Review

Oncolytic virotherapy in glioma tumors

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Abstract:

Glioma tumors are one of the most devastating cancer types. Of the different glioma tumors, glioblastoma is the most advanced stage with the worst prognosis. Current therapies are still unable to provide an effective cure. Recent advantages in oncolytic immunotherapy have generated great expectations in the cancer therapy field. The use of oncolytic viruses (OV) in cancer treatment is one of those immune-therapeutic alternatives. OV have a double oncolytic action by both, directly destroying the cancer cells, sparing the patient's life, and stimulating a tumor specific immune response to revert the ability of tumors to escape the control of the immune system. OV are one promising alternative to conventional therapies in glioma tumor treatment. Several clinical trials have proven the feasibility to use some viruses to specifically infect tumors eluding undesired toxic effects in the patient. Here we have revisited the literature in order to describe the main OV proposed so far as therapeutic alternatives to destroy glioma cells *in vitro* and trigger tumor destruction *in vivo*. Some clinical trials are exploring the use of this therapy as an alternative were other approaches provide limited hope.

Keywords: glioma; oncolytic virus; glioblastoma; virotherapy

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1. Introduction

Diffuse gliomas are the most frequent central nervous system (CNS) tumors with an infiltrative growth pattern which includes astrocytoma, oligodendrogliomas, and oligoastrocytomas [1]. These malignant tumors are classified by histology and molecular features established by the World Health Organization (WHO) [2]. Glioblastoma (GBM) categorized as WHO grade IV is the most common and lethal glioma with an incidence of 4.32 per 100,000 habitants in USA [3]. Since 2005, the treatment guidelines involves a combination of surgical intervention, radiotherapy and chemotherapy based on the DNA alkylating agent temozolomide (TMZ) [4]. Despite these aggressive therapies, unfortunately most of the tumors relapse and the majority of GBM patients die within 15 months [5]. Different factors are responsible for treatment failure, such as a high invasive and infiltrative potential, several resistance pathways and high intra- and inter-tumoral heterogeneity [6]. The presence of a subpopulation of cancer initiating cells appears to be the responsible of the tumor cell dissemination through the normal brain parenchyma[7], which contributes to gliomagenesis and recurrence [8].

Even though several strategies are being studied to overcome therapeutic resistance, the second- line treatment is not well established and different approaches are being performed [9]. Alternative radiotherapy regimens as tumor treating fields (TTFields) appears to increase the overall survival (OS) for some months [10]. The use of antiangiogenic agents as bevacizumab are able to add some quality of life, but fail to significantly increase patient´s OS [11]. Furthermore, there are no new Food and Drug Administration (FDA) validated therapies for GBM and all the current alternatives continue in research phases [12].

Many of the ongoing studies are validating the efficacy of immunotherapies including antitumor vaccine-based treatment, immune checkpoints and viral therapy. Virotherapy is considered a promising strategy for cancer treatment and could be divided into two different approaches: the use of non-replicating viruses as gene delivery vector systems and the oncolytic replicating viruses [13]. The oncolytic virotherapy (OV) that uses replicative viruses amplifies the viral progeny and the danger-associated molecular patterns (DAMPs) that triggers the innate and adaptative immune responses [14]. Tumor infection triggers both viral and tumor-specific immune responses, aiming at stimulating a tumor destruction through the induction of specific immunogenic cell death (ICD). OV can also be selected or engineered to be tumor-specific by genetic modifications that limit their pathogenicity and, or enhance tumor immunogenicity [15].

Upon viral infection, the host cell recognizes specific patterns of the virus known as pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). These receptors start the innate immune response, inducing signaling pathways that lead the expression of IFN-β and proinflammatory cytokines like IL-6, TNF-α, IL-1β among others, which promotes an antiviral state in the tumor environment [16]. However, it is known that some cancer cells are deficient in triggering this immune-mediated responses. This kind of tumor cells are more susceptible to viral replication mediated oncolysis [17]. Viral infection also can promote the antiviral response affecting the tumor microenvironment. The balance between the direct tumor destruction by the virus replication and the virus-mediated antitumoral immune-response determines OV effectiveness.

There are some constrains in the clinical application of OV that could limit the efficacy of this therapy. Some patients could have raided immune memory against specific OV from previous infections or vaccinations, which could restrict the oncolytic viral therapy. For example, systemic neutralizing antibodies could limit therapeutic viruses to access the tumor microenvironment, so most of them need an intratumoral administration [18].

Here, we review the state-of-the-art in the oncolytic virotherapies for the treatment of brain tumors (Figure 1). We present the main viruses that have been proposed for brain tumor oncolytic therapy alone or in combination with other therapeutic approaches. We focus on viruses used in preclinical studies (Table 1 and 2) and clinical trials (Table 3) performed mostly in GBM patients.

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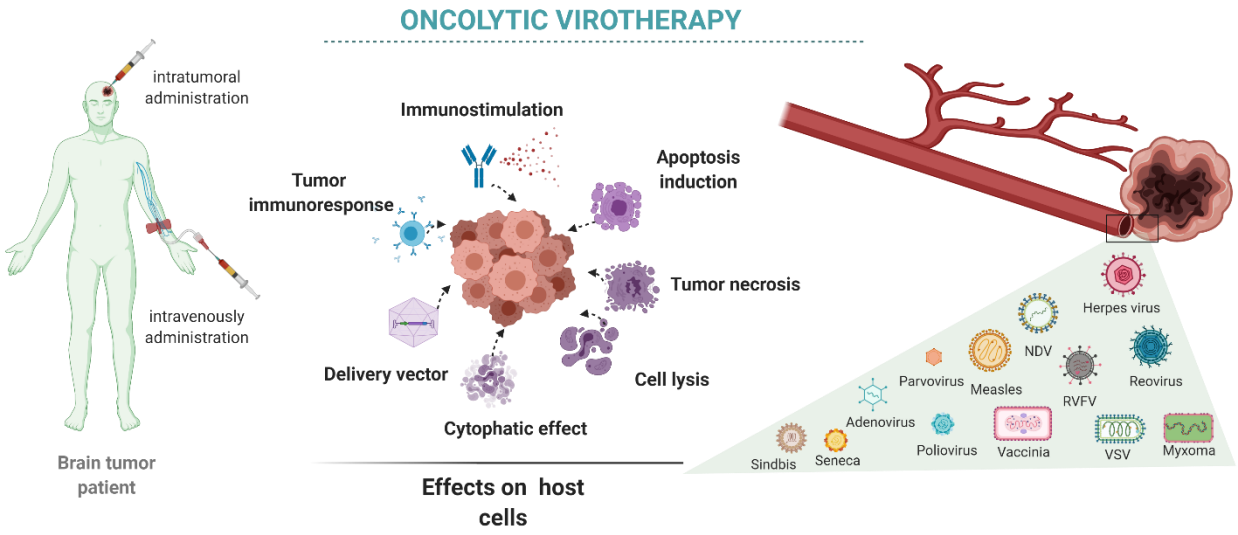

Figure 1. Oncolytic virotherapy in brain tumors: Intratumor or systemic administration of oncolytic viruses may have different oncolytic reactivity once the virus reaches the target cells. Those effects will depend on the characteristics of the tumor and the variety of available viruses and their characteristics.

2. DNA viruses proposed as glioma oncolytic agents.

2.1 Herpes Simplex Virus type I.

Herpes simplex virus Type 1 (HSV-1) is an enveloped double stranded DNA virus that belongs to *Herpesviridae* family. This virus is known by its ability to infect and replicate in neural tissue making it a candidate for glioma treatment. Natural HSV-1 entry is mediated by the binding of viral glycoprotein D (gD) to the cell surface protein CD111, also known as Nectin-1 [19]. Nectin-1 is differentially expressed in gliomas as compared to normal tissue [20]. It has been demonstrated that this virus mediates a direct lytic effect in tumor cells, but in addition, most of the *in vivo* effects propose a tumor destruction mediated by activation of tumor-specific immune responses [21].

As a neurotropic virus HSV-1 present though some toxicity and potentially may present side effects associated to normal tissue infection. The genetic attenuation of the virus can overcome this problem, allowing also the introduction of therapeutic genes. A first approximation for oncolytic attenuation of HSV-1 was HSV-1716, a modified HSV-1 strain 17 with a deletion of 759 bases in $\gamma_1$34.5 loci. $\gamma_1$34.5 is a viral antagonistic protein known to block PKR antiviral signaling in infected cells. This deletion was shown to prevent encephalitis in mice infected with the mutant virus by eliciting an abortive infection in non-tumor cells [22]. This virus has showed infectivity and oncolytic activity in cell lines and patient-derived gliomas cells [23]**.** Phase I and Ib clinical studies have demonstrated tolerance to HSV-1716 up to 10^5 pfu, and in two different studies in which 4 out of 9 and 3 out of 12 patients survived for more than 1 year [24,25]. A Phase II study has been completed but results are not published yet (NCT02031965**).**

A step forward in HSV-1 attenuation for oncolytic use was HSV-1 G207. This virus is a double mutant constructed by the insertion of *Escherichia coli* lacZ gene into the coding sequence for viral ICP6 gene and deletion of both viral copies of $\gamma_1$34.5 loci [26]. ICP6 is a ribonucleotide reductase essential for viral replication and growth in non-dividing cells. By eliminating this gene, HSV-1 G207 becomes tumor restricted. This virus showed no neurovirulence after intracerebral injection of $1x10^7$ pfu both in mouse and in owl monkeys. This OV shows also extended survival and lower tumor growth ratio in mice [27]. Phase I and Ib clinical studies demonstrated no neurovirulence in patients with GBM even at high doses ($3x10^9$ PFU) of intracerebral inoculation, an increase of six months in OS, with two of 21 patients surviving more than 5 years [28–30].

HSV-1 G47Δ is a triple mutant virus based on G207 to increase the oncolytic effect. G47Δ includes a deletion in α47 gene and herpes unique short 11 (US11) promoter. α47 is a viral antagonistic factor that inhibits MHC-I presentation. This deletion increases viral infection immunogenicity and places the lytic factor US11 under immediate-early α47 promoter, increasing tumor lysis. HSV-1 G47Δ has enhanced viral growth an displays a higher tumor lytic effect after intracerebral administration inoculation in mice [31]. Phase I clinical trial showed limited toxicity and a Phase II resulted in increased survival of treated patients [32].

Following a different strategy HSV-1 C134 was developed as a chimeric virus that not only includes deletion of $\gamma_1$34.5 loci, but also the expression of human cytomegalovirus (HCMV) IRS1 gene. This insertion increases viral replication and lytic effect when administered intrathecally in a murine GBM tumor models inducing antitumor T cell mediated immune responses, that elicits long systemic immune-memory enhancing survival [33,34]. A phase I trial in recurrent GBM is being conducted with this virus without results at this moment **(**NCT03657576).

Cytokine expressing HSV-1 is another approximation that has been explored in different studies for glioma treatment. HSV-1 expressing murine IL-12 in substitution of $\gamma_1$34.5 (M002) has been compared with other strains of HSV-1 such as R3659 and G207 in the intracranial 4C8 glioma mouse model, demonstrating an increase in animal survival, a higher tumor infiltration of CD4+, CD8+ and NK cells and a longer persistence of virus titers inside the tumor [35]. Similar results were obtained with HSV-1 R8306 in which $\gamma_1$34.5 genes were replaced by murine IL-4. This virus induced a higher infiltration of macrophages, CD4+ and CD8+ in the tumor and longer survival in mice in comparation with HSV-1 R8308, virus expressing the anti-inflammatory cytokine IL-10, or a control virus R3616 [21].

Finally, obtention of Talimogene laherparepvec (TVEC) resulted in one of the most promising modifications of HSV-1. Besides, having the double deletion of $\gamma_1$34.5, TVEC has a deletion of infected cell protein 47 (ICP47) and a double insertion replacement of GM-CSF gene into the $\gamma_1$34.5 loci. ICP47 is a viral protein that inhibits the antigen presentation through MHC-1 in infected cells, affecting the local immune response. In addition, the unique short 11 (US11) gene was translocated from its normal location, being now under ICP47 immediate early promoter control. Tumor production of GM-CSF induces a local immune activation by migration and differentiation of monocytes into macrophages and dendritic cells, increasing a T cell response against tumor epitopes in a systemic level [36, 37]. TVEC has demonstrated inhibition of tumor growth and immune stimulation in multiple tumors such as melanoma, colon or lung, inducing a protection against tumor re-challenge in mice [38]. The use of this OV has not been published in GBM patients yet.

2.2. Adenovirus

Adenoviruses are icosahedral non-enveloped viruses with double-strand DNA genome. 57 serotypes have been described in humans, some causing pathologies. In addition, other adenovirus serotypes infect different mammal species. Adenoviruses have been studied for decades, being an interesting viral vector for gene delivery. Characterization of the virus genetic elements and the possibility to manipulate them, has allowed the generation of recombinant viruses, allowing to develop several oncotherapeutic options.

Until now, better approximations into the use of Adenovirus as oncolytic therapy are the Conditionally Replicative adenovirus (CRad). Different generations of CRad have been developed within the years with promising results in gliomas in both pre-clinical and clinical studies [39].

First generation of CRad started with Onyx-015, a chimeric 2/5 Adenovirus serotype that has a deletion in the E1B-55kD gene. This protein binds and inhibits p53 in infected cells allowing viral replication. Due to this modification, Onyx-015 is deficient for replicating in non-tumor cells [40]. This virus showed a powerful antitumoral effect in both p53 wild type and p53 mutant glioma xerograph tumor models in mice, inducing a relevant tumor regression [41]. A phase I clinical trial was carried out with 24 glioma patients showing no adverse effects and regression in one and no progression of the disease in another patient [42].

A second generation of CRad was developed in order to not only decrease the infectivity of Adenovirus in no-tumor cells but also increase tumor infectivity. Delta-24 is an Adenovirus serotype 5 (Ad5) with a different mechanism than Onyx-015 to restrict replication in tumor cells. This virus has a 24 base pair deletion in E1A. This protein binds to the tumor suppression protein Rb. Delta-24 lacks this ability, which contributes to restrict virus replication to Rb-deficient tumor cells. This virus is modified also to incorporate an Arg-Gly-Asp (RGD) tripeptide. This sequence recognizes integrins present in gliomas allowing viral entry into the tumor cells [43,44]. This modified virus is called Delta-24-RGD [45]. *In vivo* studies showed a high antitumoral effect in tumors with lack in Rb pathway, while wild type cells remained resistant to infection [46]. Several phase I and II clinical trials have been carried on by using both Delta-24 virus and the Delta-24 RGD version (Table 3).

Although partial deletion of early gene E1A makes virus more restricted to Rb lacking cells, excessive accumulation of E1A protein can induce toxicity in normal cells. To overcome this situation, a recombinant virus was made inserting the cell cycle dependent E2F-1 promoter as the regulatory promoter for E1A protein. Thus, fast replicating tumor cells will preferentially express E1A as compared to non-tumor cells. This adenovirus that also includes de 24 base pair deletion of E1A and a RGD motive to improve tumor infectivity is known as ICOVIR-5 [47]. This virus showed less percentage of normal cells infected and stronger antitumoral effect in comparation to Delta-24 and Delta-24-RGD *in vitro*. A orthotopic murine model of U87 tumor cell xenografts treated with ICOVIR-5 showed longer survival than no treatment, and comparable survival rate than Delta-24-RGD [46]. Further modifications have improved these results, as ICOVIR-7 [48] or ICOVIR-15, which increase tumor cytotoxicity [49]. More relevant in the treatment of gliomas is ICOVIR-17 adenovirus, which in addition to the already mentioned modifications, express a soluble form of the human sperm hyaluronidase (PH20) regulated under the major viral late promoter (MLP) [50]. Hyaluronic acid is an abundant element of the tumor matrix and it is associated with tumor metastasis in the brain and inhibition of infiltration antitumor treatments [51,52]. This virus showed potent oncolytic activity *in vitro* as well as an increased survival in a murine GBM tumor model, in comparison with ICOVIR-15 [53]. VCN-01 is a modified ICOVIR-17 in which RGD motive has been relocated into the fiber shaft protein of the virus in order to increase infectivity. This virus shows a potent oncolytic activity against aggressive infiltrative and not infiltrative tumors both, *in vitro* and *in vivo* [54].

Immune stimulation is also another strategy that has been explored using adenoviruses to induce oncolysis and tumor regression. Delta-24-RGDOX is a modified Delta-24-RGD adenovirus that expresses the immune stimulatory OX40 ligand (OX40L) to stimulate antigen presentation in tumor cells by recruiting and activating T cells in a tumor specific way. This virus showed an efficient CD4+ and CD8+ activation in pre-clinical models [55]. It is now in a Phase I clinical trial with GBM patients (NCT03714334).

The use of CRad as a therapeutic option in GBM treatment has additional possibilities to be explored. Recent advantages in the use of high capacity adenoviral vectors provides an interesting platform to deliver therapeutic genes to the tumor environment [56]. Either alone or in combination with other treatments, the use of this non replicating viral vectors is an interesting tool that needs additional attention.

2.3. Vaccinia virus (VV)

Vaccinia is an enveloped double stranded DNA virus belonging to the *Poxviridae* family. VV can be easily modified making it an interesting tool to generate recombinant virus vectors as well as to design alternative approaches for glioma oncolytic treatment.

Strategies to increase the virus oncolytic potential are focused in enhancing apoptosis, as the recombinant rVV-p53 that has shown a higher ability to trigger apoptosis in both *in vitro* glioma cells and animal tumor models compared with wild type VV [57]. IL-12 and IL-2 are two important cytokines involved in the activation of a robust Th1 immune response. Recombinant viruses expressing these cytokines present in general an effect in halting tumor growth and promote the antitumor specific activation of the adaptive immune response. In order to avoid toxicity, the recombinant VV expressing these cytokines must be

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administered in a very low dose (10^2-10^3 pfu) [58]. Combination of different recombinant VV is another approach that has been explored in GBM treatment. Coinfection of high doses of rVV-p53 ($2x10^7$ pfu) with a low dose of a recombinant rVV-mIL12 (10 pfu) resulted in a strong tumor inhibition with an increase in immune response after intratumoral injection in a nude mouse glioma model [59]. However, this strategy must be validated in an immune competent animal tumor model to determine all its potential.

A double-deleted version of western reverse (WR) VV strain, also known as vvDD has been developed in order to increase cell lysis and at the same time limit the grow of this aggressive strain into tumor cells [60,61]. vvDD lacks thymidine kinase protein (TK), determining virus dependence on dividing cells to replicate, and Vaccinia growth factor (VGF), a secreted protein that primes surrounding cells for division and VV infection [60]. This virus has demonstrated an efficient destruction of rat and human malignant glioma tumor cells *in vitro*. Systemic delivery was able to reach solitary and multifocal tumors, increasing surveillance of animals [62]. A safety dose assay in non-human primates has shown that vvDD has not adverse effects in contrast with WR unmodified strain that produced several complications such as fever, skin rash or presence of virus in multiple organs [61]. Combination of vvDD with other GBM treatments, such as rapamycin or cyclophosphamide, seems to increase the oncolytic potential of the virus [62]. A modification of this virus expressing IL15Rα (vvDD-IL15Rα), aims at boosting the immunostimulating effect of the virus in combination to direct lytic effect, has been proven quite efficient killing of murine glioma cells *in vitro*. Intratumoral administration of this virus results in prolonged survival and a significant recruitment of NK and CD8+ T cells into the tumor. Secondary effects, such as ventriculitis-meningitis, was observed in some animals after the treatment [63].

A different strategy in the use of VV as an oncolytic virus is the combination of direct effect and a specific drug delivery system. Following this approach TG6002 was developed as a double-deleted recombinant VV virus that has been tested for the treatment of gliomas. This virus lacks TK and ribonucleotide reductase genes being able to replicate mainly in tumor cells. In addition, TG6002 has been modified to express the yeast FCU1 gene. This gene encodes cytosine deaminase and uracil phosphoribosyl transferase which transforms the pro-drug flucytosine (5-FC) into cytotoxic 5-fluorouracil (5-FU) and 5-fluoro-uridilyl monophosphate (5-FUMP). This virus can replicate in glioma cells and induce cell death *in vitro*. In addition, the systemic administration of TG6002 in an orthotopic brain tumor mouse model showed an increase of surveillance. These results were improved with oral administration of 5-FC pro-drug [64]. Currently, there are a phase I and II clinical trials using a combination of TG6002 and 5-FC in patients with GBM (NCT03294486).

2.4. Myxoma

Myxoma virus (MYXV) is an enveloped double-stranded DNA that belongs to the poxvirus family. This virus is highly pathogenic in European rabbits and has not been described to produce disease in other vertebrates [65]. However, this virus can infect and replicate in cells displaying deficiencies at the IFN system, making it a good candidate for oncolytic treatments [66].

MYXV has shown ability to infect and destroy cells in most human and rat glioma cell lines, being some of them partially resistant. This virus showed safety characteristics and showed tumor regression in an *in vivo* mouse model [67]. Part of the effect of MYXV is produced by a decrease on MHC I expression in infected glioma cells, and the consequent NK elimination [68]. One handicap of using MYXV as oncolytic virus *in vivo* is a low ability to proliferate outside the tumor injection area [67]. Oncolytic activity in this context will rely on a tumor specific immune stimulation upon virus injection. Different approaches have been used to increase expansion and effectivity of this virus. One of them is radiotherapy and TMZ pre-treatment followed by infection with MYXV, resulting in increased replication rate and decreased cell viability in GBM cell lines as compared to other non-tumor cells [69]. The use of MYXV replication-permissive cells as a delivery tool for the virus in the tumor is another strategy to colonize gliomas with the virus. Adipose-derived stem cells (ADSCs) are susceptible to MYXV replication without compromising cell viability. These cells are an excellent cargo for acting as a constant supplier of MYXV in tumors. Co-cultured Myxoma virus

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infected ADSCs with GBM cell lines results in a widespread infection and low viability of tumor cells. Intrathecal treatment of orthotopic GBM mouse models with MYXV-ADSCs, but outside of the tumor site, resulted in tumor infection and increased animal survival [70].

MYXV-M011L-KO is a modified MYXV lacking the antiapoptotic protein M11L. This virus showed a potent apoptotic effect in patient-derived GBM CSCs as compared to the wild type MYXV. MYXV-M011L-KO intratumor administration in a GBM tumor model of immunocompetent mice showed a synergistic effect with temozolomide co-treatment in prolonging animal survival [71].

2.5. Parvovirus

Parvoviridae is a family of single-stranded DNA genome. These viruses have an icosahedral capsid. So far there have been described 134 viruses which infect several hosts [72]. The rat protoparvovirus H-1 known as H-1PV is non-pathogenic in humans that binds to host cell surface receptors entering into cells by clathrin-mediated endocytosis [73]. H-1PV DNA replication occurs when active mitotic cells enter into S-phase [74], triggering a DNA damage response and cell-cycle arrest, that finally kill target cells.

It has been proposed that H-1PV only destroy cisplatin and TRAIL resistant glioma cells by inducing cathepsins B and L aggregation and decreasing the expression of their inhibitors, the cystatin B and C [75]. Complete GBM tumor regression was observed in rats models using H-1PV in an early tumor infection by Geletneky and colleagues [76]. Followed by this promising data, a phase I/IIa clinical trial was conducted in 18 recurrent GBM patients (ParvOryx01: NCT01301430) [77]. As a first approach the safety and the tolerance was evaluated, indicating a lack of toxicity and that upon intravenous administration, the virus can cross the blood brain barrier and reach the tumor [78]. Additionally, H-1PV can enhance immunogenicity within the tumor microenvironment [79]. H-1PV treated patients show an increase in tumor-infiltrating cytotoxic T cells, and induction of cathepsin B, and the expression of IFN-γ and IL-2, among other cytokines within the tumor microenvironment. These data together with the lack of toxicity and selective replication observed with Parvovirus such as LuIII and minute virus of mice (MVM) make *Parvoviridae* one good viral family candidate to treat GBM [80–83].

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Table 1. Preclinical studies of DNA viruses in glioma tumors.

Virus	*Cell lines*	*In vivo* models	Results
Herpes	Human: U87, T98G, SB18, U373 and U251 [23]	-	HASV-1716: tumor cells infection and death
	Human: U87, U373, U138 and T98G [27]	U87 i.c. and s.c. nude mice, i.c. owl monkeys	G207: Elimination of tumor cells, necrosis and no toxicit
	Human: U87 and U373 [31]	U87 s.c. in nude mice	G47Δ: increased survival, higher number of cured mice th G207.
	Human: D54, U87 and U251 Murine: N2A [33]	U87 i.c. in SCID mice	C134: reduces tumor volume and increases surveillance
	Human and murine: 12 established GBM [34]	N2A orthotopic in A/J and BALB/c mice	C134: improved replication and longer survival *in vivo*
	Murine: 4C8 [35]	4C8 i.c. gliomas in $B6D2F_1$ mice	M002: increase mice survival, infiltration of CD4+, CD8+ a NK cells, longer viral persistence in tumors
	Human: U251 and D54 [21]	GL-261 i.c. in C57BL/6	HSV-IL4: infiltration of macrophages, CD4+ and CD8+, longer survival
	Human: U87 [38]	U87 s.c. in nude mice	T-VEC: Inhibition of tumor growth, immunostimulation
Adenovirus	Human: 4 primary GBM [41]	S.c. xenograft in nude mice	ONIX-15: tumor regression
	Human: U251, U373, U87 and D54 [46]	D54 s.c. in nude mice	Delta-24: Cell death with low doses, single injection inhib tumor growth, and several injections resulted in 36% o animals with tumor regression
	Human: U251 and U87 [47]	U87 i.c. xenograft in nude mice	ICOVIR-5: Tumor cytotoxic effect *in vitro* high tumor selectivity and increase of survival *in vivo*
	Human: U87, U138, LN308, Gli36, U373, LN229 and 6 primary GBM [53]	U87 and CSCs i.c. in nude mice	ICOVIR-17: better distribution in HA tumors Longer mice survival
	Human: U87, A172, T98G, U251, U373, SNB19 and 2 GBM CSC [54]	U87 and GBM CSC i.c. xenografts in nude mice	VCN-01: Control of tumor growth One single injection improves survival in aggressive infiltrative tumor
	Human: U87 Murine: GL261 [55]	GL261 i.c. in C57BL/6	Delta-24-RGDOX: Proliferation of tumor specific T cells Sinergy with anti PD-L1
Vaccinia	Rat: C6 [57]	C6 s.c. in nude mice	Moderate cell apoptosis rVV-p53: Tumor growth control
	Rat: C6 [59]	C6 s.c. in nude mice	rVV-mIL12/mIL2: cytokine toxicity at high dose Antitumor effect NK dependent
	Rat: C6 [62]	C6 s.c. in nude mice	rVV-p53 and rVV-mL12: better tumor growth control Higher NK and macrophage infiltration
	Human: A172, U87MG and U118 Rat: RG2, F98 and C6 [61]	U87, U118 and C6 s.c. and RG2, F98 i.c. in nude mice	vvDD: control of tumor growth Sinergy with rapamycin or cyclophosphamide
	-	Rhesus macaques [63]	vvDD: no adverse effects
	Murine: GL261 [63]	GL261 i.c. in C57BL/6J	vvDD-IL15Rα: Increase of NK and CD8+ in tumor Prolonged survival
	Human: U87 and patient derived GBM [64]	U87 i.c. and s.c. in nude mice	TG6002: Prolonged survival in s.c. and i.c. Synergic effect with 5FC in i.c. model
Myxoma	Human: U87, U251, U373, U343, A172 and U118 Rat: RG2 and 9L [67]	U87 and U251 i.c. in nude mice	Regression and longer survival in both models
	Human: U87, U251, and U118 [68]	U87 orthotopic in CB-17 SCID mice	MYXV inhibits MHC-I tumor expression and promotes N mediated death
	Human: U118 and 3 patient samples Murine: GL261 Rat: T9 [69]	-	SOC co-treatment increases results of MYXV
	Human: U87 and U251 [70]	U87 orthotopic in nude mice	Increase the tumor infection rate
Parvovirus	Human: U87 Rat: RG-2 [84]	U87 i-deficient rats and RG-2 i-competent	Complete remission of the tumors
	Human: U373, U138 and 5 CSCs [75]	RGD orthotopic rats	Cathepsin B activation induce cell death in H-1PV
	Human: U87, U373, U118, MO59J and A172 Murine: GL261 [80]	U87 and U373 s.c. U87 orthotopic CB17-SCID mice	Selectively infection, no toxicity, reduce tumor volume *in*
	Human: U373, U87, SW1088, SK-N-SH Rat: C6 [81]	-	MVM p strain cytotoxic only in U373 and C6 (MVM)
	Human: U87 and MO59J [82]	-	Selectively GBM infection (MVM)
	Murine: Fibroblast L929 and A9. Astrocytoma MT539MG [83],	-	Safe for microglia (MVMp)

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i.c.; intracranial, s.c.; subcutaneous, i-deficient; immunodeficient, i-competent; immunocompetent

3. RNA viruses proposed as glioma oncolytic agents.

3.1. Reovirus

Reoviridae is a family of double stranded RNA not enveloped viruses that can cause asymptomatic or mild enteric infections in humans. Orthoreovirus also known as reovirus, has been shown to be a natural oncolytic virus because can overtake and specifically replicate in Ras pathway activated cells, commonly present in gliomas [85,86]. Reovirus treatment of mice with both subcutaneous and intracerebral tumors generated using human glioma cells, resulted in an intense and often total regression [87]. Reovirus-mediated oncolysis has been tested in preclinical models inducing both, a direct tumor lysis and also an increase of T cell infiltrate, and expression and secretion of Type I IFN in the tumor microenvironment [88]. Phase I clinical trials have been performed using not genetically modified reovirus for intratumoral treatment of malignant glioma, showing no adverse effects [89,90]. Similar results were detected in a Phase Ib clinical trial in which they observed an increase in tumor leukocyte infiltration and higher expression of IFN, caspase 3 and PD-L1 in tumors from reovirus treated patients [88].

3.2. Measles

Measles virus (MV) belongs to *Paramixoviridae*, a family of enveloped viruses with a negative single-strand RNA genome. MV fusion (F) and hemagglutinin (H) proteins have demonstrated to play a role in the antitumor activity of the virus in gliomas [91]. In this sense, MV Edmonston´s vaccine (MV-Edm) is one of the approximations that have been tried for glioma treatment. This vaccine was modified to express carcinoembryonic antigen (MV-CEA) in order to track viral gene expression *in vivo* through blood analysis since this factor can be released and detected in blood [92]. MV enters into cells by interaction of the viral H protein with the cell receptor CD46, a protein that is overexpressed in tumor cells [93]. Glioma cells infection with MV-CEA leads to a syncytial formation mediated apoptosis, while normal cells do not develop cytopathic effect. Animal models showed a significant increase in surveillance and tumor regression after intratumoral treatment with MV-CEA [92]. A phase I clinical trial has been carried out in patients with GBM showing a tolerance up to 10^7 pfu TCID50 (NCT00390299).

MV-NIS is another modification of the MV-Edm, in this case the recombinant virus expresses the human sodium iodide symporter (NIS) to improve the monitoring of MV infection *in vivo* in brain tumors with a non-invasive method by using systemic administration of ^{123}I, ^{124}I, ^{125}I, or ^{99m}Tc isotopes and measuring the isotope accumulation in virus-replicating cells. In addition this modification increases cytopathic effect of MV treatment through radiotherapy by local accumulation of ^{131}I. MV-NIS induced longer survival in mouse models and increased viral titers and cell death in comparison with MV-CEA [94].

MV-GFP-H$_{AA}$-scEGFR is a recombinant virus modified to ablate H protein recognition by the two natural viral receptors CD46 and SLAM. Instead, this virus expresses a single chain antibody that binds to EGFR fused to the C terminal end of the virus H protein. Amplification of epidermal growth factor receptor (EGFR) is one of the most frequent genetic alterations in GBM. This characteristic determines the specificity of MV-GFP-H$_{AA}$-scEGFR. *In vitro* and *in vivo* experiments showed similar results that MV-GFP, with significant regression and induction of cell apoptosis. However, administration of MV-GFP-H$_{AA}$-scEGFR at the central nervous system in CD46-expressing mice, resulted in no neurotoxicity [95].

MV-141.7 and MV-AC133 are two other recombinant viruses in which H protein has been modified to retarget the virus to the CD133 receptor. CD133 is a marker commonly expressed by GBM CSC. MV-141.7 resulted in a better survival rate, in comparison with MV-Edm, in the treatment of orthotopic glioma mouse model [96].

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3.3. Vesicular stomatitis

Vesicular stomatitis virus (VSV) is an enveloped negative strain RNA virus that belongs to *Rhabdoviridae* family. VSV entry is mediated by its Glycoprotein spike (G) and a very ubiquitous receptor, the low-density lipoprotein receptor (LDL-R), which allows the virus to enter in almost every cell type [97]. Moreover, this virus has a short replication cycle of around 3 hours, leading to a cytopathic effect that can be observed as soon as 4-6 hours after infection, making it a good candidate for treatment of a wide range of tumors [98]. The use of unmodified wild type VSV is able to kill a large variety of tumor and immortalized cells *in vitro* as well as inhibit the growth of C6 GBM in flanks of mice [99]. However, the virus can be lethal for animals if upon infection, they do not mount an efficient IFN response. In this context, the virus toxicity can be contained by administration of recombinant type I IFNs, without blocking oncolytic effects on tumor cells [100]. In order to reduce unspecific neurotoxicity, several viral modifications have been developed like the deletion of the G encoding gene in the VSV-ΔG viral vector. The cytopathic effect of this vector in glioma cells is markedly lower than unmodified VSV and does not reach as many cells as other recombinant viruses [101].

VSV$^{\Delta M51}$ is an attenuated replicating virus strain. This strain has a single amino acid deletion in the matrix protein (M), affecting the nuclear-cytoplasmic transport. This modification avoids M protein ability to block the IFN-β mRNA transport from the nucleus to the cytoplasm and thus, affecting the antagonistic activity of IFN, restricting the virus replication to tumor cells incapable to produce IFN, allowing infected normal cells to produce a normal IFN response and therefore, limiting the virus spread. This virus showed oncolytic activity against 14 glioma cell lines and 15 primary human tumor glioma cells infecting and inducing cell death in them. *In vivo* experiments showed a tumor regression and prolonged survival in U87 and U118 tumor models in mice [102].

A different strategy to attenuate the virus is the tumor-adapted VSV-rp30 (repeated passage 30. In this case this VSV was adapted to glioma cells by 30 serial passages in which the time between infection and virus recovery was reduced after every 10 passages in order to select the fastest replicant viruses. VSV-rp30 has higher replication rate on glioma cell lines and less cytotoxicity in no-tumor cells as compared to wild-type virus [82]. The virus can infect *in vivo* models after intravenous administration and destroy GBM brain tumors with tumor dissemination [103].

Another strategy to attenuate the virus is the modification of the genome to reduce the cytoplasmic tail of the G protein [104]. VSV-CT9 and VSV-CT1 are truncated G protein versions of the virus that have reduced cytoplasmic region of the G protein; from 29 amino acids to 9 or 1 respectively [105]. These two versions showed efficacy to kill *in vitro* GBM cells, being VSV-CT9 more toxic for no-tumor cells as compared to VSV-CT1. Viral toxicity for normal cells can be reduced by a co-treatment with IFNα, inhibiting viral replication in these cells, while viral titers remain high, with a small decrease, in glioma cells [101]. VSV-CT1 showed also less neurotoxicity after intracranial injection and intranasal inoculation as compared to wild type VSV [106].

Combination of two different strategies of virus attenuation resulted in VSV-CT9-M51. This mutant combine both strategies used to develop VSV $^{\Delta M51}$ and VSV-CT9. VSV-CT9-M51 showed less neurotoxicity in normal cells than both VSV-CT9 and VSV $^{\Delta M51}$ while retaining the ability to infect, spread within and kill human GBM in a mouse model after systemic administration, triggering also higher type I IFN dependent responses in the animals [106].

Another attenuation strategy that has been proposed to reduce virulence of VSV is gene rearrangement [107,108]. Introducing foreign genes such as GFP or RFP at the first position in the genome is a different way to attenuate VSV. VSV-p1-GFP and VSV-p1-RFP showed high cytopathic effect and induced death after infecting U87 GBM cells *in vitro*, having at the same time lower toxicity in non-tumor cells. VSV-p1-GFP showed promising results in animal models [101].

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Other strategies to reduce adverse effects, beyond direct attenuation of the virus, have been developed. Coinfection of VSV-CT9-M51 (intracranial) with an adeno-associated virus expressing mouse IFN-β (AAV-mIFN-β) or co-treatment with ribavirin resulted in less neurotoxicity and a OS extension in a GBM mice model [109].

3.4. Newcastle Disease Virus (NDV)

NDV is an enveloped, negative sense, single-stranded RNA virus. This virus belongs to the family *Paramyxoviridae* and mainly avian species, while has marginal pathogenicity in humans [110]. Depending of the virus pathogenicity in chickens, the different NDV strains can be divided into velogenic, mesogenic and lentogenic [111]. Following NDV infection, human cells induce the type I IFN response [112].

Although the specific tropism for cancer cells is poorly understood, it has been postulated that the small GTPase Rac1, which is involved in the maintenance of GBM stem properties [113], is required for NDV replication [114]. Preferential replication could also be explained by tumor-limited replication due to deficiencies in the type I IFN system present in many GBM patients [115].

Our group have recently observed that type I IFN-deficient GBM CSCs are more receptive for NDV replication than type I IFN competent cells. This fact was also noted in the mouse model, in which NDV treatment reduced tumor volume only in IFN-deficient bearing cells [116]. Preclinical animal models have reported an apoptotic effect using NDV in GBM treatment [117,118], and an increase in the median survival from 28 to 64 in the mouse models [119], as well as a synergistic effect with TMZ [120]. Although few studies have done in small cohorts of GBM patients, all of them have shown promising results. Csatary and colleagues treated intravenously 4 GBM patients with the mesogenic strain MTH-68/H after standard treatment and reported an increase in the OS and enhance of the quality of life [121]. Similar effects were observed in 10 patients treated with the vaccine VOL-DC composed by NDV infected dendritic cells [122]. One additional report describes that the intravenous administration of the lentogenic NDV strain OV001/HUJ achieved a complete tumor regression in one patient [123].

3.5. Seneca valley

Seneca Valley virus isolate 001 (SVV-001) is a non-enveloped positive single chain RNA virus belonging to *Picornaviridae* family. This virus was isolated and identified from a contamination of culture cells and does not produce any described disease in animals [124]. SVV-001 has showed oncolytic activity for neuroendocrine tumors [125]. *In vitro* experiments with 6 different GBM CSCs resulted in total infection and significant decrease on viability for 4 of them. Permissibility of tumor glioma cells is dependent on the presence of α2,3-linked and α2,6-linked sialic acids. Intravenous injection with SVV-001 showed infection, cell lysis and prolonged animal survival on permissive GBM intracranial xenograft in Rag2 SCID mice models [126].

3.6. Poliovirus

Poliovirus belongs to the *Picornaviridae* virus family. These encapsidated viruses have a positive single strand RNA [127]. Poliovirus can cause neurotoxicity, although Gromeier and colleagues eliminated it by replacing the internal ribosome entry site (IRES) of the poliovirus vaccine Sabin strain with the non-virulent human rhinovirus type 2 (HRV2) [128]. The resulting PVS-RIPO recombinant virus can infect and reduce glioma cells viability *in vitro* [129]. In addition, this virus can trigger cytolysis of GBM primary cultures [130]. Finally, PVS-RIPO can halt tumor growth in a murine GBM flank tumor model [131] and increased the mice OS in after intracranial virus administration [132].

The efficacy of the PVS-RIPO appears to be correlated with the CD155 expression that is known to be overexpressed in some cancers, including human GBM, specifically in CD133+ cells [133]. All these evidences prove that poliovirus is capable of inhibiting GBM tumoral growth in preclinical models.

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Therefore, an interventional clinical study (NCT01491893) with 61 recurrent GBM patients was developed corroborating that the intratumoral injection of the virus is safe and increased the survival rate [134]. With the aim to confirm this safety and test efficacy of the virus, there are currently two active clinical trials for recurrent glioma in adults and children, respectively (NCT02986178 and NCT03043391).

3.7. Sindbis

Sindbis virus is a small alphavirus of positive stranded RNA genome surrounded by a capsid protein that belongs to the *Togaviridae* family. The natural host are birds but mosquitoes act as vectors to infect mammals, including humans, through their bites [135]. The infection occurs when the virus binds to the 67-kDa high-affinity laminin receptor (LAMR) which is overexpressed in cancer cells. Sindbis virus is used as gene therapy vector, however, it has been shown oncolytic activity on cancer cells [136]. Particularly, Sindbis can replicate and propagate in U87 glioma cell line *in vitro* and *in vivo* [82]. Sindbis has also been used as a vector expressing the fusogenic membrane glycoprotein GALV.fus that increases the infectivity of the virus and the cytotoxic effect in a U87 cells GBM mouse model [137]. Sindbis vectors presents a synergistic effect when combined with chemotherapy [138], and the potential application for detection of the tumor cells in the brain parenchyma by the addition of reporter genes [139]. Future investigations are needed to find out the potential the clinical use of this virus.

3.8. RIFT Valley fever virus (RVFV)

RFVF is a single stranded RNA virus that belongs to the family *Bunyaviridae* with a wide host-range including several domestic animals and humans [140]. Although little is known about the clinical application of this virus in the field of brain tumors, there are some studies in the literature that prove its infection efficacy *in vitro* in GBM C6 rat cells [141] and U87 [142].

Table 2. Preclinical studies of RNA viruses in glioma tumors.

Virus	*Cell lines*	*In vivo* models	Results
Reovirus	Human: 24 GBM cell lines [87]	U87 and U251 intracranial and subcutaneous in SCID mice	Death in 20 out of 24 GBM lines Regression in both *in vivo* models Toxicity in nude mice
	Human: U87 and 2 patient-derived lines [88]	GL261 intracranial in C57/BL6	i.v. administration reaches brain tumors T cell tumor recruitment and cytotoxicit Synergy with anti PD-L1
Measles	Human: U87, U251, and U118 [92]	U87 intracranial and subcutaneous in nude mice	MV-CEA: regression in s.c. tumor after intravenous and intratumor administrati Regression in intracranial tumor after intratumor administration
	Human: U87, U251 and 6 patient derived GBM [94]	U251 subcutaneous and GBM intracranial in nude mice	MV-NIS: synergic effect of viro and radiotherapy
	Human: 5 patient derived GBM [95]	GBM intracranial in nude mice Mouse model Ifnar^{ko} CD46 Ge	MV-GFP-H$_{AA}$-scEGFR: Tumor regressio after intratumor administration No toxicity in CNS
	Human: primary GBM [98]	GBM intracranial in NOD/SCID	MV-141.4: better survival rate in comparis with MV-Edm
VSV	-	C6 subcutaneous nude mice [99]	Inhibition of tumor growth
	Human: U87 Rat: C6 [98]	-	VSV-ΔG: infection of cell lines Rapid lysis
	Human: 14 glioma cell lines and 15 primary gliomas [102]	U87 and U118 subcutaneous in nude mice	VSV$^{\Delta M51}$: infection and elimination of all c lines Tumor regression and prolonged surviv
	Human: U87, U118, U373 and A172 [82]	U87 subcutaneous in nude mice	VSV-rp30: increased selectivity and lyti capacity in glioblastoma cells Tumor selectivity and cytopathic effect
	Human: U87 and U118 [103]	U87 orthotopic in nude mice	VSV-rp30: infection and lysis of brain an peripheral tumors
	Human: U87, U118, U373 and A172 [101]	U87 orthotopic in nude mice	VSV-CT1 and CT2: elimination of tumor c Normal cell toxicity can be eliminated wi IFN co-treatment

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Seneca Valley	Human: U87, U118, U373 and A172 Rat: 9L [106]	U87 orthotopic in CB17-SCID mice	VSV-1p-GFP: infection and potent apopto over tumor VSV-CT1 and VSV-CT9-M51: less toxicit than wt VSV VSV-CT9-M51 is able to infect and kill tumors in brain
	Human: primary GBM [109]	Orthotopic CB17 SCID	VSV-CT9-M51: coinfection with AAV-mIF β or with ribavirin enhances oncolytic properties
	Human: primary GBM [125]	4 orthotopic models in nude mice	Partial response against glioma cells Effectivity in 2 of 4 *in vivo* tumors
	Human: GBM CSCs [126]	6 GBM CSC orthotopic nude mice	4 of 6 prolonged survival, tumor infectio and cell lysis Susceptibility dependent of sialic acid presence
NDV	Human: 6 GBM CSCs [116]	Orthotopic nude mice	NDV replication is dependent on IFN deletion
	Human: U87 and DBTRG.05MG [117]	Subcutaneous nude mice	Induce apoptosis Decrease tumor volume
	Human: A172 and U87 and 2 CSCs [118]	-	Induce apoptosis
	Murine: GL261 [119]	GL261 orthotopic mice	NDV induces ICD
	Human: T98G, LN18, U251, U87. Rat: C6 [120]	C6 in rats	Synergistic effects with TMZ Decrease tumor volumes and increase C
Poliovirus	Human: U87 [129]	-	Reduce viability
	Human: CSCs and established cell lines [132]	HTB14 orthotopic and HTB15 flanks	Tumor regression
	Human: 6 CSCs [130]	-	Cytolysis
	Human: U87, HTB14 and HTB15 [131]	HTB15 in athymic Balb/c mice	Tumor regression
Sindbis	Human: U87, U-118, U373, M059J, A172 [82]	U87 in flanks CB17-SCID mice	Effective replication and selective kill U8
RVFV	Rat: C6 [141] Human: U87 [142]	-	Infection occurs

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Table 3. Clinical trials of OV for glioma tumors.

Virus	Phase and reference	N patients	Results
Herpes	Phase I: HSV-1716 [24]	9	Two 24 months survivors Evidence of tumor infection
	Phase Ib: HSV-1716 [25]	12	Three patients clinically stable for two years
	Phase II: HSV-1716 NCT02031965	2	No results available
	Phase I: G207 [29]	21	No toxicities
	Phase Ib: G207 [29]	6	No toxicity Evidence of tumor infection
	Phase I: G207 [30]	9	No toxicities in combination with 5 Gy
	Phase I: C134 NCT03657576	24	Recruiting
Adenovirus	Phase I: ONYX-015 [42]	24	No toxicity One patient without progression and other with regression
	Phase I: Delta-24-RGD NCT03896568	36	Recruiting
	Phase I: Delta-24-RGD NCT03178032	12	No results available
	Phase II: Delta-24-RGD NCT02798406	49	Active
	Phase I: Delta-24-RGD NCT02197169	37	No toxicities
	Phase I: Delta-24-RGD NCT01956734	31	No results available
	Phase I and II: Delta-24-RGD NCT01582516 [143]	20	Virus spread in tumor, oncolytic effect and immunostimulation
	Phase I: Delta-24-RGD NCT00805376	37	20% of >3 years survivors 12% of >95% tumor regression Evidence of immunostimulation
	Phase II Delta-24-RGD (2016-001600-40)	-	Discontinued
	Phase I: Delta-24-RGD NCT03714334	24	Recruiting
	Phase I: Delta-24-RGD NCT03072134	36	No results available
	Phase I: DNX-2440 NCT03714334	24	Recruiting
Reovirus	Phase I: Reovirus [89]	12	No toxicities
	Phase I: Reovirus NCT00528684 [90]	15	One 2 years survivor One 3 years survivor
	Phase Ib: Reovirus [88]	9	Evidence of T cell tumor infiltration and upregulation of IFN and PD-1/PD-L1 axis
	Phase I: Reovirus/ Sargramostim NCT02444546	6	Active
Vaccinia	Phase I and II: TG6002 NCT03294486	78	Recruiting
Measles	Phase I: MV-CEA NCT00390299	23	No toxicities
NDV	Phase I/II: NDV-HUJ NCT01174537 [123]	14	No toxicities Complete regression in 1 patient
	Phase 0: MTH-68/H [121]	4	OS 5-9 years
	VOL-DC vaccine [122]	10	Increase OS
	Phase II: ATV-NDV vaccine [144]	23	PFS 40 weeks vs 26 weeks
Parvovirus	H-1PV [77]	18	Enhanced immunogenicity
Poliovirus	Phase I: NCT01491893 [134]	61	No neurovirulence and increase the survival rate
	Phase II: NCT02986178	122	Active
	Phase Ib: NCT03043391	12	Recruiting

5. Conclusions

The use of OV is at the front edge of the next therapeutic approach in GBM treatment. The use of viruses, as any other therapy, must deal with the stability and the specificity of the treatment without compromising patient's survival. The use of mammal adapted viruses require modifications to limit viral replication and lytic effect to the tumor cells. However, viruses adapted to more distant species may be too attenuated to be effective and require some complementary modifications to boost specific toxicity against the tumor cells.

Important issues in the use of OV in patients remain unsolved, such as complete lack of viral unspecific toxicity, DNA integration or viral latency in the host, virus restriction to the tumor cells, incomplete responses by attenuated viruses and low capacity or complexity to be genetically modified. It is also likely that a description of new viruses or modifications or the ones proposed so far, may improve current therapies.

A better definition of the tumor characteristics will determine future approximations to improve OV therapy. In recent years an impressive effort to classify GBM genetic characteristics has undercover tumor specific modifications and key target points, however much work is still needed to define the different scenarios of GBM immunocompetence and immunogenicity, in particular, those that will be involved in reacting to immunotherapeutic treatments and specifically the ones that use oncolytic viruses. The immunoprivileged characteristics of the CNS introduces an important uncertainty factor in generalizing the experiences of immune-based treatments to brain tumors. In addition, the immune characteristics of GBM cells require better animal models to understand the reactivity of different treatments. Some of the animal models used to test OV efficacy are immunocompromised and thus the important contribution of the immune response to OV remains poorly characterized. Understanding the tumor microenvironment from this angle will determine the best strategy in each specific GBM case.

Based on current data from clinical trials, DNA viruses such as modified HSV-1 and Adenovirus as well as RNA viruses such as reoviruses and NDV present promising results that require further improvements. Current versions of these viruses will need to be updated and tested in different glioma types and patient situations in order to improve glioma treatments. Additional viruses are still behind in proving some alternatives but coming results from current clinical trials may provide different possibilities to improve the current OV therapeutic options.

Likely, single treatments may not be enough in many tumors, including GBM. The combination of different treatments such as surgery, chemotherapy, radiotherapy, immunotherapy, and viral therapy will result in better prognosis. Recent approaches propose the use of a sequential stimulation of inducers to overcome the immune-tolerance of tumors. The introduction of therapeutic approaches based on trained immune stimulation may also provide a step forward in combinatorial therapies [145].

Finally, there is an urgent need to develop better immunocompetent animal models that consider different subtypes of GBM to better study and understand the best combination of treatments for this type of devastating tumors. In summary, OV is at the front edge of the next generation of glioma treatments and should be seriously considered as an option where no alternatives are available.

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Abbreviations

WHO	World Health Organization
TMZ	Temozolomide
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
GBM	Glioblastoma
OV	Oncolytic virotherapy
CNS	Central Nervous System
DAMPs	Danger-Associated molecular patterns
OS	Overall survival
ICD	Immunogenic cell death
PRRs	Pattern recognition receptors
HSV-1	Herpes simplex virus Type 1
LAMR	Laminin receptor
VV	Vaccinia virus
MYXV	Myxoma virus
NDV	Newcastle Disease virus
TVEC	Talimogene laherparepvec
TTFields	Tumor treating fields

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