumoral injection through an implanted catheter followed by en bloc resection days postimplantation to evaluate posttreatment specimens. The study demonstrated quite promising clinical results, with 20% of group A patients surviving more than 3 years posttreatment and 12% of patients demonstrating greater than 95% enhancing tumor reduction with associated more than 3 years of progression-free survival. Analysis of group B specimens postresection demonstrated direct virus-induced oncolysis with tumor infiltration by CD8 cells. Subsequent analyses of cell lines derived from these patients showed induction of immunogenic cell death after virus insertion into tumor cells. Overall, this Phase I study provided promising results demonstrating increased long-term survival in patients with recurrent high-grade gliomas due to the direct oncolytic effects of DNX-2401 adenovirus.[20]

A promising study recently published in *Neuro-Oncology Advances* found potentiating effects of the Adv Delta24-RGD on the response of a murine GBM model to anti-PD1 therapy overcoming tumor-induced immune suppression via significant recruitment of dendritic cells resulting in a robust antitumor response and survival benefit, suggesting the potential benefit of combination therapy.[21,22] Other mechanisms of action affect the function of T cells, specifically decreasing tumor-infiltrating T regulatory (Treg) cells and increasing interferon-gamma producing CD8 T cells. In addition, the oncolytic AdCMVdelta24 virus can augment systemic tumor antigen specific T cells and reprogram Treg cells to a stimulatory rather than immunosuppressive state.[23]

Reduced expression in immortalized cells/Dickkopf-3 (REIC/Dkk-3) is a tumor suppressor and therapeutic gene in many human cancers, including malignant glioma with promising results with adenovirus oncolytic therapy.[24] An adenovirus REIC vector was developed to increase REIC/Dkk-3 expression (Ad-SGE-REIC), which is currently undergoing a Phase I/IIa clinical trial for treatment of recurrent malignant glioma.[25]

Not only can the Adv vector be used to stimulate the antitumor immune response, but it also has possible applications to enhance intraoperative discernment of tumor tissue from normal brain. In 2015, Yano and colleagues[26] reported the successful use of a green fluorescent protein expressing adenovirus OBP-401 to label GBM cells to allow fluorescence guided surgery techniques to resect the murine GBM with nearly undetectable residual macroscopic tumor in the surgical bed.

Herpes Simplex Virus Type-1

Genetically engineered oncolytic Herpes Simplex Virus type 1 (oHSV), in particular, has been the focus of extensive preclinical and clinical research, offering several advantages as a therapeutic vector.[14] It is an enveloped icosahedral virus with double-stranded linear DNA that belongs to the Herpesviridae family. It is intrinsically neurotropic and does not integrate into the host cell DNA, making it an ideal vector for targeting primary brain tumors.[27,28] The deletion of essential genes required for replication in normal cells in combination with replacement of nonessential genes with foreign DNA can provide therapeutic advantages.[14,28] In addition, engineered oHSVs remain sensitive to antivirals, which contributes to its safety profile in the event of unanticipated adverse reactions.

The introduction of inactivating mutations in the $\gamma_1$34.5 neurovirulence gene, an essential gene for viral replication in normal cells in the central

nervous system, has been extensively used in oncolytic viral models.[29,30] In response to herpes simplex virus (HSV)-1 infection, normal cells activate the double-stranded RNA–dependent protein kinase R (PKR) system. This leads to phosphorylation of eukaryotic initiation factor (eIF) 2α inducing translational arrest and resulting in severe impairment of viral protein synthesis.[29] Infected cell protein 34.5 (ICP34.5), the product of $\gamma_1$34.5, reverses this process and is thus essential for successful viral replication in the central nervous system. Deletion of $\gamma_1$34.5 results in conditional viral replication within tumor cells that have low intrinsic PKR activity, such as human glioma.[5,29,30] This prevents productive infection in normal cells in the brain through PKR-mediated translational arrest while still maintaining oncolytic activity against glioma cells, which have defective signaling pathways and/or activating RAS mutations that suppress antiviral responses.[5,29,30] Clinical trials of $\gamma_1$34.5-deleted oHSV G207 (**Table 1**) have demonstrated safety with evidence of efficacy in both adults and children (**Table 2**).[14,31–37] Markert and colleagues[32] conducted a phase I trial on 21 adult patients and demonstrated safety at doses up to 3×10^9 pfu with 9 patients showing evidence of neuropathologic or radiographic response. A follow-up phase 1b trial on 6 patients with recurrent GBM receiving 2 doses of G207 totaling 1.15×10^9 pfu, with 13% of this total dose injected before tumor resection via a catheter placed stereotactically into enhancing portion of the tumor, also demonstrated safety and confirmed viral replication.[34] A third study demonstrated safety of vG207 in combination a single 5 Gy radiation dose in 9 adults with recurrent high-grade gliomas to provide in vivo synergistic viral replication based on preclinical data.[33] A clinical trial in pediatric supratentorial HGG trial is now complete and demonstrated safety of a controlled-rate infusion of intratumoral G207 up to 1×10^8 pfu (maximum planned dose) alone and combined with 5 Gy of radiation. Radiographic, neuropathologic, and/or clinical responses were seen in 11 of 12 patients. Matched pretreatment and posttreatment tissue in several patients demonstrated marked increase in tumor-infiltrating lymphocyte months after treatment with G207 (data not yet published).[31] A first-in-human trial assessing the safety of G207 alone and combined with 5 Gy of radiation in malignant cerebellar tumors, including malignant gliomas, is currently ongoing.[37]

Placing ICP34.5 or its human ortholog GADD34 under nestin promoter control (rQNestin34.5 and NG34) resulted in enhanced selectivity and efficacy compared with control virus in preclinical models.[38,39] Nestin encodes for the intermediate filament, which is a protein expressed during neuronal embryogenesis but not in the adult brain and it has been shown to be upregulated in malignant glioma, resulting in selective production of ICP34.5.[38,40] An ongoing Phase I clinical trial is currently ongoing to test the safety of these viral constructs (see **Table 1**). Another approach uses oHSV G47Δ constructed by deleting the *α47* gene, responsible for inhibiting the transporter associated with antigen presentation, from γ34.5-deficient HSV-1 vectors; leading to increased MHC class I expression in infected human cells and enhanced viral replication. Ongoing phase I-IIa clinical trials in Japan are assessing the safety and efficacy of G47Δ for the treatment of GBM.[41,42] Interim analysis of these showed that the 1-year survival rate of 13 patients was 92.3%.[42]

Pathophysiological hypoxia is a hallmark of high-grade gliomas. It fosters the glioma stemlike cell (GSC) phenotype and has been linked to tumor development, invasiveness, and resistance to chemotherapy and radiation. Although GSCs demonstrated no inherent resistance to oHSV, hypoxia may limit the oncolytic effect of some oHSVs.[43–46] To improve replication in such hostile environments without increasing neurovirulence, chimeric HSV C134 was developed to express the human cytomegalovirus (HCMV) PKR-evasion gene.[43,47] C134 is able to evade PKR-mediated protein shutoff and maintain late viral protein synthesis to significantly enhance virus replication, including in hypoxic conditions.[43] There is an ongoing clinical trial assessing the safety and therapeutic benefit of C134.[48]

In addition to direct oncolytic effects, oHSV can elicit a robust antitumor immune response.[1] Viruses with insertion of proinflammatory cytokine genes have been described, such as IL-12, which results in intratumoral production of IL-12 during viral replication to enhance targeted immune destruction.[13] IL-12 has potent antitumor properties that enhance the cytolytic activity of natural killer cells and cytotoxic T cells.[49] It also promotes the development of T_H-1 immune response, potentially eliciting a more durable antitumor effect.[49] Treatment with oHSV models producing IL-12 in combination with immune checkpoints (CTLA-4 and PD-1) have also shown promising results.[50] There are several completed and ongoing trials assessing the safety and therapeutic benefit of second-generation oHSVs (eg, IL-12 producing oHSV M032) in adults.[13,14]

Measles Virus

Measles virus (MV) is a single-stranded, negative-sense, enveloped RNA virus within the *Morbillivirus* genera of the Paramyxoviridae family. MV

Table 1
Ongoing and completed clinical trials[a]

Virus	GBM Type	Study Title	Phase	Biological	n	Duration	NCT Number and Reference	Status
Adenovirus	Recurrent	DNX-2401 (Formerly Known as Delta-24-RGD-4C) for Recurrent Malignant Gliomas	Phase I	DNX-2401	37	February 2009–February 2015	NCT00805376 [20]	Completed
	Recurrent	Safety Study of Replication-competent Adenovirus (Delta-24-RGD) in Patients With Recurrent Glioblastoma	Phase I-II	DNX-2401	20	June 2010–December 2014	NCT01582516	Completed
	Recurrent	Virus DNX2401 and Temozolomide in Recurrent Glioblastoma	Phase I	DNX2401	31	September 2013–March 2017	NCT01956734	Completed
	Recurrent	DNX-2401 With Interferon Gamma (IFN-γ) for Recurrent Glioblastoma or Gliosarcoma Brain Tumors	Phase I	DNX-2401	37	September 11, 2014–March 15, 2018	NCT02197169	Completed
	Recurrent	Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects	Phase II	DNX-2401	49	June 2016–June 2021	NCT02798406	Active, not recruiting
	Recurrent	DNX-2440 Oncolytic Adenovirus for Recurrent Glioblastoma	Phase I	DNX-2440	24	October 16, 2018–October 16, 2022	NCT03714334	Recruiting
	Recurrent	Oncolytic Adenovirus DNX-2401 in Treating Patients With Recurrent High-Grade Glioma	Phase I	DNX-2401	36	February 12, 2019–May 31, 2022	NCT03896568	Recruiting
Herpes	Recurrent	Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma	Phase I	G207	21	February 1998–May 1999	NCT00036699 [32]	Completed
	Recurrent	Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM	Phase Ib	G207	6	January 2002–August 2003	NCT00028158 [34]	Completed
	Recurrent	G207 Followed by Radiation Therapy in Malignant Glioma	Phase I	G207	9	May 2005–December 2008	NCT00157703 [33]	Completed

	Recurrent	Oncolytic HSV-1716 in Treating Younger Patients With Refractory or Recurrent High-Grade Glioma That Can Be Removed By Surgery	Phase I	HSV-1716	2	December 2013–May 2016	NCT02031965	Terminated
	Recurrent	Genetically Engineered HSV-1 Phase 1 Study for the Treatment of Recurrent Malignant Glioma	Phase I	M032 (NSC 733972)	15 of 26	September 2014–September 2023	NCT02062827	Recruiting
	Recurrent	HSV G207 Alone or With a Single Radiation Dose in Children With Progressive or Recurrent Supratentorial Brain Tumors	Phase I	G207	12	May 2016–April 2021	NCT02457845	Active, not recruiting
	Recurrent	A Study of the Treatment of Recurrent Malignant Glioma With rQNestin34.5v.2	Phase I	rQNestin34.5v.2	108	July 18, 2017–July 2022	NCT03152318	Recruiting
	Recurrent	HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Phase I	G207	15	September 12, 2019–September 1, 2024	NCT03911388	Recruiting
	Recurrent	Trial of C134 in Patients With Recurrent GBM	Phase I	C134	24	September 23, 2019–September 2024	NCT03657576	Active, not recruiting
	Recurrent	HSV G207 With a Single Radiation Dose in Children With Recurrent High-Grade Glioma	Phase II	G207	30	October 1, 2020–October 1, 2024	NCT04482933	Not yet recruiting
Measles	Recurrent	Viral Therapy in Treating Patients With Recurrent Glioblastoma Multiforme	Phase I	MV-CEA	23	October 23, 2006–November 30, 2019	NCT00390299	Completed, results not published yet
NDV	Recurrent	New Castle Disease Virus (NDV) in Glioblastoma Multiforme (GBM), Sarcoma and Neuroblastoma	Phase I-II	HUJ	0	July 2011–July 2011	NCT01174537	Withdrawn
Polio	Recurrent	PVSRIPO for Recurrent Glioblastoma (GBM)	Phase I	PVSRIPO	61	April 25, 2012–June 2021	NCT01491893 [75]	Active, not recruiting
	Recurrent	PVSRIPO in Recurrent Malignant Glioma	Phase II	PVSRIPO	122	June 1, 2017–December 2023	NCT02986178	Active, not recruiting
	Recurrent	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Phase I	PVSRIPO	12	December 5, 2017–July 1, 2021	NCT03043391	Recruiting

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Table 1
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Virus	GBM Type	Study Title	Phase	Biological	n	Duration	NCT Number and Reference	Status
Parvovirus	Recurrent	Parvovirus H-1 (ParvOryx) in Patients With Progressive Primary or Recurrent Glioblastoma Multiforme	Phase I-IIa	H-1PV	18	September 2011–May 2015	NCT01301430	Completed
Reovirus	Recurrent	A Phase I Trial of Intratumoral Administration of Reovirus in Patients With Histologically Confirmed Recurrent Malignant Gliomas	Phase I	Reolysin	12	June 2002–July 2005	N/A[89]	Completed
	Recurrent	Safety and Efficacy Study of REOLYSIN® in the Treatment of Recurrent Malignant Gliomas	Phase I	Reolysin	18	July 2006–June 2010	NCT00528684 [90]	Completed
	Recurrent	Wild-Type Reovirus in Combination With Sargramostim in Treating Younger Patients With High-Grade Relapsed or Refractory Brain Tumors	Phase I	Reolysin	6	June 21, 2015–January 1, 2025	NCT02444546	Active, not recruiting
Vaccinia	Recurrent	Safety and Efficacy of the Oncolytic Virus Armed for Local Chemotherapy, TG6002/5-FC, in Recurrent Glioblastoma Patients	Phase I-II	TG6002	78	October 12, 2017–September 2021	NCT03294486	Recruiting

Abbreviations: HSV, herpes simplex virus; MV-CEA, measles virus carcinoembryonic antigen.
[a] Citations are included only for clinical trials with published results.

Table 2
Summary viral constructs for the treatment of glioblastoma and other malignant gliomas

Viral Vector	Mechanism/Pathway Involved	Effect(s) on Tumor Cell
Adenovirus		
REIC/Dkk-3 + cRGD	Activation caspase-9; reduced expression B-catenin	Decreased proliferation rate
Antisense MMP-9	Downregulation of MMP-9 activity	Impaired tumor invasiveness
DNX-2401 + pembrolizumab	Increased epitope presentation to CD8+ T cells	Induced antiglioma immune response
AAV8 and AAV9 +IFN-B	Increase in tumor-associated microglia	Improved tumor sensitivity to chemoradiation; improved median survival
dsAAV2	Downregulation of TGF-B	Suppressed tumor growth; reduced tumor immunosuppressive effects
Herpes Virus		
G47Δ	Deletion of the γ134.5 and α47 genes and a disabling lacZ insertion within ICP6; Murine angiostatin insertion	Gain of function mutation leading to increased MHC class I expression in infected cells this resulting in enhanced viral replication
HSVtk + Flt3L	Release of HMGB1	Phagocytosis of tumor; activation of immune response
HSV-M032	Deletion in both copies of $\gamma_1$34.5 gene; Insertion of Human IL-12	Selective glioma cell replication and expression of IL-12 in infected glioma cells resulting in enhanced immune response and tumor cell lysis
HSV-G207	Deletion in both copies of $\gamma_1$34.5 gene and disabling lacZ insertion in UL39	Selective glioma cell replication
HSV-C134	Deletion in both copies of $\gamma_1$34.5 gene, expression of the HCMV TRS1 gene product	Selective and enhanced glioma cell replication
rQNestin34.5v.2	Deletion in $\gamma_1$34.5 gene and UL39; ICP-34.5 under control of synthetic nestin promoter	Selective and enhanced glioma cell replication
Lentivirus		
Sh-SirT1	Downregulation SirT1	Increased tumor sensitivity to radiotherapy
Sh-TLX	Downregulation TLX; expression of TET3	Impaired tumor growth and tumorigenicity of stem cells
GAS1 + PTEN	Decreased AKT and ERK 1/2 expression	Impaired tumor growth
Paramoxyvirus		
Measles (MV-CEA)	Attenuated strain modified to express the carcinoembryonic antigen gene	Designed to track viral gene expression in vivo via serum analysis to optimize dosing and administration schedule without resorting to histologic tissue analysis

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Table 2
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Viral Vector	Mechanism/Pathway Involved	Effect(s) on Tumor Cell
Measles (MV-NIS)	Attenuated strain modified to express human thyroidal sodium iodide symporter (NIS) gene	NIS can act as a reporter gene that enables the non-invasive tracking of viral localization, spread, gene expression and replication over time. NIS may also be used as a therapeutic transgene by allowing intracellular uptake of isotopes, such as ^{131}[I] (radiovirotherapy)
Picornavirus		
Poliovirus (PVSRIPO)	Enhanced immune cell infiltration; reduction of TIM-3 expression	Promote immune response and tumor inflammation
Retrovirus		
Toca 511	Increased delivery of 5-FC to tumor	Increased tumor sensitivity to radiotherapy

expresses a glycoprotein hemagglutinin protein H that has a high affinity for CD46 receptors shown to be overexpressed in GBM cells.[51,52] The MV Edmonston strain (MV-Edm), a well-known attenuated strain used to vaccinate humans against MV, has been further modified to express the carcinoembryonic antigen gene (MV-CEA).[53]

Phuong and colleagues[54] were the first to show that intravenous MV-CEA resulted in significantly prolonged survival and regression of in vivo glioblastoma tumor in mice bearing subcutaneous and orthotopic U87 tumors MV-CEA treated mice had no neurologic or clinical toxicity, which sparked further investigation. In subsequent studies, MV specificity for GBM was increased by developing retargeted oncolytic measles strains that invade via different receptors: epidermal growth factor receptor (MV-EGFR), EGF receptor variant III (MV-EGFRvIII), and IL-13Rα2 receptor.[55–58] Additional studies demonstrated that MV immunovirotherapy against GBMs can be enhanced with either adjuvant radiation therapy or anti-PD-1 antibody therapy.[59,60] Recombinant oncolytic MV (MV-NIS) is another example that was designed to express human thyroidal sodium iodide symporter (NIS) gene. NIS can act as a reporter gene via radiotracers and can also be used as a therapeutic transgene via radiovirotherapy, by allowing intracellular uptake 131[I] potentially enhancing the therapeutic efficacy.[61]

A phase 1 clinical trial treated 23 measles immune patients who were candidates for gross total or subtotal tumor resection of recurrent GBM with intracranial injection of MV-CEA.[62] One group received a total dose of MV-CEA ranging from 10^5 to 2×10^7 TCID50 via injection into the resection cavity. The second group of patients received one intratumoral MV-CEA injection and subsequently underwent tumor resection 5 days following this first intratumoral injection–time for projected maximum viral replication to be achieved– with a second MV-CEA injection into the resection cavity before closure. Resected tumor specimens were analyzed with in situ hybridization and immunohistochemistry.[63]

Poliovirus

Poliovirus is a positive-sense, single-stranded RNA encapsulated virus belonging to the Picornaviridae family known for its neurotoxic effects.[64] The prototype oncolytic poliovirus developed by Gromeier and colleagues,[65] PVS-RIPO, is the live attenuated poliovirus type 1 (Sabin) with its internal ribosome entry site (IRES) replaced by that of human rhinovirus type 2 (HRV2). Although this polio-rhinovirus chimera was found to possess neuronal incompetence, in vitro studies demonstrated its ability to infect and reduce glioma cell viability and trigger cytolysis of GBM primary cultures.[66–71] In subsequent animal studies, PVSRIPO was able to arrest tumor growth in both murine GBM flank tumor models and improve survival after intracranial virus administration in mice.[66,72] In addition, its efficacy was found to be correlated with CD155 expression, known to be overexpressed in human GBM.[73,74]

Indeed, its moderate success in preclinical models paved the way for a phase II clinical trial involving inoculation of 61 patients with recurrent GBM with PVS-RIPO. The results were published in a landmark article in 2018, which not only corroborated safety of intratumoral viral administration in humans but demonstrated an increase in patient survival rate from 4% to 21% at 36 months when compared with historical control groups.[75,76] Three other clinical trials on PVS-RIPO are currently ongoing assessing safety in children and combination therapy with lomustine (CCNU) and pembrolizumab.[76–78] Because clinical and radiographic responses were observed after the first cycle of chemotherapy administered for tumor progression in patients receiving PVSRIPO infusion, a second follow-up randomized trial of PVSRIPO alone or in combination with single-cycle CCNU in patients with recurrent World Health Organization grade IV malignant glioma is ongoing to further assess the potential of combination therapy CCNU.[76]

Reovirus

Another human virus that has shown oncolytic ability is the Respiratory Enteric Orphan virus or Reovirus, a segmented nonenveloped double-stranded RNA virus composed of 3 size groups. This naturally occurring virus, which is commonly isolated in the respiratory and gastrointestinal tracts of humans but causes mild to no symptoms, preferentially targets the activated RAS pathway.[79] The numerous downstream effectors induced by the RAS/RalGEF/p38 pathway in particular, have been implicated in promoting the reovirus life cycle and leading to cell death.[80–84]

Animal studies in severe combined immunodeficient (SCID) mice containing subcutaneous MG cell lines U251 N and intracerebral cell lines U251 N and U87lacZ showed a reduction in tumor burden after infection with serotype 3 (strain Dearing) live virus.[85,86] Lethality was also demonstrated in vitro in 83% of 24 established malignant glioma cell lines. The susceptibility of cells to reovirus may in part be attributed to the various ways reovirus circumvents cell defense mechanisms. For example, when 3-dimensional cultures of stem cell-like cells (GSC) from grade IV gliomas (glioblastoma) expressing junction adhesion molecule-A (JAM-A) were infected by the wild-type (wt) variant and the JAM-A independent jin-1 reovirus variant, viral entry and protein synthesis were similar.[87] JAM-A is typically used by wt reovirus for cell entry and level of expression is correlated with infectivity. These results suggest that reovirus may use alternative entry pathways for infectivity that avoid the JAM-A adhesion route. Interestingly, reovirus has been found to also upregulate PD-L1 expression lending credence to its use as part of a multifaceted tumor killing strategy with the use of PD-1/PD-L1 inhibitors.[88]

The first clinical trial using reovirus in recurrent malignant glioma demonstrated that intratumoral injection was safe.[89] Although the trial's purpose was not to show efficacy, 6 patients lived more than 6 months, 3 patients lived more than 1 year, and 1 continued to survive at 54 months. A subsequent study using convection-enhanced delivery also confirmed safety and noticed improved survival >2 years in select patients.[90] Intravenous administration of reovirus has also been evaluated in preclinical studies with promising results.[91]

ZOONOTIC ONCOLYTIC VIRUS MODELS

Newcastle Disease Virus

Newcastle disease virus (NDV) is a chicken pathogen with selective oncolytic properties applicable to various types of human cancer.[92] Molecularly, NDV is an avian paramyxovirus with a negative-stranded RNA genome.[17] Although the tumor-suppressive abilities of NDV have been extensively demonstrated through in vivo models and clinical trials, the exact mechanism is not fully understood. It is theorized that NDV achieves oncolysis via activation of a Ras pathway in addition to inducing secretion of tumor necrosis factor alpha (TNF-alpha) by mononuclear cells resulting in an enhanced antitumor immune response.[17] More recent studies suggest that the Ras-related C3 botulism toxin substrate 1 (Rac1) pathway may be the target of NDV.[92] Rac1 is involved in proliferation signaling by regulating gene transcription and G1 cell cycle progression. In GBM, Rac1 is therefore a crucial contributor to cell survival. NDV interactions with Rac1 are believed to induce cell cycle arrest along with degradation of the actin cytoskeleton and ultimately cell death.[92] Murine models have shown increased long-term survival after NDV injection due to cytotoxic T-cell infiltration.[16] However, this long-term survival benefit was not seen in immunodeficient murine models with depleted CD8 cells, stressing the importance of an intact host immune system for maximal benefit.[16] Type I interferon (IFN) expression in GBM cells also greatly impacts the effectiveness of NDV given the role of IFN in promoting an antiviral state and decreasing viral replication.[93] Nonetheless, recombinant NDV expression of an IFN antagonistic protein can overcome this protective role of IFN in GBM cells.[93]

NDV delivery to GBM cells can be targeted via mesenchymal stem cells (MSCs). This technique

takes advantage of the natural ability of MSCs to target sites of injury and inflammation, including tumors.[94] Higher rates of apoptosis were demonstrated in glioma cells when MSCs were used as the vector for NDV delivery as compared with direct NDV infection with similar virus titers. Moreover, TNF-related apoptosis-inducing ligand (TRAIL) has been identified as a key mediator in the antitumor effects of these hybrid MSCs due to synergy between TRAIL and NDV in the induction of apoptosis.[94] NDV can also potentiate the effects of TMZ. Bai and colleagues[95] found that when combined with TMZ, NDV inhibits AKT and activates AMPK, ultimately resulting in enhanced antitumor effects of TMZ and extended survival in a murine model. Clinical trials have demonstrated therapeutic efficacy and safety of autologous NDV-modified cellular vaccines or oncolytic effects in clinical trials but larger clinical trials are necessary to confirm efficacy.[96] In a phase I/II clinical trial, Freeman and colleagues[97] showed that the toxicity of NDV strain (HUJ, lentogenic) was minimal and a maximal tolerated dose was not achieved when administered intravenously to 14 patients with GBM using intrapatient dose escalation (1–11 billion infectious units) followed by 3 cycles of 55 billion infectious units with 1 patient achieving a complete response, and the others developed progressive disease.

Rodent Parvovirus

Certain members of the Parvoviridae family, a group of nonenveloped icosahedral single-stranded DNA viruses, can selectively kill malignant glioma cells while sparing normal cells in preclinical studies. These include rodent oncolytic viruses such as the Minute Virus of Mice and the more extensively studied rat parvovirus H-1PV.[98] Intratumoral and intravenous injection of H-1PV into 12 immunodeficient rats containing the U87 human glioma cell line resulted in prolonged survival and decreased tumor burden compared with controls.[99] The efficacy was in part due to a secondary viremia that resulted from progeny particles after initial tumor infection and boosted infection of remaining tumor cells. The lethality of H-1PV also extends to malignant gliomas resistant to death ligands such as TRAIL and DNA-damaging agents such as cisplatin.[100] The virus triggers accumulation of lysosomal cathepsins and downregulating cathepsin inhibitors. The orientation of certain variable regions of the capsid protein of H-1PV has also been tied to its infectivity.[101]

Studies in short-term and low-passage cultures of human grade IV and gliosarcoma cell lines also showed increased susceptibility to H-1PV at low multiplicities of infection (MOI; 1–5 infectious units per cell).[102] These cell cultures more closely parallel clinically diseased cells than do cells from long-term in vitro cell cultures. Intranasal application of H-1PV has also been shown to prolong survival in immunodeficient rats containing U87 human glioma cells versus controls. A Phase I/IIa trial of H-1PV in 18 patients demonstrated no dose-limited toxicity and widespread distribution after intratumoral and intravenous injection.[103,104]

Other Viral Vectors

Several other potential viral vectors have been described, but have not been assessed in clinical trials for GBM. Pseudorabies virus (PRV) and the Seneca Valley Virus (SVV), 2 viruses in which pigs are the natural host, have shown potential as oncolytic targets. However, intravenous infusion of PRV did not result in uptake within intracranial glioma cells.[105,106] SVV improved survival in mice bearing GBM as well as medulloblastoma and retinoblastoma models, which led to phase 1 clinical trials in adults and children with neuroendocrine tumors, which demonstrated safety, but no clear antitumor responses, and all patients rapidly developed anti-SVV antibodies and cleared the virus.[107,108] Vesicular Stomatitis Virus (VSV) and Sindbis Virus (SIN) are mosquito-borne viruses that have also shown oncolytic potential. Chimeric VSV-lymphocytic choriomeningitis virus, and VSV-Chikungunya virus mutants with replacement of the VSV glycoprotein have demonstrated tumor lysis with decreased toxicity to normal cells in glioma and intracranial melanoma mouse models.[109,110] SIN has tropism for neural cells and can cause encephalitis in mice.[17] Tropism for tumor cells is believed to be related to the high affinity laminin receptor, which is overexpressed in many tumors.[111] SIN can be a vector for introduction of hyperfusogenic membrane glycoproteins that lead to formation of syncytia and apoptosis.[112] Myxoma virus and Vaccinia virus (VV), within the Poxviridae family are the most promising candidates for malignant glioma virotherapy because they are highly immunogenic and capable of creating antitumor immunity.[113–116]

NONONCOLYTIC VIRAL VECTORS FOR GENE THERAPY OR TARGETED DRUG DELIVERY

Gene therapy has emerged as a potential treatment for malignant gliomas, whereby a vector introduces tumor suppressing or growth regulating genes into malignant cells. Multiple approaches are used for gene therapy including suicide gene, oncolytic gene, and tumor suppressor gene therapies.[117]

Viruses are a prime candidate for the introduction of gene therapies. They create a potent cytotoxic effect and are easily modified to facilitate genetic engineering.[19] Current approaches are attempting to target proteins commonly mutated or upregulated in GBM, including EGFR, PTEN, IDH-1, and p53.[118] The most common viral vectors include neurotropic retrovirus and adenoviruses. Retroviral vectors were among the first studied, and the first trial began in 1992 with a retroviral HSV-thymidine kinase (HSV-tk) with ganciclovir. HSV-tk acts as a suicide gene and converts the prodrug ganciclovir into its active form to inhibit cell division and DNA replication. The efficacy of this treatment was limited to small tumor sizes given its poor transfection efficiency.[119]

Adenoviral vectors have been used in clinical trials. An early study of an adenoviral vector with wt p53 gene (Ad-p53) showed efficacious transfection of tumor cells with minimal toxicity; however, similar to retroviral vectors, Ad-p53 demonstrated poor ability to penetrate tumor tissue widely.[120] Sandmair and colleagues[121] demonstrated increased survival time in patients receiving ganciclovir with adenovirus-delivered HSV-tk as compared with retrovirus delivery, again demonstrating poor retroviral transfection and tumor penetrance. In addition, adenovirus and HSV vectors have been used to introduce CD, which convert the prodrug 5-fluorocytosine into 5-fluorouracil, inducing apoptosis.[122] A phase I study in patients with recurrent glioma with aglatimagene besadenovec (AdV-tk), which adenoviral vector engineered to express the HSV thymidine kinase (HSV-tk) gene in conjunction with a synthetic anti-herpetic prodrug acyclic guanosine analogue administration demonstrated a safe dose range with 3 of 13 patients surviving more than 24 months.[123] A subsequent phase I trial in children treated with AdV-tk as adjuvant to surgery and radiation for pediatric malignant glioma and recurrent ependymoma also showed safety and potential efficacy.[124]

Lentiviral vectors have been used to introduce small-hairpin RNA (shRNA) to silence sirtuin 1 expression in GBM, which results in increased radiosensitivity with resultant increased tumor death.[125] Similarly, lentiviral delivery of human orphan nuclear receptor tailless (TLX) shRNA resulted in tumor growth inhibition and decreased tumorigenicity.[126,127]

CHALLENGES, LIMITATIONS, AND FUTURE DIRECTIONS

Although clinical trials have been completed or are ongoing for several oncolytic viruses, only a few have moved beyond a Phase I clinical trial.[16] Finding the ideal balance to achieve safety but also virulence to maximize efficacy remains a significant challenge.

Moreover, viral delivery remains a significant challenge, as most clinical trials have focused on intratumoral delivery. Recent trials have used stereotactic techniques to place a localized catheter into the tumor to use for administration of virus.[31,33,35,37,128] This requires a neurosurgical procedure and may limit additional doses. Thus, innovative routes of administration need to be devised, such as systemic, intrathecal, intracavitary, and intraventricular delivery. However, the challenges of systemic delivery are considerable due to the blood-brain-barrier and virus neutralizing antibodies, and the safety of these routes needs to be confirmed.[16]

Although the clinical results of several oncolytic viruses have been promising including HSV, poliovirus, and adenovirus, these studies have all been in recurrent, often heavily pretreated patients. Thus, it will be important to test immunovirotherapy in upfront regimens. Furthermore, future studies are needed to combine oncolytic viruses with other potentially synergistic approaches to maximize oncolysis an antitumor immune response such as immune checkpoint inhibitors, CAR-T therapy enhanced with bispecific T-cell engagers (BiTE), vaccines, and other immunotherapies.[14,50,129–131] For example, Saha and colleagues[129] demonstrated durable responses in an orthotopic GBM model by combining anti-PD-1 and anti-CTLA-4 antibodies with oHSV expressing IL-12. An alternate approach to systemic delivery of checkpoint inhibitors is by using oncolytic viruses carrying genetic material to express the immune checkpoint inhibitors locally.[103,132,133] In addition, CAR-T-cell therapy with bicistronic constructs can convert gliomas who have difficult-to-target surface topology to more familiar, targetable topology or help trigger enhanced immune responses with targeted, localized CD3 expression to facilitate local immunomodulation.[130]

SUMMARY

Immunovirotherapy has shown significant promise as a targeted therapy for malignant gliomas, and attempts to address several of the challenges often encountered in treatment, such as ability to treat unresectable lesions or addressing challenges encountered hypoxia, anti-inflammatory effects, and consequences of intratumoral and intertumoral heterogeneity in treatment. However, barriers related to therapeutic delivery, viral entry and replication, and immunosuppressed patients

must be overcome. Strategies such as arming viral vectors with enhancements (therapeutic transgenes, checkpoint inhibition, host antiviral immune response, improved and selective replication) and combining viruses with synergistic agents must continue to be developed and tested in the clinics so that the great therapeutic potential of oncolytic immunovirotherapy can be realized.

CLINICS CARE POINTS

- To date, no oncolytic virus has been approved by the FDA for the treatment of malignant glioma and all remain investigational treatments.
- Multiple ongoing clinical trials are currently enrolling participants, most of them available for patients with recurrent malignant gliomas.
- Oncolytic viral models engineered to alter/modulate various cellular and inflammatory pathways leading to selective replication in tumor cells, enhanced immune response, impaired tumor angiogenesis, amongst others.
- Multiple non-oncolytic viral vectors have been studied as gene therapy vectors in glioma; these varied approaches include increasing radiosensitivity via gene silencing and induction of tumor cell apoptosis in conjunction with various prodrug administrations.
- Talimogene laherparepvec (T-VEC) is the first US Food and Drug Administration (FDA)-approved oncolytic virus; and is currently indicated for advanced melanoma. T-VEC is an oHSV that and expresses human granulocyte macrophage colony-stimulating factor (GM-CSF) to active the immune system and has specific genetic deletions that result in improved capacity for MHC presentation.

ACKNOWLEDGMENT

The manuscript was supported in part by National Cancer Institute grants R01CA222903, R01CA217179 and a grant from Gateway for Cancer Research (all JMM -Last Author). And supported in part by the National Institutes of Health (Training Grant R25 TW009337) funded by the Fogarty International Center, National Institutes of Health Office of the Director, and National Institute of Mental Health. (DEO - First author).

DISCLOSURES

Dr J.M. Markert holds equity (<8%) in Aettis, Inc. (a company that holds stocks of oncolytic virus); Treovir, Inc (25%), a company holding intellectual property and funding clinical trials of oncolytic virus for pediatric brain tumors. A company that Dr J.M. Markert formerly held equity in (<8%) Catherex, Inc., was purchased in a structured buyout. Dr J.M. Markert has served as a consultant for Imugene. He also holds a fraction of the IP associated with oncolytic virus C134, which is licensed by Mustang Biotech.

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