Oncolytic Viral Therapy of Malignant Glioma

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Summary: Novel approaches to treatment of malignant glioma, the most frequently occurring primary brain tumor, have included the use of a wide range of oncolytic viral vectors. These vectors, either naturally tumor-selective, or engineered as such, have shown promise in the handful of phase I and phase II clinical trials conducted in recent years. The strategies developed for each of the different viruses currently being studied and the history of their development are summarized here. In addition, the results of clinical trials in patients and their implication for future trials are also discussed. **Key Words:** Oncolytic viral therapy, malignant glioma, G207, HSV 1716, ONYX-015 adenovirus, Reolysin, vaccinia, Newcastle disease virus, measles virus.

INTRODUCTION

Malignant glioma is the most frequently occurring primary brain tumor. Prognosis is abysmal, despite surgical resection and chemotherapy/radiation therapy, with time to progression averaging 6 months and median survival of one year. Investigations into the use of oncolytic viruses (OVs) for the treatment of a variety of malignancies, including malignant glioma, were initiated more than half a century ago after anecdotal observations that some cancer patients experienced periods of remission after suffering from an acute illness of viral etiology, or after being inoculated with attenuated viral vaccines. Perhaps the most well-known case report came from de Pace in 1912 that described a patient whose cervical carcinoma regressed after receiving Pasteur's attenuated rabies vaccine strain after a dog bite.[1] Subsequently, adenoviruses (earlier referred to as adenoidal-pharyngeal-conjunctival, or APC viruses) were inoculated in 30 patients with epidermoid cervical carcinomas.[2] In this study, 65% of treated tumors had areas of necrosis after intratumoral inoculation. Naturally-occurring viruses evaluated for the treatment of acute leukemia include Newcastle disease virus, Sendai, Semliki forest virus and influenza viruses.[3] Cases of Burkitt's lymphoma[4] and Hodgkin's disease[5] have partially responded to treatment with the measles virus. In the late 1970s, the mumps virus was administered for various human cancers, with one study enrolling 200 patients.[6,7]

Until the early 1990s, safety concerns limited the use of these nonattenuated, replication-competent viruses as primary anti-tumor therapy. Instead, replication-defective viruses, including engineered adenovirus and retrovirus vectors, were evaluated as gene therapy vectors for cancer therapy. However, numerous problems were associated with these vectors, including adequate virus delivery and distribution, insufficient levels of both gene transfer and gene expression, and lack of prolonged efficacy. More recently, advancements in molecular biology, combined with research in novel experimental therapies for cancer treatment has renewed interest in applying OVs for glioma therapy. A history of these advancements with respect to CNS malignancies, and a summary of the different OVs under evaluation, is provided.

There are numerous advantages of oncolytic virotherapy. First, if not already naturally discriminating, OVs can be genetically engineered to be selective for mitotically active, neoplastic cells. This is especially appealing for glioma therapy, as the tumor-adjacent, quiescent neurons remain resistant and intact after treatment. Second, most of the viruses discussed herein, and all those tested in phase I clinical trials for malignant glioma thus far, are replication-competent in addition to being oncolytic. This means that tumor killing is not limited to the initial target cell. Rather, its tumor-targeted cytolytic advantage can spread to surrounding cells that escaped initial infection. This latter property theoreti-

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cally allows for OVs to seek out and destroy tumor cells distal from the initial treatment site. Third, some OVs have large genomes enabling the introduction of one or more foreign genes, allowing them to act as gene therapy vectors for augmentation of the antitumor effect. M002 (discussed as follows) is one example of an oncolytic herpes simplex virus (HSV) engineered for expression of murine interleukin (IL)-12 heterodimers specifically in tumor cells only. Finally, any viruses that demonstrate efficacy in the treatment of brain tumors can be used clinically in combination with standard treatment modalities.

History of oncolytic viral therapy

In 1991, Martuza et al.[8] reported that the engineered HSV thymidine kinase-deleted mutant, *dl*sptk, was replication-attenuated in nondividing cells, such as neurons. The *tk* gene deletion required the virus to rely on mitotically active cells to supply both thymidine kinase and nucleotide pools for DNA replication. The *dl*sptk virus displayed a promising therapeutic profile in the treatment of glioma in animal studies. However, the tk gene inactivation also rendered this mutant resistant to antiviral agents, such as acyclovir, which targets the viral thymidine kinase. Lost susceptibility to the viral tk-targeted drugs, combined with an undesirable toxicity level at high titers, prevented advancement of this OV into clinical trials for malignant glioma. Nevertheless, these "proof of concept" studies led to the development of the first generation of clinically safe HSV vectors, G207, and HSV1716, as described in more detail as follows.

As with any novel therapy, safety after administration in humans is the highest priority, but in addition, OVs must demonstrate potent anti-tumor activity either alone or combined with existing standard therapies, such as de-bulking surgery, radiation, and chemotherapy. During the past 2 decades, a number of promising OVs have been developed that have demonstrated anti-glioma activity in preclinical studies. These include polio virus-derived vectors, vaccinia virus, Newcastle disease virus, and most recently, measles virus vectors. In addition, phase I clinical trials have been completed for several OVs, including HSV-1 (G207 and HSV1716),[9,10] a conditionally replicative adenovirus (CRAd) termed Onyx-015,[11] and a reovirus, REOLYSIN (Oncolytics Biotech Inc, Calgary, Ontario, Canada).[12] A number of these vectors have already demonstrated their safety after administration in patients, including some in combination with prior debulking surgery or in combination with radiation.[13,14] Yet, major hurdles remain, which includes efficient delivery of OVs, both into the primary tumor mass, as well as delivery to tumor cells distal from the primary site; tumor-specific genotypes resistant to certain OV therapies; and persistence of viral replication and expression of foreign genes introduced to augment OV activity by multiple mechanisms. Strategies to overcome these hurdles are currently under intense investigation and will be discussed at the end of this review.

HERPES SIMPLEX VIRUS-1

Herpes simplex virus type 1 (HSV-1) is a well-studied, neurotropic virus with essential and nonessential genes that have been established.[15] The genes involved in its oncolytic properties are distinct from the genes for neurovirulence, and manipulation of the viral genome allows continued oncolysis with conditional replication in cancer cells. Its sensitivity to acyclovir and ganciclovir gives HSV a distinct advantage over other oncolytic vectors in that it adds to its safety profile when used in human clinical trials.

After the development of HSV-1 virus *dlsptk* as previously described, other HSV vectors have been constructed that are avirulent in the normal brain, but can proliferate in actively cycling cells due to different mutations. HSV-1 mutant hrR3 contains an inactivating *lacZ* insertion into the U_L39 locus encoding the large subunit of ribonucleotide reductase (infected cell protein 6),[16] required for the synthesis of nucleotides in a postmitotic cellular environment, such as neurons, which would otherwise not support HSV proliferation. The U_L39 mutation is complemented *in trans*[17] via the cellular version of the enzyme in malignant gliomas and other cycling cells, such that hrR3 can still infect these rapidly dividing cells.[18] In addition, this mutation increases susceptibility of the virus to anti-viral therapy with acyclovir and ganciclovir.[19]

Because of its large genome (>150 kb), up to 30 kbp of HSV genome can be replaced with foreign DNA while still retaining the ability of the virus to replicate. After infection with wild-type HSV-1, double stranded RNA is produced, and this RNA is sensed by protein kinase R (PKR) through an intracellular stress response. PKR phosphorylates eukaryotic initiation factor alpha (eIF-2α), which turns off protein synthesis. Expression of infected cell protein 34.5, a gamma-1 gene, leads to dephosphorylation of eukaryotic initiation factor alpha (eIF-2α), restoring protein synthesis. Infected cell protein 34.5 is expressed from the $\gamma_1$34.5 gene, which is present in two copies and located in the inverted repeat regions flanking the unique long segment of the viral genome. Restoration of protein synthesis occurs when infected cell protein 34.5 recruits protein phosphatase-1a, leading to eukaryotic initiation factor alpha (eIF-2α) dephosphorylation.[20] R3616, constructed by Chou et al.,[21] lacks both copies of the $\gamma_1$34.5 gene and was derived from the wild-type virus, HSV-1 (F) strain. Deletion or disruption of both copies of the $\gamma_1$34.5 gene severely limits virus replication, unless its target is a tumor cell that possesses a complementing mutation,

such as *ras* overexpression,[22] or alterations in PKR and other cell-signaling pathways.[23] Deletion of this gene also removes part of the latency-activated transcripts, which are encoded on the complementary antisense DNA strands. As such, these viruses are impaired in their ability to establish latency after infection.[24] R3616 was the parent virus for G207, which has now completed two phase 1 clinical trials in the United States. Results of a third trial are pending.

Herpes simplex viruses: clinical trials

The first generation oncolytic herpes simplex virus vectors (OHSV) that have already completed phase 1 or early phase 2 clinical trials in patients have one or both of these attenuating mutations ($\gamma_1$34.5 deletion and U_L39 disruption). G207, developed by Mineta et al.'s[25] group, combined both of these strategies and was constructed on HSV-1 (F) wild-type background. HSV1716, in contrast, only lacks copies of its $\gamma_1$34.5 gene- the UL39 sequences remain intact. HSV1716 is derived from wild-type strain 17 and was constructed by MacLean et al.[26]

G207. G207 has deletions of both copies of its $\gamma_1$34.5 gene in addition to a *lacZ* insertion into the U_L39 locus.[25] These two mutations improve the safety of G207 by rendering it susceptible to standard anti-HSV therapy, and by impairing ability of the virus to establish latency within an infected cell. Preclinical studies demonstrated that G207 was safe in neurotoxicity studies in mice and nonhuman primates, and efficacious against the U87 human malignant glioma xenograft model in immunocompromised mice.[27] New World owl monkeys (*Aotus nancymae*) were used for the primate neurotoxicity studies due to their exquisite sensitivity to HSV infection.[28] These monkeys showed no clinical signs of HSV-induced illness after G207 inoculation (up to 1×10^9 plaque forming units [PFU] of G207 injected).

Three phase I trials have been completed to date for G207 treatment alone or in combination with radiation. In the initial trial, a total of 21 subjects were enrolled, all of whom had recurrence of tumor diagnosed on CT or MRI after standard therapy (resection or biopsy followed by radiation). Each patient received a stereotactic intratumoral injection of G207 within the enhancing portion of the tumor, starting at a dose of 1×10^6 PFU in the first cohort, 1×10^7 PFU in the second cohort, and a dose escalation in half-log increments for each subsequent cohort (three patients per cohort). A maximally tolerated dose could not be established; even at the highest dose (3×10^9 PFU). CT scans 30 days after virus inoculation showed reduced enhancement in eight patients, with eight patients who survived 9 or more months posttreatment, and a single glioblastoma multiforme (GBM) patient remained alive 5.5 years after inoculation without evidence of disease.[9]

The objectives of the second phase Ib G207 trial were three-fold: 1) to confirm the safety and tolerability of intratumoral inoculation of G207, as well as inoculation into the brain surrounding the tumor; 2) to demonstrate active replication inside the tumor; and 3) to demonstrate the safety of multiple-dose delivery of the virus. Six patients with recurrent GBM were enrolled in the study. A catheter was stereotactically implanted into the tumor, and 13% of a total dose of 1.15×10^9 PFU of G207 was injected through the catheter. Either 2 or 5 days later, the tumor was resected en bloc with the catheter in place, and the remainder of the G207 was injected into the tumor bed. Radiological and pathological evidence of anti-tumor activity was seen. Evidence of HSV replication in situ was demonstrated. HSV encephalitis did not develop in any patient, although one patient experienced transient fever, delirium, and hemiparesis, which entirely resolved within 12 hours on high-dose dexamethasone; no acyclovir was necessary. These symptoms were attributed to inadvertent inoculation of the virus into the ventricular system. Overall, G207 appeared to be safe both for multiple-dose delivery and for direct inoculation into the brain surrounding the tumor cavity.[13]

HSV1716. Concurrent with the G207 studies in the United States, HSV1716, derived from HSV strain 17, was being tested in clinical trials in Glasgow, United Kingdom. Like G207, HSV1716 also lacks both copies of the $\gamma_1$34.5 gene, but its UL39 gene remains intact.[26] In the initial HSV1716 phase I trial, nine patients (eight GBM and one AA) who had all undergone prior surgery and radiotherapy, with most who had received chemotherapy, had an initial dose of 1×10^3 PFU stereotactically inoculated into the enhancing portion of the tumor. This dose was increased by 1 log in each of three cohorts, with three patients per cohort, ending at a maximum dose of 1×10^5 PFU. At the highest dose, no signs of encephalitis or other dose-limiting toxicities were encountered, and no maximally tolerated dose was reached.[10]

To validate safety and demonstrate in situ viral replication, a second phase I trial was conducted. In this trial, 12 patients (11 GBM and 1 AA) received 1×10^6 PFU of HSV1716 via direct intratumoral inoculation prior to surgical resection 4 to 9 days later. Again, this virus was proven safe and replication within the tumor was supported by PCR detection of the virus in two of the resected tumors.[14]

Recently, according to their website, Crusade Laboratories has received regulatory approval to begin Europe-wide phase III clinical trials of HSV1716 in glioma patients after first recurrence postresection and radiotherapy, with satisfactory results potentially leading to license and marketing authorization for glioma therapy.

M002/M032. OHSV engineered to express transgenes (including interleukins, such as IL-4 or IL-12) have been shown to enhance tumor killing in both syn-

geneic murine brain tumor models and human glioma xenograft tumor models.[29,30] M002 and M032, like G207, are both derived from HSV-1 (F) strain with deletion of both $\gamma_1$34.5 gene copies, but in contrast, the UL39 gene remains intact in M002 and M032. A bicistronic expression cassette encoding IL-12 p40 and p35 subunits from either murine (M002) or human (M032) origin, and separated by an internal ribosome entry sequence (IRES), were introduced into both 34.5 deleted sites.[30] A lot of M032 has been produced using current good manufacturing practices through the National Cancer Institute Rapid Access to Intervention Development program for eventual use in phase I clinical trials in humans.

Next generation HSV. A number of different strategies are currently being used to enhance anti-tumor potential of OHSV vectors. These include introduction of foreign genes for pro-drug conversion,[31–33] tumor-specific gene expression,[34,35] and expression of other viral proteins that restore efficient viral replication,[36,37] to name a few. Genetic manipulation of HSV proteins that mediate virus entry, combined with new discoveries related to the mechanism of tumor cell resistance to OHSV therapies have led to the engineering of novel tumor-targeted HSV vectors. For example, an OHSV was constructed to specifically target the IL-13Rα2 receptor,[38] found abundantly on high-grade astrocytomas, but not on normal human tissues.[39] Derivatives of this IL13Ra2-targeted virus have been constructed, which are no longer capable of binding to the normal HSV entry receptor, nectin 1.[40,41] Other tumor-targeting strategies under investigation for OHSV vectors include the use of tumor-specific promoters[35,42,43] or radiation-inducible promoters[44] for the expression of genes that optimize the tumor cell microenvironment for efficient viral replication.

Malignant gliomas will likely require a broad approach that incorporates current treatment strategies with novel therapeutics, including OHSV. Dual treatment regimens combining OHSV therapy with ionizing radiation or chemotherapies are all strategies currently under intensive investigation.[45–49]

In summary, the G207 and HSV1716 clinical trials reinforce the safety and potential benefit that OHSV vectors may offer to patients with malignant glioma.

CONDITIONALLY REPLICATING ADENOVIRUSES (CRAds)

Human adenovirus serotype 5 (Ad5) has served as the platform for a multitude of oncolytic viral agents. This nonenveloped DNA virus is not associated with any serious disease and has a well-characterized genome of approximately 36 kb that allows for relatively easy manipulation.[50] Recent years have witnessed a rapid expansion in the number of strategies with CRAds intended to treat glioma, illustrating the variety of strategies by which this might be accomplished. An overview of the CRAds that have been developed, as well as those that have advanced or those that will soon be advanced to clinical trials, is summarized as follows.

ONYX-015

ONYX-015 is an adenovirus made conditionally replicative by deletion of the E1B-55k gene, which is responsible for binding cellular p53. This interaction normally prevents apoptosis of the infected cell, permitting continued viral replication. Although this CRAd was intended to replicate selectively within p53-deficient cells, it has since been shown that other functions of E1B-55k are responsible for its cancer-selective replication.[51] ONYX-015 was among the first CRAds to be described and has been utilized in clinical trials for head and neck cancer. More recently, human glioma xenografts were shown to be susceptible to ONYX-015 replication *in vivo,*[52] an effect that was enhanced by radiation therapy[53] These findings led to a phase I clinical trial for recurrent glioma, in which ONYX-015 was administered to the tumor bed after surgical resection. The virus was well-tolerated in this trial, with no evidence of toxicity. However, efficacy was not determined.[11]

Ad-Δ24 and derivatives

Other partial deletions of the Ad5 genome have also been used to generate CRAds. Fueyo et al.[54] described a CRAd in which the E1A gene was partially deleted. This CRAd (designated as Ad-Δ24) has a 24-base pair deletion within E1A that renders the protein unable to bind cellular Rb, thus limiting its replication to cells with disregulated cell cycles. Ad-Δ24 was more oncolytic than an E1B-deleted CRAd in a panel of glioma cell lines tested, and was more effective in suppressing tumor growth in both intracranial and subcutaneous models of glioma.[55] In addition, because Ad-Δ24 increases expression and activity of topoisomerase I in glioma cells, its antitumor effect was shown to be synergistically improved in an experimental murine glioma model when administered along with the topoisomerase I inhibitor irinotecan.[56]

The Ad-Δ24 system has itself served as the platform for a number of modifications, including the addition of transgenes whose expression should augment its oncolytic potency. Some examples include p53,[57,58] a humanized form of the pro-drug converting enzyme yeast cytosine deaminase,[59] and the tissue inhibitor of matrix metalloproteinase-3.[60]

Other modifications of Ad-Δ24 vectors have focused on the fiber knob to target glioma-specific receptors. Adenovirus infection depends on initial binding of the knob portion of the fiber capsid protein with the coxsackievirus and adenovirus receptor on the cell surface, followed by a secondary binding of Arg-Gly-Asp (RGD)

motifs in the viral capsid to cell surface integrins. Many tumor cell types, including gliomas, express low levels of coxsackievirus and adenovirus receptor, but high levels of integrins. Inclusion of an RGD peptide in the fiber knob retargets initial binding of the virus to cell surface integrins.[61] In addition, Ad-Δ24-RGD can infect glioma stem cells isolated from human tumor specimens and can induce autophagy in these cells.[62]

Finally, the Ad-Δ24 system has also been modified by those seeking to enhance the selectivity of its replication. This has been achieved either by deletion of additional portions of the genome,[55,63,64] or by the addition of exogenous promoters, such as the E2F1[65] and tyrosinase promoter.[66]

CRAd-survivin

The CRAd-survivin system includes several oncolytic adenoviruses, in which replication is controlled by the survivin promoter.[67–72] Survivin is an inhibitor of apoptosis protein, which is normally active only during embryogenesis. These survivin-controlled CRAds have incorporated a number of different fiber genes to enhance their infection of glioma cells. Examples include RGD-modified fiber,[72] a chimeric fiber with Ad3 knob[69] and the inclusion of a poly-lysine motif.[70] In addition, the activity of the survivin promoter is induced by radiation, thereby increasing viral replication and resulting in a synergistic antitumor effect when virus administration is combined with radiation therapy.[67]

Oncolytic adenoviruses-additional therapeutic strategies

In addition to the three systems previously detailed, a variety of other strategies for the generation of glioma-targeted CRAds have also been described. Bieler et al.[73] used an adenovirus with a partial deletion of E1A in combination with irinotecan and trichostatin A (a histone deacetylase inhibitor that upregulates coxsackievirus and adenovirus receptor expression) in a three-pronged strategy to improve replication and destroy drug-resistant glioma cells. This virus also acts synergistically to inhibit tumor growth *in vivo* when used in conjunction with radiation.[74] Other groups have developed CRAds for which replication was dependent upon a hypoxia response element,[75] the glial fibrillary acidic protein promoter[76] or the hTERT promoter.[77,78] Hoffmann et al.[79,80] constructed a CRAd with the E1A and E4 genes under control of the glial fibrillary acidic protein and Ki67 promoters, respectively.

In some cases, CRAds have been infectivity-enhanced through the inclusion of an RGD-modified fiber[78] or a chimeric fiber consisting of the Ad5 fiber tail and the Ad35 shaft and knob (referred to as 5/35), which retargets the virus to CD46.[79–81] Several of these CRAds have demonstrated enhanced antitumor activity when administered with chemotherapeutic agents, such as temozolomide,[78,80] RAD001,[78] and carmustine.[75] Finally, inclusion of cytotoxic transgenes, such as thymidine kinase[82] or TRAIL[81] in CRAds has also been investigated.

Ongoing studies with CRAd vector systems continue to investigate the optimal combination of viral mutations with standard therapies for glioma therapy. Table 1 summarizes all of the CRAd vectors described herein.

REOVIRUS

Reoviruses are nonenveloped RNA viruses that usually do not cause serious disease, but they may be associated with very mild gastrointestinal or respiratory symptoms that can resolve without further incidence.[83] As discussed earlier in this review, under normal cellular conditions after viral infection, the PKR pathway is activated as a result of the intracellular double-stranded RNA that is produced. PKR activation results in host protein synthesis shutoff, and consequently in viral replication. In many tumor cells, activation of this pathway is blocked in cells in which the Ras signaling pathway has been upregulated via EGFR[84] and PDGFR[85] mutations commonly found in malignant gliomas, which permits productive infection of reoviruses. The naturally discriminatory phenotype of reovirus for cells with unrestrained Ras pathway activity, combined with its mild disease profile in humans led to its evaluation as an OV for therapy of multiple tumor types, including glioma. Early studies showed that of the multiple brain tumors specimens tested *ex vivo* for vulnerability to reovirus, all glioma specimens were killed, as well as 20 of 24 glioma cell culture lines, but none of the meningiomas tested were susceptible.[86] Experimental models of glioma using U87 and U251N cell lines *in vitro* and *in vivo* demonstrated the efficacy of reovirus and confirmed its natural limitation to neoplastic cells.[87] However, serious toxicity involving severe hind limb necrosis at the injection site, myocarditis, and eventual virus-mediated death, if administered intracranially, occurred in immunocompromised severe combined immunodeficiency (SCID) mice treated with reovirus. Such toxicity has not been seen in other, non-SCID models.[87,88] Although immunocompromised by their tumor and glucocorticoid use, toxicity in otherwise immune competent cancer patients was predicted to be minimal due to the known mild disease profile of reovirus in humans. After direct inoculation in primates, no significant toxicities were observed,[89] paving the way for phase 1 trials in patients. Two phase I dose escalation trials, in which reovirus (Reolysin) was injected intratumorally in patients suffering from recurrent malignant glioma, have been conducted in Canada (University of Calgary) and in the United States (University of Alabama at Birmingham, Ohio State University, and Cedars-Sinai Medical Center).[12] The second

Table 1. *Oncolytic Adenoviruses for Glioma Therapy*

Agent	Replication Control	Infection Control	Receptor Target	Transgene: Promoter	Location
ONYX-015[52]	ΔE1B-55k	None (wt fiber)	CAR		
Ad-Δ24[54]	Δ24	None (wt fiber)	CAR		
Ad-Δ24-p53[57]	Δ24	None (wt fiber)	CAR	p53: SVE	E3
Ad-Δ24-hyCD[59]	Δ24	None (wt fiber)	CAR	hyCD: CMVi.e.	E3
Ad-Δ24-TIMP[60]	Δ24	None (wt fiber)	CAR	TIMP3:CMVi.e.	E3
Ad-Δ24-RGD[61]	Δ24	RGD fiber	$\alpha v\beta 3$, $\alpha v\beta 5$ integrins, CAR		
CB1[63]	Δ24, ΔE1B-55k	None (wt fiber)	CAR		
Ad-Δ24/39[55]	Δ24, Δ39	None (wt fiber)	CAR		
Ad-2/24CMV[64]	CMV, Δ2, Δ24	None (wt fiber)	CAR		
ICOVIR-5[65]	E2F1, Δ24	RGD fiber	$\alpha v\beta 3$, $\alpha v\beta 5$ integrins, CAR		
Ad-24TYR[66]	Tyrosinase, Δ24	None (wt fiber)	CAR		
Ad-24CMV[64]	CMV, Δ24	None (wt fiber)	CAR		
CRAd-survivin-RGD[72]	Survivin	RGD fiber	$\alpha v\beta 3$, $\alpha v\beta 5$ integrins, CAR		
CRAd-survivin-5/3[69]	Survivin	5/3 Chimeric fiber	CD46, CD80, CD86		
CRAd-survivin-pk[70]	Survivin	pk7 fiber	HSPG		
dl520[73]	Del. of E1A 13S	None (wt fiber)	CAR		
HYPR-Ad[75]	Hypoxia response element	None (wt fiber)	CAR		
Ad5-gfa2(B)3-E1[76]	GFAP with B enhancer	None (wt fiber)	CAR		
hTERT-Ad[77]	hTERT	None (wt fiber)	CAR	EGFP:nativeE1B	E1
hTERT-Ad-RGD[78]	hTERT	RGD fiber	$\alpha v\beta 3$, $\alpha v\beta 5$ integrins, CAR	EGFP:nativeE1B	E1
Ad5/35.GΔ · ki[79]	GFAP (E1A), Ki67 (E4)	5/35 Chimeric fiber	CD46		
Ad5/Ad35.IR-E1A/TRAIL[81]	Inverted repeats, RSV	5/35 Chimeric fiber	CD46	TRAIL:IRS, RSV	E1
IG.Ad5.E1+.E3TK[82]	NA (wild-type)	None (wt fiber)	CAR	hsvTK:nativeE3	E3

wt = wild type.

study is still underway at the time of this writing. In the Calgary study, up to 1×10^9 PFU of virus was administered in the highest dose group, which was well tolerated. No maximally tolerated dose was defined in this study. These results warrant continued investigation of reovirus in efficacy studies alone or in combination with currently defined standard of care therapies, including radiation and chemotherapy.

PARAMYXOVIRUS

Newcastle disease virus

The avian paramyxovirus, Newcastle disease virus (NDV), is a highly contagious disease of chickens, turkeys, and many wild birds. On occasions of human infection, NDV can cause cause mild flu-like symptoms, laryngitis, and conjunctivitis.[90] Although both the lytic and nonlytic strains can be cytotoxic,[91] it is the lytic strains that have been more extensively investigated as anti-neoplastic agents after the virus was found to show enhanced replication efficiency in cancer cells as compared with replication in normal cells.[92,93] The NDV vector 73-T was one of the early vectors to show efficacy in a number of animal tumor models after direct administration into human neuroblastoma[94] and fibrosarcoma[95] xenografts in mice. Efficacy was also demonstrated after intraperitoneal administration for treatment of a variety of carcinomas.[96] Another NDV attenuated vector, PV 701, has been tested in several solid tumors, but not tumors of CNS origin, for safety after intravenous administration using different dosing regimens.[97–99]

Two NDV strains have been evaluated in early phase I/II clinical trials of patients with recurrent GBM: MTH-68/H and NDV-HUJ. The MTH-68/H NDV strain has been utilized by a Hungarian group for the treatment of a number of cancers.[100–102] In 1999, a case was reported of a 14 year-old boy diagnosed with a recurrent GBM who was treated intravenously with MTH-68/H daily, beginning in April 1996.[101] The tumor reportedly shrunk between November 1996 and September 1998, after which chemotherapy was discontinued, and at last report the boy had been receiving only MTH-68/H injections. In 2004, four additional case studies using MTH-68/H in one adult and three pediatric GBM patients were reported with substantial increase survival rates ranging

between 5 and 9 years.[103] Finally, MTH-68/H therapy combined with valproic acid (an antiepileptic drug, also shown to have anti-tumor activity) was evaluated in a pediatric patient with anaplastic astrocytoma unresponsive to irradiation and chemotherapy.[104]

The most recent NDV vector to be assessed in a phase I/II clinical trial of recurrent GBM is the lentogenic NDV strain HUJ.[105] Lentogenic NDV strains cause mild or asymptomatic illness in poultry that is limited to the respiratory tract. The NDV vectors previously described (73-T, PV701 and MTH-68H) are all mesogenic strains (moderately pathogenic), and use of these strains poses increased the risk of undesirable side effects. The safety and anti-tumor activity of the lentogenic NDV-HUJ vector was assessed after intravenous administration of multiple doses. A total of 14 patients, including one pediatric patient, were enrolled. All patients had MRI confirmed recurrent GBM. No major side effects were observed, and one patient had a complete tumor response. Although replication of the HUJ strain is limited in humans, infectious particles were recovered for up to 9 days after dosing in the patients, and suggests that limited replication is occurring, possibly in the tumor tissue. Further studies are warranted using this attenuated strain.

Another NDV vector being developed deserves discussion here. Zulkifli et al.[106] evaluated the V4UPM strain, a modified V4 strain, which developed as a thermostable feed pellet vaccine for poultry for oncolytic activity against two glioma cell lines (DBTRG.05MG and U-87MG), both in vitro and in vivo in subcutaneous flank tumors. The U-87MG was very susceptible to V4UPM, both in vitro and in vivo after a single virus dose. The DBTRG.05MG cell line was more resistant to the NDV vector, but oncolysis still occurred. When a single intratumoral administration to the latter tumors did not result in regression, a second higher dose of virus was administered. Despite this, the tumor grew persistently, although the overall tumor volume was significantly reduced as compared to the control treatment group alone.

Measles virus

Another member of the Paramyxoviridae family, measles virus (MV) represents a fairly recent addition to the growing panel of oncolytic viral vectors being evaluated for glioma therapy. The MV receptor, CD46, is upregulated in many glioma cell lines, allowing for preferential tumor targeting.[107,108] The measles virus vector MV-carcinoembryonic antigen (CEA), engineered to overexpress the human carcinoembryonic antigen, has demonstrated infection of a number of human glioma cell lines and xenografts.[108] Toxicology studies using MV-CEA in rhesus monkeys, including MRI imaging 4 to 5 months after direct intracranial administration, demonstrated no evidence of neurological dysfunction.[109] The authors indicated that a phase I study to test the safety of MV-CEA, after administration both intratumorally and into the resection bed, is underway.

POXVIRUSES

Vaccinia virus

Vaccinia virus, a member of the Poxviridae family, has been recently considered for use as an oncolytic vector for glioma therapy, in part due to results from a phase III trial in patients with stage III melanoma.[110] Administered as a tumor vaccine, a polyvalent vaccinia melanoma oncolysate revealed an increase in the disease-free interval and overall survival in treated patients. Similar to the initial thymidine kinase deletion mutants in HSV-1, tk inactivation in vaccinia virus limits replication to transformed cells.[111,112] A recombinant vaccinia virus engineered to express p53 (rVV-p53) was demonstrated to inhibit growth of a number of human and murine glioma cell lines alone[113] or in combination with radiation therapy in the rat C6 glioma model.[114] Recombinant VV that express IL-2 or IL-12 have enhanced viral-mediated oncolysis as compared with viruses that do not express one of these cytokines.[115,116]

Myxoma virus

Another poxviridae family member being considered for oncolytic viral therapy is the myxoma virus, with tropism that is restricted to European rabbits. The myxoma virus is nonpathogenic in humans.[117] The myxoma viruses has been shown to have oncolytic activity against a number of human glioma and medulloblastoma cell lines in vitro and increased tumor reduction *in vivo*.[118,119] Combination of intratumoral injection of myxoma virus with rapamycin treatment increased intratumoral viral replication and prolonged survival of tumor-bearing mice.[119] The oncolytic activity of the myxoma virus in syngeneic tumors in immunocompetent animals remains to be determined.

POLIOVIRUS

Recombinant PV: PV-RIPO

The neuropathogenicity of poliovirus (PV) can be attenuated by mutations within the IRES sequence located in the 5′ untranslated region of its genome.[120] Substitution of the PV-1 Mahoney IRES sequence with the IRES sequence from human rhinovirus type 2 was described to severely attenuate it while maintaining its ability to replicate in non-neuronal cells.[120] This mutant, referred to as PV-1-RIPO, was demonstrated to be safe for intracranial administration.[121,122] PV recombinants such as PV-1-RIPO are naturally tropic for GBM due to expression of the poliovirus receptor CD155 on these tumors.[122,123] Subsequently, PVS-RIPO (derived from the Sabin vac-

cine strain, not the wild type PV-1 Mahoney strain) was shown to be genetically stable after *in vivo* passage in GBM xenografts, alleviating concerns regarding its phenotypic stability in the context of its replication in malignant glioma. This finding, supported by lack of sequence changes after serial passage,[124] supports consideration of PVS-RIPO for advancement into clinical trials in patients with recurrent GBM.

Poliovirus-derived replicons

Replicons are oncolytic poliovirus derivatives, engineered with a deletion in the capsid (P1) protein, which prevents release of new infectious particles after a single replication cycle.[125,126] Safety was established using transgenic mice expressing the human poliovirus receptor that were given both wild-type polio virus to compare to replicon administration both intracranially and intraspinally.[127] Replicons have demonstrated oncolytic activity in a number of CNS-derived tumors *in vitro*,[128] as well as increased survival of SCID mice bearing intracranial tumors of the human malignant glioma cell line, known as D54-MG.

RHABDOVIRUS

Vesicular stomatitis virus

Vesicular stomatitis virus (VSV) is part of the Rhabdoviridae family, which are negative-sense, nonsegmented RNA viruses with a genetic organization that is similar to the Paramyxoviridae family, both of which are part of the Mononegavirales order. VSV infection in humans is rare, and usually asymptomatic in those cases.[129] When compared as one of nine potential new OVs for glioma therapy, the VSV variant, termed VSV-rp30, was superior to the other candidates regarding replication, spread, and ability to lyse tumor cells.[130] This virus was selected for adaptation to optimal replication in malignant glioma cells after numerous serial passages. A recent report showed that intravenous administration of VSV-rp30 in experimental murine models of both human and mouse intracranial tumors specifically targeted those tumors, as compared to control viruses which did not. Tumors outside the brain were also targeted by VSV-rp30, suggesting that this virus may be an effective target against migrating tumor cells as well.[131]

Table 2. *Summary of Oncolytic Viruses in Glioma Clinical Therapy Pipeline*

Virus	Name	Tumor-targeting Mutation(s)	Clinical Trial Status	References
HSV-1	G207	$\gamma_1$34.5 genes deleted, *lacZ* insertion in UL39	Two Phase I studies complete; phase II trial with IR underway	9, 13
	HSV-1716	$\gamma_1$34.5 genes deleted	Phase I/II studies complete; phase III in planning stages	10, 14
	M032	$\gamma_1$34.5 genes deleted, human(M032) IL-12 transgene inserted	Phase I planning stage	30
Adenovirus	ONYX-015	E1B-55kD deleted	Phase I	11
	Ad-Δ24-RGD	24 bp deletion in E1A/RGD fiber	Phase I in progress	61
Reovirus	Reolysin	Naturally tumor-selective	Phase I in progress	12
Newcastle disease virus	MTH-68/H	Attenuated NDV strain (mesogenic)	Pilot phase I	101–104
	NDV-HUJ	Attenuated NDV strain (lentogenic)	Phase I	105
	V4UPM	Attenuated NDV strain	Preclinical	106
Measles virus	MV-CEA	Overexpresses human carcino-embryonic antigen	Phase I trial underway	109
Vaccinia virus	rVV-p53	p53 gene insertion	Preclinical	113, 114
Myxoma virus		Naturally tumor-selective	Preclinical	119
Polio virus	PV-RIPO	Native IRES substituted with IRES from human rhinovirus type 2	Preclinical	121, 122
Vesicular stomatitis virus	VSV-rp30	Passaged, naturally tumor-selective	Preclinical	131

HSV = herpes simplex virus; IL = interleukin; IRES internal ribosome entry sequence; MV = measle virus; NDV = Newcastle disease virus; PV = poliovirus; RIPO = oncolytic poliovirus; VSV = vesicular stomatitis virus; VV = vaccinia virus.

CONCLUSIONS AND FUTURE OF OV THERAPY

The study of viruses for glioma therapy has become increasingly popular within the past 2 decades, as more sophisticated genetic manipulations have led to the development of safer and more tumor-specific viruses. The safety of several of these tumor-targeted viruses have been confirmed by several phase I clinical trials for the HSVs G207[9,13] and HSV1716,[10,14,132] adenovirus ONYX-015,[11] as well as for the Reolysin reovirus trial,[12] Newcastle disease virus vectors MTH-68/H,[103,104] and NDV-HUJ.[105] Table 2 summarizes the different types of oncolytic viral vectors currently in preclinical studies and in phase I and phase II clinical trials for glioma therapy.

Several recent studies indicate that supplementing glioma treatment regimens incorporating chemotherapy or radiotherapy with viral therapy may lead to synergistic antitumor effects. Collectively, the clinically-proven safety of several of these viruses, along with their efficacy in preclinical studies and their evidence of improved tumor oncolysis when administered with standard treatments, support further investigation of these agents in the hope that they may develop into efficacious therapies for these recalcitrant tumors.

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