

Reviews

Advancing glioblastoma treatment with oncolytic virotherapy

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ABSTRACT:

Glioblastoma is a highly invasive brain tumor with few therapeutic options for those affected. Since the 1990's, when an oral chemotherapy drug named temozolomide was introduced, the standard of care for glioblastoma has remained largely unchanged. A pressing need for novel therapeutics has been met with innovative research that aims to tackle glioblastoma with virotherapy. The discovery of oncolytic viruses that can selectively infect and eradicate tumors has revolutionized the therapeutic landscape. Since the first proof-of-principle experiments, the number of candidate viruses being evaluated for their oncolytic activity has exploded. This opportunity has been met with cautious optimism; rigorous safety testing has ensured that only the safest and most targeted viruses progress to clinical trials. Meanwhile, several groups have been constructing immune-stimulating recombinant viruses and evaluating their potential to enhance the oncolytic virotherapy movement. Simultaneously, numerous studies are assessing the efficacy of virus administration with concomitant immunotherapies. The results have brought astounding promise to the future of a glioblastoma treatment that is eager to be translated into the clinic.

INTRODUCTION

Glioblastoma is the most lethal and most common primary brain tumor in adults¹. The disease is characterized by rapid and invasive growth and a correspondingly dismal prognosis². Approximately 1,000 Canadians are diagnosed with glioblastoma annually and undergo maximal surgical resection followed by adjuvant chemotherapy and radiotherapy³. Despite these interventions, 90% of patients experience tumor recurrence within seven months, and fewer than 5% of patients survive five years after their initial diagnosis⁴. In recent years, key genomic and molecular aberrations that underlie glioblastoma pathogenesis have been uncovered, aiding prognosis and casting light on therapeutically-targetable signaling pathways⁵. Division by clinical subtype has noted primary (*de novo*) tumors to frequently harbor *EGFR* amplification and *PTEN* loss, while secondary tumors that develop from lower grade astrocytoma

often bear *TP53* mutations, *RB* loss, *PDGFRA* amplification, and *PTEN* loss. Further molecular classification has identified five subtypes (classical, mesenchymal, neural, proneural, and G-CIMP), each with distinct transcriptional signatures. The same investigations have provided evidence that extensive intratumoral heterogeneity is a hurdle to be overcome in the search for tumor-specific therapies. This suggests that a multifaceted treatment approach is not only favorable, but likely necessary, to advance the current standard of care⁶. Indeed, novel therapies for glioblastoma have taken advantage of this idea to target multiple aspects of tumor biology using small-molecule inhibitors, immunotherapy, gene therapy, oncolytic viruses, and more, often in conjunction with radiotherapy and chemotherapy⁷. This review summarizes the current standard of care for patients diagnosed with glioblastoma and elaborates on one emerging biotherapeutic agent, oncolytic viruses, currently undergoing evaluation for therapeutic efficacy.

The Path to Current Therapies

The current standard of care for glioblastoma patients consists of maximal surgical resection, radiotherapy, and concomitant temozolomide (TMZ)³. While this regime extends patient survival, the mortality rate for glioblastoma remains startlingly high⁸. However, the challenge in bringing more targeted therapeutics to the clinic is credited to several important factors: 1) intratumoral genetic heterogeneity; 2) tumor infiltration into intricate brain regions, making complete surgical resection unachievable in many cases; 3) physiological isolation of the tumor due to the blood-brain barrier; and 4) challenges identifying self-renewing glioblastoma stem cells (GSCs)^{6,9}.

TMZ, the major first-line therapy for glioblastoma, was first used to treat primary brain tumors in 1993^{10,11}. TMZ's cytotoxic activity comes from its ability to transfer a methyl group to DNA, with greatest anti-tumor activity observed upon methylation at the O⁶ position of guanine. The DNA repair response is initiated soon after but, unable to find a corresponding base, leaves long-lasting nicks in the genetic strand. These nicks accumulate and block the cell cycle at the G₂-M DNA damage checkpoint, which ultimately triggers apoptosis when cells cannot proceed through mitosis. However, the enzyme O-6-methylguanine-DNA methyltransferase (MGMT) plays a primary role in resistance to TMZ by transferring methylguanine's methyl group to a cysteine residue in the active site of MGMT⁷. Effectively, this reverses the cytotoxic lesion created by TMZ activity and allows the cell to proceed through mitosis unharmed. In line with this, the methylation status of *MGMT* has been correlated with responsiveness to therapy¹². Indeed, glioblastoma patients with epigenetic silencing of the *MGMT* gene by promoter methylation were observed to have a median survival of 21⁷ months after TMZ and radiotherapy treatment, compared to only 15.3 months in glioblastoma patients with silenced *MGMT* who received only radiotherapy¹³.

Since the introduction of TMZ, several therapeutic options have been discovered and reserved for salvage treatment after recurrence. These include bevacizumab, a vascular endothelial growth factor (VEGF)-targeting monoclonal antibody that inhibits tumor angiogenesis and vascular permeability; a PCV (procarbazine, lomustine, vincristine) cocktail with a high toxicity profile and similar mechanism of action to TMZ; and irinotecan, another chemotherapeutic that has been combined with bevacizumab to extend progression-free survival¹⁴. Despite the availability of these therapies, they have done little to extend overall survival for patients with glioblastoma, often work in only a subset of patients, and come with a host of toxicities and side-effects that reduce quality of life both during and after treatment¹⁵. Thus, while it is true that clinicians' therapeutic repertoire has expanded, the advent of biotherapeutics, namely immunotherapy and oncolytic virotherapy, suggests that more targeted, long-lasting approaches are on the horizon.

Oncolytic Virotherapy

The first report of a genetically modified oncolytic virus (OV) being used to treat cultured glioblastoma cells was pioneered in 1991¹⁶. The study used a herpes simplex virus (HSV) mutated in the thymidine kinase (TK) gene, named HSV-*dlsptk*, to specifically infect and kill U87 human glioblastoma cells while leaving non-dividing neurons unharmed. Since then, HSV and other candidate viruses have been further engineered to reduce neurotoxicity and enhance oncolytic activity. Notably, many of these modifications have armed OV's with immunostimulatory genes that, when expressed, enhance the host's anti-tumor immune response⁹. For example, a γ _{34.5}-deleted HSV with resulting attenuated neurovirulence has been engineered to express *IL-12* to stimulate helper T cell activity and enhance glioblastoma cell killing¹⁷. Approaches such as this have been so successful that several clinical trials are evaluating the safety and efficacy of OV's in a clinical setting (reviewed in (16)).

Unlike gene therapy which employs replication-incompetent viruses to deliver genes, OV's exercise a self-amplifying effect by replicating within cancer cells, triggering cell lysis, and spreading to nearby cells¹⁸. This is possible because malignant transformation activates signaling pathways that oppose cellular antiviral responses, such as the Wnt/ β -catenin and EGFR/Ras/MAPK pathways^{19,20}. One of the most notable alterations that occurs during tumorigenesis is loss of interferon (IFN) signaling, which is normally implicated in growth suppression, recognition of viral infection, and presentation to the host immune system²¹. While loss of IFN signaling confers proliferative, angiogenic, and immune-evasive advantages upon cancerous cells, it simultaneously impairs their ability to sense and clear viral pathogens²². This allows OV's to selectively infect and lyse cancerous cells without harming surrounding healthy cells. The resulting cell lysis contributes to the immunogenic properties of OV's by releasing tumor-associated antigens that can be recognized by the immune system and used to stimulate a long-term anti-tumor response²³. Recombinant OV's have also been engineered to express molecules that augment the virus' lytic activity and enhance the resulting immune response¹⁶. The properties of recombinant oncolytic viruses are summarized in Figure 1. A non-exhaustive list of several promising candidates for clinical OV therapy is described below and presented in Table 1.

Herpes Simplex Virus

Herpes simplex virus (HSV) is a double-stranded DNA virus with inherent neurotropism²⁴. HSV was the first OV to be experimentally applied to the treatment of glioblastoma, and it has since undergone extensive genetic modification to attenuate its infectivity in healthy cells and enhance its oncolytic properties^{25,26}. These modifications include mutations to the γ _{34.5}, UL39, and ICP47 genes, which ultimately limit viral infection, replication, and viral-mediated lysis to tumor cells. In addition to enhancing selectivity, HSV variants have been armed with therapeutic transgenes that generate an anti-tumor immune response by recruiting helper T cells, reducing regulatory T cell infiltration, and inhibiting angiogenesis in the local

Table 1. Oncolytic viruses undergoing evaluation for glioblastoma treatment (adapted from Wollman et al).¹⁶

Virus	Mechanism(s) of Selective Activity	Current Trial Stage	Host Species
Herpes Simplex Virus (HSV)	Mutations to TK, γ_{34.5}, and U₃₉ restrict viral replication and protein translation to tumors with compensating signaling	Clinical – Phase I/II	Human
Poliovirus (PV)	Selective binding to cell surface CD155 (Necl-5) – highly expressed in glioblastoma	Clinical – Phase I/II	Human
Reovirus (RV)	Viral genetic makeup limits replication to tumor cells with overactive Ras signaling to block PKR activation	Clinical – Phase I/II	Mammal
Zika Virus (ZKV)	Specificity for interferon signaling responses to infection (under investigation)	Preclinical – <i>In Vivo</i>	Human
Newcastle Disease Virus (NDV)	Tumor-specific regulation of IFN-α and induction of downstream genes	Clinical – Phase I/II	Avian
Vesicular Stomatitis Virus (VSV)	Dependent upon impaired interferon signaling in cancer cells	Preclinical – <i>In Vivo</i>	Livestock
Measles Virus (MV)	Selectively binds to cell surface CD46 which is overexpressed in glioblastoma	Clinical – Phase I	Human
Adenovirus (AV)	Mutations to E1B and E1A restrict viral replication to cells with defective p53 or Rb signaling	Clinical – Phase I/II	Human
Vaccinia Virus (VV)	Mutations to TK and VGF restrict viral propagation to tumors with compensating EGFR activity and nucleotide excess	Preclinical – <i>In Vivo</i>	Cow/Horse
Myxoma Virus (MXV)	Limited to cancer cells with defective interferon signaling and favors cells with overactive Akt signaling	Preclinical – <i>In Vivo</i>	Rabbit

microenvironment²⁷. An example of this is dvB7lg, a recombinant HSV expressing secreted soluble B7-1, which stimulates T cell activity and activate T cells in an anergic state²⁸. Similarly, a HSV variant expressing interleukin-12 (IL-12), named M032, and tested in a phase I clinical study promotes the tumor-killing activity of natural killer cells and cytotoxic T cells, while also interfering with tumor-induced angiogenic activity²⁹. Recently, the HSV T-Vec (*talimogene laherparepvec*) received FDA approval for use in advanced melanoma, highlighting the potential for this family of viruses to be adapted to other cancer types³⁰.

Poliovirus

Poliovirus (PV) is a single-stranded, positive-sense RNA virus that normally causes the human disease poliomyelitis. The virus is capable of entering cells by using capsid proteins VP1, VP2, and VP3 to bind to the PV receptor CD155 (Necl-5, nectin-like molecule 5), which has been shown to be highly expressed in glioblastoma patient explants and cell lines³¹. Unmodified PV's neurotoxicity further stems from the 5' end of its genome which bears an internal ribosomal entry site (IRES) essential for viral protein translation³². To test the efficacy of PV in glioblastoma, a recombinant oncolytic

PV, termed PVS-RIPO, combines the non-pathogenic IRES from human rhinovirus type 2 with the genome of the serotype 1 (Sabin) vaccine strain of PV³³. Together, these components dampen PV's neurovirulence and preclude viral replication and translation from occurring in healthy neuronal cells, but not glioblastoma cells³⁴. Pre-clinical studies of glioblastoma xenografts in mice demonstrate that PVS-RIPO is capable of completely eliminating tumors *in vivo* and promoting a host response to virus-induced tumor cell lysis³⁴. Indeed, intratumoral PVS-RIPO administration is observed to induce infiltration of dendritic cells, helper T cells, and cytotoxic T cells into the tumor microenvironment³⁵. Phase I trials are currently ongoing at Duke University (USA) to assess the safety and optimal dose of PVS-RIPO when delivered intracerebrally; to date, the virus has not demonstrated dangerous side effects in patients and does not spontaneously convert back to its wild-type neurovirulent form^{36,37}. Recombinant PVS-RIPO has demonstrated a 24-month survival rate of 24% and has been exceptionally made rapidly available to patients with glioblastoma while it undergoes further evaluation.

Reovirus

Orthoreovirus (RV) is a member of the reovirus family and has a double stranded RNA genome. This genetic structure, along with its transcripts, rapidly causes activation of the PKR pathway upon viral entry into cells, leading to the inhibition of viral RNA translation and induction of apoptosis³⁸. As a consequence, RV shows a tropism for cells with overactive Ras signaling pathways which block PKR function and allow the virus to survive in the host cell¹⁶. Past studies have demonstrated that over 16 glioblastoma cell lines are susceptible to RV-mediated infection and killing, making this OV a promising, and selective, therapeutic agent³⁹. RV administration, like other OV therapeutics, has been tested via direct intralesional injection, which is associated with several technical and safety challenges. However, a recent clinical study in patients with glioblastoma demonstrated that RV could be delivered via a single-dose intravenous drip and successfully crossed the blood-brain barrier – a first for OV delivery⁴⁰. Administration of RV increased leukocyte infiltration into the tumor site, which was supported by elevated mRNA levels of *CCL3* and *CCL4*, attractants of CD4+ helper T cells and CD8+ cytotoxic T cells, in whole-tumor RNA extracts. Immunohistochemical analysis of resected brain tumor samples also revealed increased expression of apoptotic markers, such as cleaved caspase 3, in RV-treated patients, as well as programmed death-ligand 1 (PD-L1) on both RV-treated glioblastoma samples and tumor infiltrating lymphocytes. To assess the efficacy of combining intravenous RV with programmed death 1 receptor (PD1)/PD-L1 axis blockade, an immunocompetent mouse model of glioblastoma was treated with either GM-CSF/RV followed by PD1 antibody treatment, virotherapy alone, or checkpoint blockade alone. In the combination approach, mice demonstrated significantly greater intratumoral inflammatory infiltrate, which included active helper and cytotoxic T cells. Mice treated with the combination therapy also survived significantly longer than their counterparts administered either individual regimen. An initial phase I clinical trial to determine the maximum tolerated dose

(MTD) of RV administered intratumorally was performed in 12 patients in 2008⁴¹. Impressively, no MTD was reached and there was no significant toxicity associated with treatment; in turn, patient survival was extended by up to six years.

Zika Virus

Zika virus (ZIKV) is a RNA virus of the flavivirus genus, which includes West Nile virus, dengue, and yellow fever viruses. ZIKV most commonly infects neural progenitor cells (NPCs) in the developing central nervous system, which has led to an outbreak of ZIKV-induced fetal microcephaly in recent years⁴². Upon infection by ZIKV, NPCs differentiate, lose their ability to proliferate, and subsequently undergo cell death. In adults, the effects of ZIKV infection are less severe, with rare cases of meningoencephalitis and Guillain-Barré syndrome being observed. Because NPCs share similar features with glioblastoma stem cells (GSCs), ZIKV's tropism for NPCs has been leveraged against glioblastoma⁴³. In a recent study, ZIKV was found to selectively infect GSCs *in vitro*, inhibit their ability to proliferate and self-renew, and increase apoptotic activity. The same effect was observed when patient-derived glioblastoma samples were inoculated with ZIKV, but did not occur with normal neural tissue specimens. To test the effects of ZIKV *in vivo*, mouse models of glioma were infected with a mouse-adapted ZIKV strain, named ZIKV-Dakar. This led to substantial tumor regression and prolonged survival in treated mice, but did not affect the growth of non-cancerous neural cells. To enhance the virus' safety profile, ZIKV is being engineered to bear mutations that will attenuate its capacity to infect differentiated neural cells, which would make it a novel platform for glioblastoma-targeted OV therapy.

Additional Viruses

Looking beyond the viruses described above, there are several other OVs at various stages of either pre-clinical or clinical evaluation.

Newcastle Disease Virus (NDV) is a single-stranded, negative-sense RNA virus derived from the avian paramyxovirus 1 family. While the mechanisms underlying NDV's oncoselectivity in glioblastoma are not well defined, it has been demonstrated to have substantial antitumor activity both *in vitro* and *in vivo*⁴⁴. Based on these reports, NDV has been accelerated to phase I and phase II clinical trials, with Freeman et al. reporting the first phase I trial of the highly attenuated NDV-HUJ strain being administered to patients with recurrent glioblastoma⁴⁵. The success of this trial in extending patient survival led the same group to initiate a larger phase I/II clinical trial that is currently ongoing.

Vesicular stomatitis virus (VSV) was identified as a potential OV candidate in an early study demonstrating its sensitivity to IFN-signaling, thus making it a prime candidate for treating IFN-impaired cancer cells⁴⁶. Indeed, the same group later replaced glycoprotein G, VSV's key mediator of neurovirulence, with arenavirus glycoprotein LCMV-GP (rVSV(GP)), thus attenuating

its neurotoxicity in healthy neural tissue. This led to tumor-specific lytic activity of rVSV(GP) in both glioblastoma cell lines and mouse xenograft models of glioblastoma⁴⁷. More recently, a chimeric VSV (VSV-LASV-GPC), encoding proteins from VSV and Lassa virus, also showed selective toxicity of glioblastoma tumors in mice⁴⁸. Importantly, this virus had the capacity to migrate within the brain, specifically infect and destroy a contralateral tumor, and leave healthy tissue unharmed.

The measles virus (MV) is a negative-sense, single-stranded RNA virus that normally causes infection of the respiratory system in humans. In 2003, Phuong et al. discovered that the highly attenuated Edmonston strain of MV, typically used for vaccination, displayed antitumor activity when grown with U87 glioblastoma cells⁴⁹. Indeed, MV's oncolytic and safety profile in animal models led to initiation of an ongoing phase I clinical trial for its safety in patients with glioblastoma⁵⁰. Recent efforts to enhance the tumor specificity of MV have engineered strains of MV expressing antibodies targeting EGFRvIII, a common mutation of the *EGFR* gene in glioblastoma, as well as IL-13, which targets the IL-13 receptor α2, a frequently overexpressed gene in glioblastoma^{51,52}. To further augment MV's oncolytic activity, the virus has also been combined with anti-PD-1 blockade therapy⁵³. The results of this study demonstrated a significant survival benefit conferred by the combined treatment in a syngeneic orthotopic mouse model of glioblastoma; this was attributed to an influx of inflammatory infiltrate, predominantly composed of CD8+ cytotoxic T cells. Thus, a dual immunovirotherapy approach may lead to the most efficacious and long-term response in a clinical setting.

CONCLUSION

While numerous preclinical studies have successfully treated glioblastoma using OVs, what remains is a safe, efficacious, and long-term survival response to be observed in clinical trials. In the coming years, we expect to see the results of many phase I clinical trials that are currently ongoing – the sheer volume of which holds promise that we will see successful OVs progress to phase II. Indeed, the number of viruses being evaluated as potential therapies has exploded; as repurposed oncolytic ZIKV demonstrates, these can also come from unexpected sources.

Since the first experiments using OVs, the scientific community has overcome numerous hurdles on the path to clinical viability. This is most evident in the stark contrast between initial studies which used unmodified, intracranially-administered viruses and more recent efforts that use genetically-enhanced, intravenously-delivered viruses that cross the blood-brain barrier to reach CNS tumors. Encouragingly, safety trials have rarely demonstrated adverse side effects of OV administration; the most common toxicities relate to the inflammatory response initiated upon viral infection and produce many of the same symptoms. Despite these accomplishments, several barriers still exist to the widespread adoption of OV therapy for the treatment of glioblastoma. These

include developing a minimally invasive method of OV delivery, likely through intravenous administration that is successful for a range of OVs; maximizing OV intratumoral distribution in order to achieve clinical efficacy; and evading host-mediated antivirus immunity while generating antitumor immunity to provide sustained tumor control. Current pre-clinical evaluations of OVs use animal models that aim to replicate the genetic and histological changes seen in human glioblastoma. Since many of these models do not faithfully mimic the complex nature of the human disease, this presents an additional challenge that often manifests in unsuccessful clinical trials. Indeed, achieving a pre-clinical model that is representative of the heterogeneous nature of glioblastoma should be a goal in parallel to OV development. An inability to overcome one or more of these hurdles has hindered OV translation in the past, leading to poor outcomes, a lack of success in pre-clinical and clinical trials, and discontinuation of particular OV approaches. These considerations will be important to bear in mind for future OV development.

One of the most important findings gained over the course of OV studies is the capacity to generate a long-term anti-tumor immune response secondary to OV infection. This has been accomplished by combining OVs with existing immunotherapies which, as studies have shown, can significantly improve the outcome of either treatment alone. Alternatively, recombinant viruses have been created to express immune-stimulating molecules, and it is likely that combining both of these approaches will generate the most efficacious, long-term response in patients. Indeed, the only OV to exist on the market today is a recombinant virus expressing GM-CSF to stimulate the immune system. T-Vec is gaining widespread adoption for the treatment of advanced melanoma, and holds promise that other cancer-targeting OVs will follow in its footsteps.

The field of OV therapy is expanding at an outstanding rate, but such rapid growth warrants a review of our mechanistic understanding of how OVs work. In this regard, many questions remain to be answered. A thorough exploration of OV intratumoral activity, optimal mode of delivery, interactions with the immune system, and integration with immunotherapy will benefit this understanding and accelerate our path to the clinic. OV therapy has gained significant momentum and will have a remarkable impact on the future of cancer treatment.

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Figure 1. Summary of Key OV Modifications.

Recombinant OVs are made to selectively infect and lyse cancer cells while also stimulating an anti-tumor immune response.

Past OVs have harnessed some, or all, of these modifications to enhance the virus' oncolytic properties and optimally target glioblastoma tumors.

