HHS Public Access

Author manuscript

Handb Clin Neurol. Author manuscript; available in PMC 2024 September 30.

Published in final edited form as:

Handb Clin Neurol. 2024 ; 205: 297–315. doi:10.1016/B978-0-323-90120-8.00009-5.

Cell and gene therapy in Neuro-oncology

Maria Luisa Varela[1,2,3], **Andrea Comba**[1,2,3], **Syed M Faisal**[1,2,3], **Anna Argento**[1,2,3,4], **Jorge A Peña Aguelo**[5], **Marianela Candolfi**[5], **Maria G Castro**[1,2,3], **Pedro R Lowenstein**[1,2,3,4]

[1]Dept. of Neurosurgery, University of Michigan Medical School, Ann Arbor, MI, USA

[2]Dept. of Cell and Developmental Biology, University of Michigan Medical School, Ann Arbor, MI, USA

[3]Rogel Cancer Center, University of Michigan Medical School, Ann Arbor, MI, USA

[4]Dept. of Biomedical Engineering, University of Michigan, Ann Arbor, MI, USA

[5]Instituto de Investigaciones Biomédicas (INBIOMED, UBA-CONICET), Facultad de Medicina, Universidad de Buenos Aires, Buenos Aires, Argentina

Abstract

The majority of primary brain tumors are gliomas, among which Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. GBM has a median survival of 18–24months, and despite extensive research it remains incurable, thus novel therapies are urgently needed.

The current standard of care is a combination of surgery, radiation, and chemotherapy, but still remains ineffective due to the invasive nature and high recurrence of gliomas. Gene therapy is a versatile treatment strategy investigated for multiple tumor types including GBM. In gene therapy, a variety of vectors are employed to deliver genes designed for different anti-tumoral effects. Also, over the past decades stem cell biology has provided a new approach to cancer therapies. Stem cells can be used as regenerative medicine, therapeutic carriers, drug targeting, and generation of immune cells. Stem cell-based therapy allows targeted therapy that spares healthy brain tissue as well establish a long-term antitumor response by stimulating the immune system and delivering prodrug, metabolizing genes, or even oncolytic viruses.

This chapter describes the latest developments and the current trends in gene and cell-based therapy against GBM from both pre-clinical and clinical perspectives, including different gene therapy delivery systems, molecular targets, and stem cell therapies.

Keywords

Gene therapy; Viral vectors; Stem cells; Nanoparticles; Glioblastoma; High-grade glioma

*Correspondence to: pedrol@umich.edu.

Introduction

More than 120 brain tumor types have been identified, and they can be divided into benign or and malignant tumors, including brain metastasis. Primary brain tumors cover a wide range of neoplasms with a broad clinical impact on patients including for e.g. surgically curable conditions, like pilocytic astrocytoma, as well as highly invasive and incurable tumors, like glioblastoma multiforme. Approximately 29.7% of all primary tumors are malignant (Ostrom et al., 2020). The most commonly occurring tumors in the central nervous system (CNS) are gliomas. They account for approximately 25.1% of all primary brain tumors and other CNS tumors, and 80.8% of malignant brain tumors (Ostrom et al., 2020). Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. GBM has high intertumoral as well as intratumoral heterogeneity, and it is characterized by uncontrolled cellular proliferation, with extensive angiogenesis, microvascular proliferation, and highly invasive features (Comba et al., 2021). Primary malignant gliomas' current standard of care (SOC) treatment consists of maximal safe surgical resection, followed by concomitant external beam radiation and chemotherapy with Temozolomide (TMZ)(Stupp et al., 2005). Nonetheless, GBM has a median survival of 18–24 months, and despite extensive clinical and basic research it remains incurable; thus novel therapies are urgently needed.

Neuro-oncology focuses on the treatment of both primary and metastatic brain tumors, and one of its biggest challenges is to find therapies that can actually reach the tumor behind the blood brain barrier (BBB). Surgical resection rarely achieves a complete removal of every tumor cell, due to their invasive features, and since chemotherapy cannot effectively get to the brain and reach therapeutic concentrations, these tumors reappear. The presence of the BBB plays a critical role in the limiting the delivery of anti-tumor agents. Tight junctions present in the endothelial cells of the brain capillaries restrict molecules from the blood to the brain (Tan et al., 2020). Although the BBB is altered at the tumor site itself, endothelial cells still keep drugs from entering the brain (Sarkaria et al., 2017). Different strategies of treatment delivery have been studied trying to overcome this challenge, with so far limited clinical success.

Gene therapy arises as a form of drug delivery, in which genetic material employs cellular machinery to treat a disease. There are different vectors to potentially deliver therapeutic nucleic acids, or gene therapy, i.e., viral vectors, nanoparticles (NP) and stem cells. In this section we will review the different vectors, the therapeutic approaches to treat gliomas, as well as the clinical stage of their current use.

Viral Vectors

Adenovirus Vector

Virus applications for cancer therapy have been tested preclinically and clinically since the 1950s (Kelly and Russell, 2007). Adenoviruses (AdV) belong to the *Adenoviridae* family. They were isolated from human adenoids in 1953 and were exploited as an important tool for molecular biology studies, such as splicing. Adenoviruses are effective vehicles for gene delivery; they are distinguished by a large size of 80–110 nm diameter, are

non-enveloped, and their genome is a linear double-stranded DNA molecule of 26–45 kb within an icosahedral protein capsid; it is relatively stable to physical and chemical changes enabling their survival outside the body. AdV are infectious for several vertebrates including humans; there are 49 distinct varieties causing pneumonia, bronchiolitis, gastrointestinal infections, etc. (Patel, 2011). The major advantage of these viruses is their easy genetic manipulation, effortless transfection in almost all dividing and non-dividing cell types, growth to high titers, minimal biosafety hazards, and high transduction efficiency. They can deliver transgenes of approximately 8 kb, and up to 30 kb in the newer generation helper-dependent vectors. However, one of the main disadvantages is the immune reactivity that they generate (Bin Umair et al., 2022).

The Adenovirus genome comprises genes controlling two different phases of the virus life cycle, Early and Late. Early genes are involved in the entry to the host cell and delivery of the genome to the nucleus and replication of viral DNA (regions E1 to E4). Late genes direct transcription/translation of genes related to structural parts of the virus (regions L1 to L5). Different types of Adv vectors have been established for use in gene therapy approaches. The First-generation vectors have a deletion of the E1a and E1b genes and the insertion of up to 3–4 kb of foreign DNA or deletion of E1 and E3 and insertion of 5–6 kb DNA of transgenic sequences of interest such as therapeutic or reporter genes. The problem with the first-generation vectors was the high immunogenicity against transduced cells. To overcome some of the first-generation vector limitations a second generation of adenovectors were generated using the deletion of E1 and E2 or E1 and E4 genes. Although second generation of Ad-vectors present decreased hepatotoxicity and greater stability *in vivo* they still present virus-immunological challenges and construction is more complicated (Patel, 2011). The last generation of adenoviral vectors are called gutless or helper virus-dependent in which the entire viral coding sequences are removed reducing immunogenicity and enhancing safety. These vectors conserve the end part of the genome, the ITRs sequence required for DNA replication, and packaging signal (Ψ) of the virus (Patel, 2011; Lee et al., 2017a; Bin Umair et al., 2022).

Due to glioma treatment resistance, gene therapy using virus was intended as a promising approach. Different virotherapy approaches for GBM treatment have been developed using Adenovirus, including suicide gene therapy, immune stimulatory gene therapy, and oncolytic viral therapy. Currently, there are several Phase-I and II clinical trials examining the efficacy of Ad-vectors in different types of adult and childhood gliomas.

The suicide gene therapy is one of the main gene therapy approaches investigated for glioma treatment at the pre-clinical and clinical levels. The method is based on the delivery of suicide genes which can convert a nontoxic prodrug to an active toxic compound, causing death of transduced tumor cells. The approach is lethal for replicating cells, targeting brain tumor cells, without necessarily damaging normal, non-dividing brain cells. Adenoviral vectors have been used for suicide gene therapy when expressing herpes simplex virus-thymidine kinase (HSV-TK), followed by ganciclovir (GCV) treatment. HSV-TK phosphorylates GCV, and monophosphorylated GCV gets further phosphorylated to generate triphosphate-GCV (3P-GCV). When cells divide, 3P-GCV is incorporated into new DNA strands halting DNA replication, and therefore inducing apoptosis. Non-replicative vectors

are delivered directly to the tumor site, making a non-toxic therapy valuable for glioma treatment (Hossain et al., 2020).

A randomized phase II clinical trial (NCT00870181) evaluated the safety and efficacy of the viral therapy using Adv-TK combined with GCV in patients with recurrent grade III and IV malignant gliomas. The study was deemed to be safe, and displayed improvement of the progression free survival at 6 months (PFS-6), PFS and overall survival (OS) in patients treated with Adv-TK compared with standard-of-care treatment (Ji et al., 2016).

A Phase II, prospective clinical trial (NCT00589875), evaluated the effectiveness and safety of gene-mediated cytotoxic immunotherapy (GMCI) in newly diagnosed malignant gliomas. The approach used AdV-TK along with valacyclovir (valine ester of GCV that was developed to improve bioavailability of GCV in the brain), combined with standard radiation and chemotherapy. The study reported improvement in patients with greater tumor resection, with a median OS of 25 months in combined therapy versus 16.9 months for standard of care (SOC) alone and 3-year survival of 32% vs 6%, respectively (Wheeler et al., 2016). On the other hand, a Phase I clinical trial for pediatric brain tumors (NCT00634231), combining AdV-TK intratumoral injection and GMCI along with standard of care, showed acceptable safety outcomes and a survival of more than 24 months in 3 of the 8 patients and 2 patients showed no tumor progression at 37.3 and 47.7 months after therapy (Kieran et al., 2019). Several completed or ongoing clinical trials for newly diagnosed or recurrent GBM used adenovirus HSV1-TK/valacyclovir (NCT03596086 and NCT03603405), or HSV1-TK/valacyclovir in combination with AdV-Flt3L (NCT01811992), or with Nivolumab (NCT03576612). However, prospective randomized studies using adenovirus therapy in combination with other therapies will be necessary to assess the actual efficacy of these approaches and improve the clinical outcomes for glioma patients.

Furthermore, administration of non-replicative adenoviral vector-based gene therapies have been used as immune boost therapies to enhance the antitumor immune response. A novel approaches using Adv-vectors expressing Human interleukin-12 (IL-12) under the control of the RheoSwitch System® (Ad-RTS-hiL-12) combined with the activator veledimexin (VDX) was developed (Barrett et al., 2018). A multicenter Phase I clinical trial (NCT02026271) for recurrent high-grade gliomas stated acceptable tolerability and promising preliminary results showing an increased tumor infiltration of lymphocytes supporting the immunological response to hIL-12 in recurrent glioblastomas (Chiocca et al., 2019). However, corticosteroids negatively affected the overall survival correlated with VDX dose, expanding this trial to a Phase I substudy (NCT03679754) to evaluate hIL-12 as a monotherapy in patients with recurrent or progressive GBM.

Another Phase I dose escalation, multi-center clinical trial (NCT03636477) used Ad-RTS-hIL-12/VDX gene therapy in combination with immune check point inhibitors drug (Nivolumab) for treatment of recurrent glioblastomas. This approach showed safety outcomes of the combination immunotherapy and appears headed to an ongoing phase II clinical trial of Ad-RTS-hIL-12 + Veledimex in Combination with Cemiplimab-rwlc in patients with recurrent glioblastoma (NCT04006119) (Chiocca et al., 2022).

A phase-I clinical trial (NCT03330197) had been expanded to pediatric brain tumors to evaluate the effect of Ad-RTS-hIL-12/VDX therapy but terminated in 2021 due to slow accrual.

Oncolytic adenovirus therapy is another approach investigated for the treatment of glioblastomas. This therapy is based on the engineered modification of AdV to conditionally replicate in tumor cells and induce cell lysis, exposing cancer cell antigens and finally stimulating the immune response (Ghajar-Rahimi et al., 2022; Kiyokawa and Wakimoto, 2019).

The oncolytic adenovirus DNX-2401 (Tasadenoturev) was created to target viral replication to tumor cells with mutations in the retinoblastoma pathway (Fueyo et al., 2000). Several clinical trials have been recently completed or are ongoing with this agent. The first clinical study using DNX-2401 was a Phase I, dose-escalation study with a biological end point in patients with recurrent high-grade gliomas (NCT00805376). The study reported that 20% of patients survived for more than 3 years and 3 of total 37 patients had more than 95% of tumor reduction resulting in >3 years of PFS from treatment. Therefore, DNX-2401 treatment likely induced an antitumor response due to the oncolytic effects, followed by induction of an immune-mediated response. Results supported the safety of the study (Lang et al., 2018).

In the Phase Ib study, the addition of IFN-γ expression did not improve patient survival (TARGET-I; NCT02197169) (Lang et al., 2017). Similarly, another Phase I/II clinical trial using DNX-2401, delivered via catheters within the tumor mass, have been performed in the Netherlands (NCT01582516). The analysis of the cerebrospinal fluid (CSF) from the study patients showed that DNX-2401 treatment increased the levels of some cytokines such as tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6) and Interferon gamma (IFN-γ). *In vitro* treatment with patients' CSF increased the levels of CD64 (M1 pro-inflammatory polarization) in macrophages (van den Bossche et al., 2018). Other studies used the combination of DNX-2401 with immune therapies. A current ongoing Phase II clinical trial (CAPTIVE, NCT02798406) is examining the effect of Pembrolizumab, an anti-PD-1 antibody, treatment in combination with the oncolytic DNX-2401 treatment. Moreover, a Phase I trial combining oncolytic DNX-2401 therapy and TMZ is ongoing for recurrent glioblastoma (NCT01956734). Further, a first Phase I trial is testing a third generation of DNX-2440 expressing the co-stimulatory OX40L, for recurrent glioblastoma (NCT03714334). For pediatric brain tumors (DIPG), a Phase I clinical trial evaluating DNX-2401 treatment (NCT03178032) is currently ongoing.

Our lab has focused on improving the delivery of therapeutic transgenes into the brain for the last 20 years. We have developed a first generation recombinant adenoviral vectors to deliver the conditionally cytotoxic enzyme HSV1-Thymidine kinase (Ad.TK) and the cytokine Flt3L (Ad.Flt3L) for the treatment of brain cancer (Ali et al., 2004; Ali et al., 2005). These vectors exhibit a very high transduction efficiency in glioma cells from all species, from rodents, to dogs and humans (Candolfi et al., 2006; Candolfi et al., 2007a; Candolfi et al., 2007b). Ad.TK-transduced cells are capable of phosphorylating the nucleotide analog GCV. Phosphorylated GCV is introduced into the nascent DNA

chain, but it blocks elongation, leading to apoptosis of replicating cells that increase the availability of tumor antigens and Damage-associated molecular patterns (DAMPs) in the tumor microenvironment (Candolfi et al., 2009; Curtin et al., 2009). On the other hand, Ad.Flt3L-transduced cells release Flt3L, which recruits dendritic cells into the tumor (Curtin et al., 2006).

Extensive preclinical research from our lab has proven that this combination therapy triggers antitumor immunity that leads to glioma eradication and immunological memory in relevant glioma models even in combination with standard chemotherapy (Candolfi et al., 2009; Curtin et al., 2009; Ali et al., 2005; Nunez et al., 2019; Ghulam Muhammad et al., 2009; King et al., 2011; Kadiyala et al., 2021). In view of the excellent neuropathology profile of these vectors and their robust antitumor efficacy in experimental glioma, the FDA cleared a Phase I dose escalation trial that was performed in patients with newly diagnosed, resectable malignant gliomas (NCT01811992). During the surgical resection of the tumor patients received increasing doses of both vectors in the tumor cavity, which was followed by cycles of valacyclovir (2) and TMZ in combination with radiotherapy (Lowenstein et al., 2019). The treatment was well tolerated, and the maximum tolerated dose (MTD) was not reached. The analysis of preliminary data suggested that this treatment provides clinically significant survival and warrants further clinical evaluation of this strategy.

In spite of the promising results obtained in experimental glioma and in GBM patients using this first-generation adenoviral platform, this strategy could be further improved by using novel generation vectors that escape the immune system, as first-generation adenoviral vectors remain immunogenic. Although intracranial administration of these vectors in naïve rodents that have not been previously expose to viral antigens systemically leads to stable long term expression of Ad-encoded transgenes (Barcia et al., 2007), a high percentage of human patients exhibit preexistent anti-Ad immunity due to previous infections and vaccination (Fausther-Bovendo and Kobinger, 2014). We have shown that pre-existing anti-Ad immunity leads to reduced Ad-mediated transgene expression in the brain that lasts less than 2 weeks (Barcia et al., 2007). Thus, we have developed high-capacity adenoviral vectors, which are devoid of all Ad genes, which makes them virtually invisible to the immune system and allow much larger payload capacity. We used these vectors for the delivery of TK and Flt3L in glioma models and found that they lead to robust therapeutic efficacy even in animals that were preimmunized against adenoviral vectors (King et al., 2008).

The larger cloning capacity of these vectors allows for the inclusion of both therapeutic genes within one vector or to add regulatory sequences, such as inducible promoters (Puntel et al., 2010). Inducible promoters allow controlling transgene expression by the addition/depletion of the inducer (Candolfi et al., 2006), allowing cessation of transgene expression in the event of adverse effects. We have included a TetOn switch within a High-Capacity Ad in order to control the expression of Flt3L, showing very tight control of gene expression both in vitro and in vivo (VanderVeen et al., 2016; VanderVeen et al., 2013; Xiong et al., 2006). Although high-capacity Ads have not yet been injected in the brain of human patients, preclinical evaluation of their neuropathology and biodistribution (Muhammad et al., 2010; Puntel et al., 2010; Muhammad et al., 2012) suggests that these vectors may

be useful tools for therapeutic transgene expression in the brain even in the context of preexisting anti-Ad immunity and warrant further evaluation in clinical trials.

HSV Vector

Herpes virus simplex 1 (HSV-1) are human infectious virus from the Herpesviridae family. They were the first oncolytic virus engineered to treat brain tumors (Martuza et al., 1991). HSV is a double-stranded DNA, enveloped virus with a 152 kb viral genome. The genome of HSV virus contains 74 known ORFs and two unique regions called long region (UL) containing 56 viral genes and a short region (US) containing 12 genes. Oncolytic HSV displays various advantages that makes them attractive for gene therapy use. HSV are highly infectious and able to infect many cell types *in vitro*; many genes are nonessential for growth *in vitro* and can be deleted to insert exogenous transgenes. HSV-1 can be grown to high titer and purity, and minimal requirements for replication and packaging (Patel, 2011). HSV-1 is neurotropic that facilitates the selective targeting of neural pathologies. Moreover, deletion of the γ34.5 gene minimizes the damage of non-tumoral brain cells. Also, HSV-1 induces cell death and also initiates and improves the antitumor immune response (Wilcox and Longnecker, 2016; Alayo et al., 2020). Due to all these advantages, currently there are several undergoing clinical trials using oncolytic HSV-1 therapies for brain tumors.

Most of the clinical trials based on HSV-1 used HSV-G207, a vector that includes the deletion of two copies of γ34.5 and has inserted the E. Coli ß-galactosidase gene, (LacZ) in the UL39 gene that inhibits DNA synthesis and replication of HSV-1 in normal cells. Moreover, this vector contains the thymidine-kinase (TK) gene. The mechanism of action of this therapy is based on the ability to induce glioma cell death by oncolysis and by enhancing the tumor infiltration of cytotoxic immune effectors. Two Phase I and II clinical trials have been completed (NCT00157703 and NCT00028158). Radiographic results showed safety and potential treatment response of a single dose HSV treatment and increased with radiation treatment (Markert et al., 2014; Markert et al., 2000; Markert et al., 2009).

The use of the oncolytic HSV-G207 in progressive supratentorial pediatric brain tumors is under investigation. A phase I clinical trial (NCT02457845) using intratumoral inoculation of G207 in combination with radiation showed no dose limiting toxic effects and clinical, radiographic and pathological responses in 11 of 12 patients and significantly increased lymphocyte tumor infiltration (Friedman et al., 2021). Currently, other Phase II clinical trial will assess the effect of HSV-G207 and a single radiation dose in pediatric recurrent high-grade glioma (NCT04482933) and a Phase I clinical trial in recurrent cerebellar brain tumors (NCT03911388).

A modified HSV-G207, G47Δ, is a third-generation oncolytic herpes constructed by deleting the α47 gene and overlapping US11 promoter from parental G207, which is a second-generation oncolytic HSV-1 with deletions in both copies of the γ34.5 gene and an inactivation of the ICP6 gene (Todo et al., 2001). This oncolytic virus has been tested in a phase 2 trial in Japan (UMIN000015995). Recent reports indicate that employing HSV G47Δ produces a survival benefit and good safety profile in patients with residual or recurrent GBM. The administration consisted in intratumoral injection using MRI-guided

stereotactic surgery at intervals of 5–14 d for the first and second doses, and up to six doses at intervals of 4 ± 2 weeks for the third and subsequent doses. Overall median survival was 20.2 (16.8–23.6) months after G47Δ initiation and 28.8 (20.1–37.5) months from the initial surgery (Todo et al., 2022). Since the median survival of recurrent GBM after standard of care treatment is ~6.0 months, these results show a remarkable improvement (Ballman et al., 2007).

Further evolution of HSV-207 includes a modification (M032-HSV-1) by insertion of the proinflammatory cytokine IL-12 to increase the activation of the adaptive immune response. A phase I clinical trial (NCT02062827) using this approach for the treatment of recurrent high-grade gliomas is currently in progress.

Additionally, a phase I clinical trial using rQNestin γ34.5v2 is currently active (NCT03152318) for recurrent high-grade gliomas. This therapy is a variation of previous approaches, as the oncolytic HSV vector includes one deletion of the γ34.5 gene and the second copy of this gene is placed under the control of the nestin promoter, that thus enables the viral replication in infected glioma tumor cells expressing high levels of nestin, without allowing replication in normal cells (Chiocca et al., 2020). Patients received intratumoral injection during surgery with and without preoperative administration of cyclophosphamide. Preliminary results showed that CCL2 and IL-10 production were detected in the serum of patients under the treatment (Otani et al., 2022). Further results will inform the safety and efficacy of oncolytic rQNestin γ34.5v2 and the effect of cyclophosphamide on innate immune response.

Retroviral vectors

Retroviruses are single-stranded positive-sense RNA-containing viruses. The retroviral genome consists of two copies of single-stranded highly condensed RNA molecules of 7–12 kb (Goff, 1992). The RNA genome is encapsulated in an icosahedral protein shell and a lipoprotein envelope (Cepko and Pear, 2001; Shimotohno, 1983; Weiss, 1987; Goff, 1992). The murine leukemia virus-derived replication-competent gamma-retroviruses can incorporate up to 8 kb of foreign DNA and transfect only proliferating mitotic tumor cells (Tai and Kasahara, 2008). Retroviral vectors, which encode herpes simplex virus-thymidine kinase (HSV-TK), were the first delivery systems tested in clinical trials for the treatment of brain cancer (NCT00001328). The glioma cell expressing HSV-TK dies after being contact with the prodrug GCV (Cytovene) (Rainov, 2000; Ram et al., 1997). The trial's preliminary findings revealed anti-glioma activity in patients with smaller tumors and recurrent glioma (Ram et al., 1997). In the long run, the system's efficiency was lower than expectations and OS did not support further developments of this approach.

Vocimagene amiretrorepvec (Toca 511) is a tumor-targeted, non-lytic replication-competent gamma-retroviral vector expressing cytosine deaminase (CD). The prodrug 5-flucytosine (5-FC) can be activated by CD, generating 5-fluorouracil (5-FU). This molecule inhibits thymidylate synthase and incorporation of this metabolite into RNA and DNA, leads to cell death (Longley et al., 2003). Toca 511 and a prolonged-release formulation of 5-FC in combination with TMZ improved treatment efficacy against TMZ-sensitive glioma (Huang et al., 2013; Ostertag et al., 2012; Tai et al., 2005). The combined treatment (Toca 511

+ 5-FC) was found to sensitize the glioma to radiation therapy (Takahashi et al., 2014). Multiple clinical trials for the treatment of patients with recurrent high-grade glioma (rHGG) use this combination (NCT01156584; NCT01156584; NCT01985256). In a phase I trial, rHGG patients treated with Toca 511 plus Toca FC showed promising treatment responses, and the combination treatment will be evaluated further in a randomized phase III clinical trial (Cloughesy et al., 2018). The treatment failed to meet expectations. There were no significant differences between the with Toca 511 plus FC group and the active control group (mOS: 11.10 vs. 12.22 months)(Cloughesy et al., 2020)

Lentiviral vectors

Lentiviruses are positive-sense, single-stranded RNA viruses that have been widely studied for their potential application against GBM (Del Vecchio et al., 2019). Unlike retrovirus, lentiviral vectors (LV) integrate into the host genome but are less prone to insertional mutagenesis. Because LV can replicate in non-dividing cells, they offer an appealing option for extending transgene expression in CNS terminally differentiated cells (Kay, 2011). The most well-known lentivirus is HIV-1-derived, which was initially observed to transduce lymphocytes (Parolin et al., 1994) and non-dividing cells (Naldini et al., 1996). In addition, bovine immunodeficiency virus (BIV) (Berkowitz et al., 2001a), simian and feline (FIV) immunodeficiency virus (Barraza and Poeschla, 2008), equine infectious anemia virus (Poeschla, 2003), maedi-visna virus (MVV) (Berkowitz et al., 2001b), and caprine arthritis encephalitis virus (Mselli-Lakhal et al., 1998) have also been used as sources for gene-therapy vectors. When compared to gamma-retroviruses, LV have a substantially bigger capacity for transgenes (up to 18 kb) (Kumar et al., 2001), and third-generation HIV-based vectors with increased transduction efficiency and safety have been created. These vectors can be manipulated to achieve tissue tropism through pseudotyping and demonstrate low immunogenicity due to a lack of viral protein synthesis expression (Del Vecchio et al., 2019).

GBM cells, including glioma stem cells, have a higher transduction efficiency than normal brain cells, when infected with LV pseudotyped with Lymphocytic choriomeningitis virus glycoproteins (Miletic et al., 2004; Huszthy et al., 2009). LVs are the preferred vectors for silencing RNA (Luan et al., 2015) or creating T cells to express chimeric antigen receptors specific for GBM antigens (Yu et al., 2017). LV with a p2A peptide-enabled dual expression systems allows expression of the tumor suppressor proteins, growth arrest-specific (GAS)-1 and Phosphatase and tensin homolog (PTEN), to be expressed under the control of a CMV promoter (Sanchez-Hernandez et al., 2018). This vector suppressed the growth of human GBM cells *in vitro* and slowed the progression of glioma in a xenograft model of human GBM (Sanchez-Hernandez et al., 2018). Human glioma stem cell tumorigenicity in mice was inhibited by an LV encoding an shRNA specific the orphan nuclear receptor TLX (NR2E1). This receptor plays essential roles in neurogenesis during early embryogenesis and perform crucial functions in maintaining stemness and controlling the differentiation of adult neural stem cells in the central nervous system. It is required for neural stem cell renewal, and induces the expression of DNA hydroxylase ten-eleven translocation 3 (TET3), a potent tumor suppressor downstream of TLX (Cui et al., 2016). The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) 9

systems have also been encoded using LV. Ablation of the Transcriptional Enhancer Factor 1 (TEAD1) decreased human GBM cell movement and altered the migratory and epithelial-mesenchymal transition (EMT) transcriptome signatures using this approach (Tome-Garcia et al., 2018).

In preclinical gene-therapy models, LV have been exploited for the expression of several RNA molecules, including miRNAs, AntagomiRs, siRNAs, and shRNAs, with encouraging results for their application in humans (Scherr et al., 2007; Sun et al., 2008; Zeng et al., 2016; Scherr et al., 2010; Song et al., 2017; Alt et al., 2018). In orthotopic glioma xenograft models, LV-mediated silencing of the vascular endothelial growth factor (VEGF)/VEGF receptor (VEGFR) signaling pathway was associated with smaller tumor size, loss of matrix metalloproteinase 9 (MMP9) immunoreactivity and increased tumor necrosis (Szabo et al., 2016).

A phase I clinical trial using LV vectors to encode chimeric antigen receptor (CAR) recognizing tumor antigens epidermal growth factor receptor variant III(EGFRVIII), Interleukin-13 receptor α2 (IL13Rα2), human epidermal growth factor receptor 2 (Her-2), ephrin type-A receptor 2 (EphA2), prominin-1 (CD133), ganglioside g2 (GD2), and administered with or without anti-PDL1 antibody, is currently evaluating the safety and efficacy of personalized CAR-T-cells immunotherapy for patients with recurrent malignant gliomas (NCT03423992). Autologous T cells transduced with LV redirected to EGFRVIII-with a CAR in patients with EGFRVIII+ glioblastoma is also in phase I clinical trial (NCT02209376) and results show improved treatment efficacy of EGFRvIII-directed strategies (O'Rourke et al., 2017; Johnson et al., 2015).

Adeno-associated virus vectors

Adeno-associated viruses (AAV) are small ssDNA non-enveloped viruses with replication defects that belong to the non-pathogenic parvovirus family (Li et al., 2005; Stilwell and Samulski, 2003; McCown, 2005). AAV are non-autonomous, and require a helper virus, such as adenovirus or HSV, to replicate inside the host cell (van Putten et al., 2010). AAV has several benefits, i.e., it is able to infect a wide variety of dividing and nondividing cells with high efficiency (Santiago-Ortiz and Schaffer, 2016), furthermore its smaller size, which allows it to better infiltrate solid tumors like gliomas (Enger et al., 2002). In human and mouse GBM models, a single cerebral injection of AAV encoding human IFN-β showed enhanced glioma cell death and boosted long-term survival (GuhaSarkar et al., 2017; Yoshida et al., 2002). In an orthotopic xenograft mouse model of invasive glioma, intracranial AAV designed to secrete soluble tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL), coupled with lanatoside C prolongs median survival (Crommentuijn et al., 2016). In a human glioblastoma cell line in vitro, recombinant adeno-associated virus (rAAV) encoding Tissue Factor Pathway Inhibitor 2 (TFPI-2) suppresses invasion, angiogenesis, and tumor formation (Yanamandra et al., 2005).

In the absence of helper virus, AAV genomes establish latency and remain largely as episomes. Only a small percentage of AAV vector genomes integrate into the host cell's genome, thereby nuclear copy number is reduced with each cell division, and AAV vectors have been preferred for short-term transgenic expression (Maier et al., 2010; Asad et al.,

2017; Li et al., 2005). In preclinical models of GBM, the AAV-mediated genetic alteration of brain tumor cells with genes expressing anti-tumor proteins has demonstrated encouraging outcomes, despite the low transduction efficiency of these tumors. A method for rapidly engineering AAV vectors was developed by selecting a chimeric AAV capsid library generated by DNA shuffling of distinct cap genes with improved transduction efficiency that results in enhanced treatment efficacy (Zolotukhin et al., 2013; Maguire et al., 2010; GuhaSarkar et al., 2016). Reduced immunogenicity compared to adenoviruses vector, high titer production, and the option of pseudotyping are all advantages of AAV-based vectors. In the near future, the clinical evaluation of these vectors for the treatment of glioblastomas is anticipated to be prompted by the encouraging outcomes of preclinical gene therapy research employing AAVs (Riyad and Weber, 2021).

Newcastle Disease Virus, Measles virus, and Baculovirus vectors

Newcastle disease virus (NDV) is a highly pathogenic avian ssRNA virus with a 16 kb genome, but usually causes only mild laryngitis and conjunctivitis in humans (Nelson, 1999; Galinski, 1991; Ahmad et al., 2015). NDV in birds can be classified as lentogenic (avirulent), mesogenic (intermediate), or velogenic (virulent), according to its pathogenicity (Zamarin and Palese, 2012). NDV-based vectors have a natural affinity for tumor cells, as well as oncolytic and immunostimulatory capabilities (Schirrmacher et al., 2019). When compared to TMZ alone, supplementary treatment with the LaSota strain of the naturally oncolytic NDV promotes enhanced apoptosis in glioma cells (Bai et al., 2018). Recombinant NDV vectors were developed using reverse genetics as potentially efficient cancer therapy agents (Vigil et al., 2007). NDV has been shown to induce tumor-specific oncolysis, but this mechanism is still ill defined. Oncolytic viruses preferentially target and eliminate cancer cells in an immunogenic manner, thereby facilitating the development of therapeutically relevant tumor-specific immune responses (Pol et al., 2018; Zitvogel et al., 2008). A Phase I/II trial of intravenous NDV-HUJ oncolytic virus was evaluated in patients with recurrent GBM, sarcoma and neuroblastoma (NCT01174537) (Freeman et al., 2006), though this trial was eventually withdrawn.

Measles virus (MV) is an enveloped ssRNA virus that causes measles and, very rarely, encephalitis (Ulasov et al., 2006). Two major proteins of MV support the oncolytic activity of MV, which shows promising efficacy in glioma therapy. The MV hemagglutinin glycoprotein has a high affinity for receptors such as nectin-4 and CD46 on host cells (Dorig et al., 1993; Muhlebach et al., 2011). The CD46 receptor is vastly overexpressed in neoplastic cells, including glioma (Dorig et al., 1993). The fusion protein of MV mediates cell-cell fusion, inducing apoptosis of these cells. The highly attenuated Edmonston strain (MV-Edm) with expression of carcinoembryonic antigen (CEA) is highly pathogenic and has potential therapeutic efficacy in gliomas (Galanis et al., 2001; Msaouel et al., 2013). No dose-limiting toxicities were seen in a dose-escalating phase I clinical trial of MV-CEA (NCT00390299) in patients with recurrent GBM (Msaouel et al., 2013). Initially, anticancer activity against gliomas by genetically engineered oncolytic measles virus strains was demonstrated by Phuong et al. *in vivo* as well as *in vitro* (Phuong et al., 2003). Recently, modified MV-NIS was evaluated in a phase I clinical trial (NCT02962167) for the treatment

of children and young adults with recurrent medulloblastoma or recurrent atypical teratoid rhabdoid tumors (ATRT) (Ghajar-Rahimi et al., 2022; Estevez-Ordonez et al., 2021).

Baculovirus (BV) vectors are another potential virus to be used for gene therapy. BV are enveloped dsDNAs which generally infect insects and do not replicate in human cells, which makes them very safe (van Oers et al., 2015). There is no pre-existing human anti-BV immunity, making them potentially effective for human therapies (Ang et al., 2016). BV has a DNA genome of approximately 134 kb, comparatively easy to engineer and can harbor large transgenes, but has not yet been evaluated in clinical trials (Dautzenberg et al., 2017; van Oers et al., 2015). The BV avoids some of the drawbacks of mammalian viral vectors while also providing another option for cancer gene therapy, BV encoding diphtheria toxin A gene showed enhanced efficacy against malignant glioma (Wang et al., 2006).

All key viral vector clinical trials are summarized in Table 1.

Non- viral vectors

Nanoparticles and Liposomes

Non-viral vectors such as liposomes, nanoparticles (NP), and polymeric vectors have been increasingly studied for GBM therapies due to their lower risks of immunogenicity and cytotoxicity compared to viral vectors (Caffery et al., 2019). Liposomes are 400 nm – 2.5 μm spherical vesicles that encapsulate a liquid phase, often containing chemotherapeutic drugs (Malam et al., 2009). They are non-toxic, biocompatible, and biodegradable. Nanoparticles range from 1 – 1000 nm in diameter and can be fabricated using nanoprecipitation, double emulsion solvent evaporation, or lithography. They are often synthesized from biocompatible polymers which are made to contain pharmacological compounds, and can be functionalized to selectively bind to different target sites where they eventually degrade over several days or weeks. Nanoparticles are also uniquely able to target multiple ligands such as antibodies, peptides, small molecules, and cell surface proteins. These vectors are also promising due to their ability to cross the BBB and release tumoricidal drugs specifically at GBM tumors (Wiwatchaitawee et al., 2021). Although there are no non-viral vectors currently FDA approved for the treatment of GBM, several have entered clinical trials as described below.

One of the most promising liposomal gene therapies for GBM is SGT-53, a tumor-targeting immunoliposome complex (SGT) encapsulating wild-type p53 plasmid DNA (Senzer et al., 2013). SGT-53 is able to cross the BBB to efficiently deliver p53 cDNA to GBM cells, resulting in wtp53 restoration. An initial phase 1 clinical trial (NCT00470613) in 2013 showed minimal side effects of SGT-53 therapy in patients with advanced solid tumors of varying cancers (Senzer et al., 2013). Significantly, it exhibited strong anticancer activity in 7 of 11 patients and showed an accumulation of p53 transgene in the targeted metastatic tumors but not normal skin tissue. Recent work with SGT-53 has focused on combination treatments with SOC GBM therapeutics. SGT-53 and temozolomide (TMZ) combination therapy improves chemosensitivity, resulting in a sensibilization of tumor cells to TMZ, and apoptosis in a GBM mouse model (Kim et al., 2015). It also has been shown to diminish, and potentially reverse, the development of TMZ resistance *in vitro* and *in vivo*. However,

a TMZ and SGT-53 co-therapy Phase II Clinical Trial began in 2015 but was terminated in 2021 due to poor enrollment. SGT-53 combined with Docetaxel has undergone a Phase 1b study (treating multiple cancers, including glioblastoma) which showed low toxicity and high clinical activity in 12 patients, with 3 patients achieving RECIST-verified partial responses of tumor reduction and 2 patients reaching stable disease state (Pirollo et al., 2016). Furthermore, combination therapy of an anti-PD1 antibody and SGT-53 in GL261 glioblastoma mouse tumor models showed increased anti-tumor efficacy by sensitizing the previously anti-PD1 resistant tumor to anti-PD1 (Kim et al., 2018). More clinical work is necessary to bring these therapies to Phase II and III clinical trials and perhaps FDA-approval.

Another liposomal-based nano-therapeutic is being developed by Tellingen et al., 2-BBB Medicines in The Netherlands, and 2X Oncology, Inc. in Cambridge, MA (Gaillard et al., 2014). Glutathione pegylated liposomal doxorubicin (2B3–101) is an existing liposome functionalized with an additional glutathione coating to enhance delivery across the BBB. 2B3–101 has been shown to significantly inhibit GBM growth in a xenograft model and is undergoing two focused Phase 2 trials in metastatic breast cancer and GBM (NCT01386580).

Nanoparticles are also being explored for GBM therapies. While none are currently FDA-approved for the treatment of gliomas, a lipid nanoparticle containing siRNAs called Patisiran was FDA approved in 2018 for treating a neurodegenerative disease, transthyretin-mediated amyloidosis (Caffery et al., 2019). Nanoparticles are comprised of a metal or carbon backbone containing or coated with anti-GBM components like chemotherapeutic drugs or immune cells (Alphandery, 2020). They are also fabricated to easily pass through the BBB and target GBM cellular receptors or angiogenic blood vessels. NU-0129 is a spherical gold nanoparticle functionalized with siRNAs targeting the oncoprotein BCL-2-like protein 12 (Bcl2L12) (Senzer et al., 2013) (Kumthekar et al., 2021). A 2019 Phase 0 clinical trial of eight GBM patients found NU-0129 crossed the BBB, accumulated in the tumor, and reduced Bcl2L12 protein abundance and did not cause short or long-term toxicity (NCT03020017). A synthetic protein nanoparticle (SPNP) comprised of polymerized human serum albumin (HSA) and oligo ethylene glycol (OEG) was developed in 2020 by (Gregory et al., 2020). The SPNPs contained siRNA against Signal Transducer and Activation of Transcription 3 factor (STAT3i) and were equipped with cell-penetrating peptide iRGD. The SPNPs penetrated the BBB, distributed throughout the tumor and delivered siRNA against STAT3. In GBM mouse models, the SPNP alongside ionized radiation was found to result in tumor regression and long-term survival in 87.5% of mice.

RNA nanoparticles are also being used to deliver nucleic acid sequences to tumor cells; targeted inhibition of oncogenic miRNA-21 by three-way-junction (3WJ)-based RNA nanoparticles (RNP) was recently shown by Croce et al in 2017 (Lee et al., 2017b). Continuing this work, Lee et. al created a new RNP called FA-3WJ-LNA-miR21 which when directed towards glioma cells, decreased their expression of miR-21, overall resulting in increased glioma cell death (Yoo et al., 2021). These studies show the clinical benefit of FA-3WJ RNP-based gene therapy targeting oncogenic miRNAs but have not yet entered clinical trials.

Stem cells

Stem cells are undifferentiated multipotent progenitor cells, which have the capacity to self-renew, migrate, and differentiate. There are different types of stem cells; mainly they can be clustered into 2 groups: Embryonic Stem Cells (**ESC**) and Adult Stem Cells (**ASC**).

ESC are pluripotent cells, which means they can become any cell type except those in the placenta. This property makes them a powerful tool but the only way they can be obtained is isolation from the inner mass cells of embryos. Its origin brings up ethical considerations and it is because of these concerns that therapeutic use has been restricted. To avoid this, in 2006 a protocol was developed to establish pluripotent stem cells from somatic cells by the addition of defined factors, also known as Yamanaka factors (Takahashi and Yamanaka, 2006). These induced Pluripotent Stem Cells (**iPSC**) are non-embryonic cells reprogramed to become ESC-like, sharing ESC pluripotentiality, being able to generate ectoderm, mesoderm, and endoderm, and with indefinite replication capacity (Takahashi and Yamanaka, 2006).

ASC are undifferentiated precursors that are present in all differentiated tissue and can repopulate it (Prentice, 2019; Clevers, 2015). This group of cells includes Hematopoietic Stem Cells (**HSC**), Mesenchymal Stem Cells (**MSC**), and Neural Stem Cells (**NSC**). HSC can be obtained from peripheral blood, umbilical cord blood, or bone marrow, being the most accessible adult stem cell. They can give rise to both myeloid and lymphoid lineages (Seita and Weissman, 2010). MSC are stromal cell that can be isolated from many different tissues, including umbilical cord, endometrial polyps, menses blood, bone marrow, adipose tissue, and endometrium. They can give rise to osteocytes, chondrocytes, monocytes and adipocytes (Ding et al., 2011). The only endogenous brain stem cells are NSC. This population can be found mainly in the subventricular zone (SVZ) of the forebrain, and hippocampus. Its multipotentiality allows them to give rise to neurons, astrocytes, and oligodendrocytes (Gage, 2000).

Stem cell function is involved in cell repair in local tissue, but they have also showed ability to target and migrate to tumor tissue, including across the BBB, making them a useful vector for gene delivery (Aboody et al., 2000; Bechmann et al., 2007). Implantation or injection of progenitor cells, carrying genetic modifications to produce an anti-tumor response has advantages as these cells can home to tumor cells. Stem cell therapies involve administration of exogenous or mobilization of endogenous stem cells (Carbajal et al., 2010; Benmelouka et al., 2021). Both MSC and NSC have displayed tropism to malignant gliomas (Spaeth et al., 2008; Aboody et al., 2000).

Glioma extra cellular matrix (ECM), as well as secreted factors, direct cell migration of several cell types (Carey-Ewend et al., 2021). Tumors present similarities with chronic injury, like hypoxia, and inflammation (Dvorak, 1986). Both, angiogenic and proinflammatory factors are produced by microglia and astrocytes in brain tumors (Müller et al., 2006).The chemokines produced by them, as well as tumor cells and infiltrating tumor cells, such as VEGF, HIF1α, CCL-25, IL-6, CXCL16, and CXCL12, attract stem cells (Jung et al., 2013; Rattigan et al., 2010; Xu et al., 2012). Studies using mouse models have demonstrated that both MSC and NSC migrate to brain tumors (Stuckey and

Shah, 2014). NCS express CCR2, which induces migration to Monocyte chemoattractant protein-1(MCP-1/ CCL2), which is highly expressed in brain neoplastic lesions (Magge et al., 2009). The lack of major histocompatibility complex type II (MHC II) in NSC allows the implantation of these cells which are able to escape host immune responses upon transplantation (Molina-Holgado and Molina-Holgado, 2010). Although there are several chemokines mediating stem cell tropism to brain tumors, there is still much to learn and the molecular mechanisms involved. Nevertheless, the migratory potential of stem cells strongly supports their therapeutic use, especially to possibly target tumor cells located far from the main tumor mass.

One of the most explored pathways for stem cell-based therapy induced cytotoxicity is the use of tumor necrosis factor-related apoptosis inducing ligand (TRAIL) interaction with death receptor (DR), which is expressed by tumor cells, and when ligated activates caspase-mediated apoptosis. This is supported by the fact that GBM exhibits TRAIL-mediated apoptosis (Sasportas et al., 2009; Hingtgen et al., 2010; Menon et al., 2009; Kauer et al., 2012). Delivery of soluble TRAIL (sTRAIL) deployed by NSCs in xenograft models of glioma showed an improved survival with a significant reduction of tumor burden (Balyasnikova et al., 2011). Although TRAIL has been studied in animal models it has not reached yet human trials.

Another approach that uses stem cells is enzymatic pro-drug activation. NSC can be used to deliver enzymes that activate prodrugs present in the tumor microenvironment. NSC can be modified to express HSV1-TK, which then can catalyze conversion of GCV after administration. Also, 5-FC pro drug can be activated by cytosine deaminase, generating cytotoxic 5-FU. Both mechanisms of prodrug activation, HSV1-TK plus GCV and CD plus 5-FC, have been described above in the adenovirus section and retroviral section, respectively. Another modification to employ enzymatic pro-drug activation is the induction of the expression of rabbit carboxylesterase in stem cells, that can convert irinotecan into toxic topoisomerase-1 inhibitor SN-38 by (Aboody, 2016).

A different approach to use stem cells, and to benefit from their tropism is delivery of oncolytic virus. In murine xenograft model of glioma, human MSC transfected with replication-competent oncolytic adenovirus (CRAd) exhibited the ability to migrate to the brain, when injected away from tumor site, and deliver CRAd to glioma cells (Sonabend et al., 2008). This approach of stem cell-based therapy could improve oncolytic virotherapy for glioma. Stem cells infected with replication competent virus is an innovative way of delivering virus into the tumor, to produce viral infection followed by tumor cell death.

The only stem cell procedure currently approved by the FDA is HSC administration, which is employed to treat multiple myelomas, leukemia, and blood disorders (Copelan, 2006). The first human study employing stem cells for neuro-oncology was performed by Portnow et al.(Portnow et al., 2017). This study involved 15 patients with recurrent GBM, which received a single dose, injected intracranially, of genetically modified NSC, expressing CD, plus prodrug 5-FC, as a single dose (NCT01172964). It showed no difference in overall survival. Nonetheless, this study establishes the safety of NSC, and the efficacy of the stem cells to locally produce chemotherapy, i.e., 5-FU (Portnow et al., 2017). Brain autopsy

proved that NSCs were not tumorigenic and also that they had migrated to distant tumor sites. This was a proof-of-concept of the ability of NSCs to target brain tumors and locally produce chemotherapy. The follow up study showed dose-escalation, multiple-treatment round of NSC-CD plus 5-FC, in combination with 5-FC and folinic acid (Leucovorin) (NCT02015819), aiming to determine the establish a dose to take forward for phase 2.

Another enzyme pro-drug system being explored in clinical trials is the use of NSC modified to express carboxylesterase plus the prodrug irinotecan. A currently active, not yet recruiting, phase 1 clinical trial for recurrent high-grade glioma will have 2 administrations of carboxylesterase-expressing allogeneic NSC on days 1 and 15 (NCT02192359). There are new clinical trials to study stem cell-based therapy to improve viral delivery. In this regard, Phase 1 clinical trial (NCT03072134) for newly diagnosed malignant glioma employed NSC expressing CRAd-S-pk7, an oncolytic adenovirus. NSC-CRAd-S-pk7 were implanted intracranially after resection. This trial was able to prove the safety of the treatment and is moving to a Phase 2/3 clinical trial (Fares et al., 2021). A soon to be recruiting Phase 1 clinical trial (NCT05139056) will use NSC-CRAd-S-pk7 in recurrent high-grade glioma, administered intracerebrally over 10 minutes once weekly for up to 4 doses. Another viral therapy used in combination with stem cells is a phase 1 clinical trial (NCT03896568) employing bone marrow derived MSC, loaded with oncolytic adenovirus DNX-2401, in patients with recurrent GBM, gliosarcoma, or wild-type IDH-1 anaplastic astrocytoma. No results have been reported to date.

In 1- to 21-year-old patients, a currently recruiting clinical phase ½ trial, to treat Diffuse Intrinsic Pontine Glioma (DIPG) employing AloCELYVIR, bone marrow-derived allogenic MSC infected with an oncolytic adenovirus, ICOVIR-5 (NCT04758533). Patients will receive weekly infusion of AloCELYVIR for 8 weeks. Results should be forthcoming.

All key non-viral vector clinical trials are summarized in Table 2.

Conclusions

Although gene therapy for human disease was proposed by Friedmann in 1972 (Friedmann and Roblin, 1972), and has currently extensive preclinical data supporting it, treatment of neurological and neuro-oncological disease continues to enter the clinical arena. The first gene therapy product approved by a government agency was Gendicine, a recombinant human p53 adenovirus. It was approved in 2003 by the China Food and Drug Administration (CFDA), to treat head and neck cancer. In Western countries, the first gene therapy arrived in 2012, when the European Commission approved Glybera, an AAV1 viral vector that delivers an intact copy of the human lipoprotein lipase (LPL) gene to reverse lipoprotein lipase deficiency (LPLD). The U.S. approved the first gene therapy treatments in 2015. The first oncolytic viral therapy approved by the FDA was Talimogene laherparepvec, which is a first-in-class, genetically modified, herpes simplex virus type 1–based oncolytic immunotherapy approved for the local treatment of unresectable cutaneous, subcutaneous and nodal lesions in patients with melanoma recurrent after initial surgery. In 2017, the first chimeric antigen receptor (CAR) T-cell immunotherapy was approved by the FDA. Tisagenlecleucel, genetically modified autologous T-cells expressing chimaeric antigen

receptor that targets B-cells (CD19), was approved to treat pediatric and young adult patients with acute lymphoblastic leukemia. Furthermore, by the end of 2017, the FDA approved the first gene therapy to target a disease caused by mutations in a specific gene. Inherited retinal disease (IRD), generated by mutations in both copies of retinal pigment epithelium-specific 65(RPE65), is currently treated with a subretinal injection of AAV2 that delivers a functional copy of this enzyme. This is the first AAV therapy approved in the U.S. In 2019, The FDA approved he first gene therapy approved to treat children less than two years of age with spinal muscular atrophy (SMA), using AAV9-mediated gene delivery of intact SMN1. Each year, the FDA is incorporating more cellular and gene therapies, and also repurposing approved therapies. Gene therapy is a promising and versatile tool in neuro-oncology because it can be locally administered during initial tumor resection and minimizes the risk of systemic toxicity. High grade gliomas are highly heterogenous and infiltrative. Genome editing aims to overcome therapeutic resistance and reduce recurrence. Viral therapy provides a treatment that is able to reach infiltrating cells which cannot be resected. Over the years different vectors employing a variety of genome editing strategies have been proven to be safe (Figure 1). Stem cell-based therapies are currently emerging as a safe and effective way to achieve gene delivery. It is emerging clinically as new mechanisms to activate enzyme-prodrug systems, and through the use of oncolytic viruses (Figure 1). Stem cell-based therapy can further improve gene therapies, by providing additional tumor tropism. Preclinical data even support the use of combined nanoparticles, and stem cells as carriers. In final summary, we believe that using gene therapies, nanoparticles, and stem cells, alone and in combination offer the greatest promise to achieve the successful treatment of glioblastoma multiforme, and other brain tumors.

References

Aboody KS (2016). Optimization of a Neural Stem Cell-mediated Carboxylesterase/Irinotecan Gene Therapy for Metastatic Neuroblastoma

Aboody KS, Brown A, Rainov NG, et al. (2000). Neural stem cells display extensive tropism for pathology in adult brain: evidence from intracranial gliomas. Proc Natl Acad Sci U S A 97: 12846–12851. [PubMed: 11070094]

Ahmad U, Ahmed I, Keong YY, et al. (2015). Inhibitory and apoptosis-inducing effects of Newcastle disease virus strain AF2240 on mammary carcinoma cell line. Biomed Res Int 2015: 127828. [PubMed: 25821783]

Alayo QA, Ito H, Passaro C, et al. (2020). Glioblastoma infiltration of both tumor- and virus-antigen specific cytotoxic T cells correlates with experimental virotherapy responses. Sci Rep 10: 5095. [PubMed: 32198420]

Ali S, Curtin JF, Zirger JM, et al. (2004). Inflammatory and anti-glioma effects of an adenovirus expressing human soluble Fms-like tyrosine kinase 3 ligand (hsFlt3L): treatment with hsFlt3L inhibits intracranial glioma progression. Molecular therapy : the journal of the American Society of Gene Therapy 10: 1071–1084. [PubMed: 15564139]

Ali S, King GD, Curtin JF, et al. (2005). Combined immunostimulation and conditional cytotoxic gene therapy provide long-term survival in a large glioma model. Cancer research 65: 7194–7204. [PubMed: 16103070]

Alphandery E (2020). Nano-Therapies for Glioblastoma Treatment. Cancers (Basel) 12.

Alt EU, Barabadi Z, Pfnur A, et al. (2018). TRAF3IP2, a novel therapeutic target in glioblastoma multiforme. Oncotarget 9: 29772–29788. [PubMed: 30038719]

Ang WX, Zhao Y, Kwang T, et al. (2016). Local Immune Stimulation by Intravesical Instillation of Baculovirus to Enable Bladder Cancer Therapy. Sci Rep 6: 27455. [PubMed: 27273619]

Asad AS, Moreno Ayala MA, Gottardo MF, et al. (2017). Viral gene therapy for breast cancer: progress and challenges. Expert Opin Biol Ther 17: 945–959. [PubMed: 28604109]

Bai Y, Chen Y, Hong X, et al. (2018). Newcastle disease virus enhances the growth-inhibiting and proapoptotic effects of temozolomide on glioblastoma cells in vitro and in vivo. Sci Rep 8: 11470. [PubMed: 30065314]

Ballman KV, Buckner JC, Brown PD, et al. (2007). The relationship between six-month progression-free survival and 12-month overall survival end points for phase II trials in patients with glioblastoma multiforme. Neuro-oncology 9: 29–38. [PubMed: 17108063]

Balyasnikova IV, Ferguson SD, Han Y, et al. (2011). Therapeutic effect of neural stem cells expressing TRAIL and bortezomib in mice with glioma xenografts. Cancer Letters 310: 148–159. [PubMed: 21802840]

Barcia C, Jimenez-Dalmaroni M, Kroeger KM, et al. (2007). One-year expression from high-capacity adenoviral vectors in the brains of animals with pre-existing anti-adenoviral immunity: clinical implications. Molecular therapy : the journal of the American Society of Gene Therapy 15: 2154–2163. [PubMed: 17895861]

Barraza RA & Poeschla EM (2008). Human gene therapy vectors derived from feline lentiviruses. Vet Immunol Immunopathol 123: 23–31. [PubMed: 18289699]

Barrett JA, Cai H, Miao J, et al. (2018). Regulated intratumoral expression of IL-12 using a RheoSwitch Therapeutic System(®) (RTS(®)) gene switch as gene therapy for the treatment of glioma. Cancer Gene Ther 25: 106–116. [PubMed: 29755109]

Bechmann I, Galea I & Perry VH (2007). What is the blood–brain barrier (not)? Trends in Immunology 28: 5–11. [PubMed: 17140851]

Benmelouka AY, Munir M, Sayed A, et al. (2021). Neural Stem Cell-Based Therapies and Glioblastoma Management: Current Evidence and Clinical Challenges. Int J Mol Sci 22.

Berkowitz R, Ilves H, Lin WY, et al. (2001a). Construction and molecular analysis of gene transfer systems derived from bovine immunodeficiency virus. J Virol 75: 3371–3382. [PubMed: 11238863]

Berkowitz RD, Ilves H, Plavec I, et al. (2001b). Gene transfer systems derived from Visna virus: analysis of virus production and infectivity. Virology 279: 116–129. [PubMed: 11145895]

Bin Umair M, Akusa FN, Kashif H, et al. (2022). Viruses as tools in gene therapy, vaccine development, and cancer treatment. Arch Virol 167: 1387–1404. [PubMed: 35462594]

Caffery B, Lee JS & Alexander-Bryant AA (2019). Vectors for Glioblastoma Gene Therapy: Viral & Non-Viral Delivery Strategies. Nanomaterials (Basel) 9.

Candolfi M, Curtin JF, Xiong WD, et al. (2006). Effective high-capacity gutless adenoviral vectors mediate transgene expression in human glioma cells. Molecular therapy : the journal of the American Society of Gene Therapy 14: 371–381. [PubMed: 16798098]

Candolfi M, Kroeger KM, Pluhar GE, et al. (2007a). Adenoviral-mediated gene transfer into the canine brain in vivo. Neurosurgery 60: 167–177; discussion 178. [PubMed: 17228266]

Candolfi M, Pluhar GE, Kroeger K, et al. (2007b). Optimization of adenoviral vector-mediated transgene expression in the canine brain in vivo, and in canine glioma cells in vitro. Neuro-oncology 9: 245–258. [PubMed: 17522335]

Candolfi M, Yagiz K, Foulad D, et al. (2009). Release of HMGB1 in response to proapoptotic glioma killing strategies: efficacy and neurotoxicity. Clinical cancer research : an official journal of the American Association for Cancer Research 15: 4401–4414. [PubMed: 19570774]

Carbajal KS, Schaumburg C, Strieter R, et al. (2010). Migration of engrafted neural stem cells is mediated by CXCL12 signaling through CXCR4 in a viral model of multiple sclerosis. Proc Natl Acad Sci U S A 107: 11068–11073. [PubMed: 20534452]

Carey-Ewend AG, Hagler SB, Bomba HN, et al. (2021). Developing Bioinspired Three-Dimensional Models of Brain Cancer to Evaluate Tumor-Homing Neural Stem Cell Therapy. Tissue Eng Part A 27: 857–866. [PubMed: 32907497]

Cepko C & Pear W (2001). Overview of the retrovirus transduction system. Curr Protoc Mol Biol Chapter 9: Unit9 9.

Chiocca EA, Gelb AB, Chen CC, et al. (2022). Combined immunotherapy with controlled interleukin-12 gene therapy and immune checkpoint blockade in recurrent glioblastoma: An open-label, multi-institutional phase I trial. Neuro Oncol 24: 951–963. [PubMed: 34850166]

Chiocca EA, Nakashima H, Kasai K, et al. (2020). Preclinical Toxicology of rQNestin34.5v.2: An Oncolytic Herpes Virus with Transcriptional Regulation of the ICP34.5 Neurovirulence Gene. Mol Ther Methods Clin Dev 17: 871–893. [PubMed: 32373649]

Chiocca EA, Yu JS, Lukas RV, et al. (2019). Regulatable interleukin-12 gene therapy in patients with recurrent high-grade glioma: Results of a phase 1 trial. Sci Transl Med 11.

Clevers H (2015). What is an adult stem cell? Science 350: 1319–1320. [PubMed: 26659043]

Cloughesy TF, Landolfi J, Vogelbaum MA, et al. (2018). Durable complete responses in some recurrent high-grade glioma patients treated with Toca 511 + Toca FC. Neuro Oncol 20: 1383–1392. [PubMed: 29762717]

Cloughesy TF, Petrecca K, Walbert T, et al. (2020). Effect of Vocimagene Amiretrorepvec in Combination With Flucytosine vs Standard of Care on Survival Following Tumor Resection in Patients With Recurrent High-Grade Glioma: A Randomized Clinical Trial. JAMA Oncol 6: 1939–1946. [PubMed: 33119048]

Comba A, Faisal SM, Varela ML, et al. (2021). Uncovering Spatiotemporal Heterogeneity of High-Grade Gliomas: From Disease Biology to Therapeutic Implications. Frontiers in Oncology 11.

Copelan EA (2006). Hematopoietic Stem-Cell Transplantation. New England Journal of Medicine 354: 1813–1826. [PubMed: 16641398]

Crommentuijn MH, Maguire CA, Niers JM, et al. (2016). Intracranial AAV-sTRAIL combined with lanatoside C prolongs survival in an orthotopic xenograft mouse model of invasive glioblastoma. Mol Oncol 10: 625–634. [PubMed: 26708508]

Cui Q, Yang S, Ye P, et al. (2016). Downregulation of TLX induces TET3 expression and inhibits glioblastoma stem cell self-renewal and tumorigenesis. Nat Commun 7: 10637. [PubMed: 26838672]

Curtin JF, King GD, Barcia C, et al. (2006). Fms-like tyrosine kinase 3 ligand recruits plasmacytoid dendritic cells to the brain. Journal of immunology 176: 3566–3577.

Curtin JF, Liu N, Candolfi M, et al. (2009). HMGB1 mediates endogenous TLR2 activation and brain tumor regression. PLoS medicine 6: e10. [PubMed: 19143470]

Dautzenberg IJC, van den Hengel SK, de Vrij J, et al. (2017). Baculovirus-assisted Reovirus Infection in Monolayer and Spheroid Cultures of Glioma cells. Sci Rep 7: 17654. [PubMed: 29247249]

Del Vecchio C, Calistri A, Parolin C, et al. (2019). Lentiviral Vectors as Tools for the Study and Treatment of Glioblastoma. Cancers (Basel) 11.

Ding DC, Shyu WC & Lin SZ (2011). Mesenchymal stem cells. Cell Transplant 20: 5–14. [PubMed: 21396235]

Dorig RE, Marcil A, Chopra A, et al. (1993). The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell 75: 295–305. [PubMed: 8402913]

Dvorak HF (1986). Tumors: Wounds That Do Not Heal. New England Journal of Medicine 315: 1650–1659. [PubMed: 3537791]

Enger PO, Thorsen F, Lonning PE, et al. (2002). Adeno-associated viral vectors penetrate human solid tumor tissue in vivo more effectively than adenoviral vectors. Hum Gene Ther 13: 1115–1125. [PubMed: 12067444]

Estevez-Ordonez D, Chagoya G, Salehani A, et al. (2021). Immunovirotherapy for the Treatment of Glioblastoma and Other Malignant Gliomas. Neurosurg Clin N Am 32: 265–281. [PubMed: 33781507]

Fares J, Ahmed AU, Ulasov IV, et al. (2021). Neural stem cell delivery of an oncolytic adenovirus in newly diagnosed malignant glioma: a first-in-human, phase 1, dose-escalation trial. The Lancet Oncology 22: 1103–1114. [PubMed: 34214495]

Fausther-Bovendo H & Kobinger GP (2014). Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important? Hum Vaccin Immunother 10: 2875–2884. [PubMed: 25483662]

Freeman AI, Zakay-Rones Z, Gomori JM, et al. (2006). Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. Mol Ther 13: 221–228. [PubMed: 16257582]

Friedman GK, Johnston JM, Bag AK, et al. (2021). Oncolytic HSV-1 G207 Immunovirotherapy for Pediatric High-Grade Gliomas. N Engl J Med 384: 1613–1622. [PubMed: 33838625]

Friedmann T & Roblin R (1972). Gene Therapy for Human Genetic Disease? Science 175: 949–955. [PubMed: 5061866]

Fueyo J, Gomez-Manzano C, Alemany R, et al. (2000). A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo. Oncogene 19: 2–12. [PubMed: 10644974]

Gage FH (2000). Mammalian neural stem cells. Science 287: 1433–1438. [PubMed: 10688783]

Gaillard PJ, Appeldoorn CC, Dorland R, et al. (2014). Pharmacokinetics, brain delivery, and efficacy in brain tumor-bearing mice of glutathione pegylated liposomal doxorubicin (2B3–101). PLoS One 9: e82331. [PubMed: 24416140]

Galanis E, Bateman A, Johnson K, et al. (2001). Use of viral fusogenic membrane glycoproteins as novel therapeutic transgenes in gliomas. Hum Gene Ther 12: 811–821. [PubMed: 11339897]

Galinski MS (1991). Paramyxoviridae: transcription and replication. Adv Virus Res 39: 129–162. [PubMed: 2038953]

Ghajar-Rahimi G, Kang KD, Totsch SK, et al. (2022). Clinical advances in oncolytic virotherapy for pediatric brain tumors. Pharmacol Ther 239: 108193. [PubMed: 35487285]

Ghulam Muhammad AK, Candolfi M, King GD, et al. (2009). Antiglioma immunological memory in response to conditional cytotoxic/immune-stimulatory gene therapy: humoral and cellular immunity lead to tumor regression. Clinical cancer research : an official journal of the American Association for Cancer Research 15: 6113–6127. [PubMed: 19789315]

Goff SP (1992). Genetics of retroviral integration. Annu Rev Genet 26: 527–544. [PubMed: 1482125]

Gregory JV, Kadiyala P, Doherty R, et al. (2020). Systemic brain tumor delivery of synthetic protein nanoparticles for glioblastoma therapy. Nat Commun 11: 5687. [PubMed: 33173024]

GuhaSarkar D, Neiswender J, Su Q, et al. (2017). Intracranial AAV-IFN-beta gene therapy eliminates invasive xenograft glioblastoma and improves survival in orthotopic syngeneic murine model. Mol Oncol 11: 180–193. [PubMed: 28098415]

GuhaSarkar D, Su Q, Gao G, et al. (2016). Systemic AAV9-IFNbeta gene delivery treats highly invasive glioblastoma. Neuro Oncol 18: 1508–1518. [PubMed: 27194146]

Hingtgen SD, Kasmieh R, van de Water J, et al. (2010). A Novel Molecule Integrating Therapeutic and Diagnostic Activities Reveals Multiple Aspects of Stem Cell-based Therapy. Stem Cells 28: 832–841. [PubMed: 20127797]

Hossain JA, Marchini A, Fehse B, et al. (2020). Suicide gene therapy for the treatment of high-grade glioma: past lessons, present trends, and future prospects. Neurooncol Adv 2: vdaa013. [PubMed: 32642680]

Huang TT, Hlavaty J, Ostertag D, et al. (2013). Toca 511 gene transfer and 5-fluorocytosine in combination with temozolomide demonstrates synergistic therapeutic efficacy in a temozolomide-sensitive glioblastoma model. Cancer Gene Ther 20: 544–551. [PubMed: 23969884]

Huszthy PC, Giroglou T, Tsinkalovsky O, et al. (2009). Remission of invasive, cancer stem-like glioblastoma xenografts using lentiviral vector-mediated suicide gene therapy. PLoS One 4: e6314. [PubMed: 19617915]

Ji N, Weng D, Liu C, et al. (2016). Adenovirus-mediated delivery of herpes simplex virus thymidine kinase administration improves outcome of recurrent high-grade glioma. Oncotarget 7: 4369–4378. [PubMed: 26716896]

Johnson LA, Scholler J, Ohkuri T, et al. (2015). Rational development and characterization of humanized anti-EGFR variant III chimeric antigen receptor T cells for glioblastoma. Sci Transl Med 7: 275ra222.

Jung Y, Kim JK, Shiozawa Y, et al. (2013). Recruitment of mesenchymal stem cells into prostate tumours promotes metastasis. Nature Communications 4: 1795.

Kadiyala P, Carney SV, Gauss JC, et al. (2021). Inhibition of 2-hydroxyglutarate elicits metabolic reprogramming and mutant IDH1 glioma immunity in mice. The Journal of clinical investigation 131.

Kauer TM, Figueiredo J-L, Hingtgen S, et al. (2012). Encapsulated therapeutic stem cells implanted in the tumor resection cavity induce cell death in gliomas. Nature Neuroscience 15: 197–204.

Kay MA (2011). State-of-the-art gene-based therapies: the road ahead. Nat Rev Genet 12: 316–328. [PubMed: 21468099]

Kelly E & Russell SJ (2007). History of oncolytic viruses: genesis to genetic engineering. Mol Ther 15: 651–659. [PubMed: 17299401]

Kieran MW, Goumnerova L, Manley P, et al. (2019). Phase I study of gene-mediated cytotoxic immunotherapy with AdV-tk as adjuvant to surgery and radiation for pediatric malignant glioma and recurrent ependymoma. Neuro Oncol 21: 537–546. [PubMed: 30883662]

Kim SS, Harford JB, Moghe M, et al. (2018). Combination with SGT-53 overcomes tumor resistance to a checkpoint inhibitor. Oncoimmunology 7: e1484982. [PubMed: 30288347]

Kim SS, Rait A, Kim E, et al. (2015). A tumor-targeting p53 nanodelivery system limits chemoresistance to temozolomide prolonging survival in a mouse model of glioblastoma multiforme. Nanomedicine 11: 301–311. [PubMed: 25240597]

King GD, Muhammad AK, Larocque D, et al. (2011). Combined Flt3L/TK gene therapy induces immunological surveillance which mediates an immune response against a surrogate brain tumor neoantigen. Molecular therapy : the journal of the American Society of Gene Therapy 19: 1793–1801. [PubMed: 21505426]

King GD, Muhammad AK, Xiong W, et al. (2008). High-capacity adenovirus vector-mediated anti-glioma gene therapy in the presence of systemic antiadenovirus immunity. Journal of virology 82: 4680–4684. [PubMed: 18287240]

Kiyokawa J & Wakimoto H (2019). Preclinical And Clinical Development Of Oncolytic Adenovirus For The Treatment Of Malignant Glioma. Oncolytic Virother 8: 27–37. [PubMed: 31750274]

Kumar M, Keller B, Makalou N, et al. (2001). Systematic determination of the packaging limit of lentiviral vectors. Hum Gene Ther 12: 1893–1905. [PubMed: 11589831]

Kumthekar P, Ko CH, Paunesku T, et al. (2021). A first-in-human phase 0 clinical study of RNA interference-based spherical nucleic acids in patients with recurrent glioblastoma. Sci Transl Med 13.

Lang FF, Conrad C, Gomez-Manzano C, et al. (2018). Phase I Study of DNX-2401 (Delta-24-RGD) Oncolytic Adenovirus: Replication and Immunotherapeutic Effects in Recurrent Malignant Glioma. J Clin Oncol 36: 1419–1427. [PubMed: 29432077]

Lang FF, Tran ND, Puduvalli VK, et al. (2017). Phase 1b open-label randomized study of the oncolytic adenovirus DNX-2401 administered with or without interferon gamma for recurrent glioblastoma. Journal of Clinical Oncology 35: 2002–2002.

Lee CS, Bishop ES, Zhang R, et al. (2017a). Adenovirus-Mediated Gene Delivery: Potential Applications for Gene and Cell-Based Therapies in the New Era of Personalized Medicine. Genes Dis 4: 43–63. [PubMed: 28944281]

Lee TJ, Yoo JY, Shu D, et al. (2017b). RNA Nanoparticle-Based Targeted Therapy for Glioblastoma through Inhibition of Oncogenic miR-21. Mol Ther 25: 1544–1555. [PubMed: 28109960]

Li C, Bowles DE, van Dyke T, et al. (2005). Adeno-associated virus vectors: potential applications for cancer gene therapy. Cancer Gene Ther 12: 913–925. [PubMed: 15962012]

Lowenstein PR, Orringer DA, Sagher O, et al. (2019). First-in-human phase I trial of the combination of two adenoviral vectors expressing HSV1-TK and FLT3L for the treatment of newly diagnosed resectable malignant glioma: Initial results from the therapeutic reprogramming of the brain immune system. Meeting Abstract | 2019 ASCO Annual Meeting I 37.

Luan Y, Zhang S, Zuo L, et al. (2015). Overexpression of miR-100 inhibits cell proliferation, migration, and chemosensitivity in human glioblastoma through FGFR3. Onco Targets Ther 8: 3391–3400. [PubMed: 26604796]

Magge SN, Malik SZ, Royo NC, et al. (2009). Role of monocyte chemoattractant protein-1 (MCP-1/CCL2) in migration of neural progenitor cells toward glial tumors. Journal of Neuroscience Research 87: 1547–1555. [PubMed: 19125409]

Maguire CA, Gianni D, Meijer DH, et al. (2010). Directed evolution of adeno-associated virus for glioma cell transduction. J Neurooncol 96: 337–347. [PubMed: 19618115]

Maier P, von Kalle C & Laufs S (2010). Retroviral vectors for gene therapy. Future Microbiol 5: 1507–1523. [PubMed: 21073311]

Malam Y, Loizidou M & Seifalian AM (2009). Liposomes and nanoparticles: nanosized vehicles for drug delivery in cancer. Trends Pharmacol Sci 30: 592–599. [PubMed: 19837467]

Markert JM, Liechty PG, Wang W, et al. (2009). Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. Mol Ther 17: 199–207. [PubMed: 18957964]

Markert JM, Medlock MD, Rabkin SD, et al. (2000). Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther 7: 867–874. [PubMed: 10845725]

Markert JM, Razdan SN, Kuo HC, et al. (2014). A phase 1 trial of oncolytic HSV-1, G207, given in combination with radiation for recurrent GBM demonstrates safety and radiographic responses. Mol Ther 22: 1048–1055. [PubMed: 24572293]

Martuza RL, Malick A, Markert JM, et al. (1991). Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science 252: 854–856. [PubMed: 1851332]

McCown TJ (2005). Adeno-associated virus (AAV) vectors in the CNS. Curr Gene Ther 5: 333–338. [PubMed: 15975010]

Menon LG, Kelly K, Yang HW, et al. (2009). Human Bone Marrow-Derived Mesenchymal Stromal Cells Expressing S-TRAIL as a Cellular Delivery Vehicle for Human Glioma Therapy. Stem Cells 27: 2320–2330. [PubMed: 19544410]

Miletic H, Fischer YH, Neumann H, et al. (2004). Selective transduction of malignant glioma by lentiviral vectors pseudotyped with lymphocytic choriomeningitis virus glycoproteins. Hum Gene Ther 15: 1091–1100. [PubMed: 15610609]

Molina-Holgado E & Molina-Holgado F (2010). Mending the broken brain: neuroimmune interactions in neurogenesis. Journal of Neurochemistry 114: 1277–1290. [PubMed: 20534007]

Msaouel P, Opyrchal M, Domingo Musibay E, et al. (2013). Oncolytic measles virus strains as novel anticancer agents. Expert Opin Biol Ther 13: 483–502. [PubMed: 23289598]

Mselli-Lakhal L, Favier C, Da Silva Teixeira MF, et al. (1998). Defective RNA packaging is responsible for low transduction efficiency of CAEV-based vectors. Arch Virol 143: 681–695. [PubMed: 9638141]

Muhammad AK, Xiong W, Puntel M, et al. (2012). Safety profile of gutless adenovirus vectors delivered into the normal brain parenchyma: implications for a glioma phase 1 clinical trial. Hum Gene Ther Methods 23: 271–284. [PubMed: 22950971]

Muhammad AKMG, Puntel M, Candolfi M, et al. (2010). Study of the efficacy, biodistribution, and safety profile of therapeutic gutless adenovirus vectors as a prelude to a phase I clinical trial for glioblastoma. Clinical pharmacology and therapeutics 88: 204–213. [PubMed: 20164833]

Muhlebach MD, Mateo M, Sinn PL, et al. (2011). Adherens junction protein nectin-4 is the epithelial receptor for measles virus. Nature 480: 530–533. [PubMed: 22048310]

Müller F-J, Snyder EY & Loring JF (2006). Gene therapy: can neural stem cells deliver? Nature Reviews Neuroscience 7: 75–84. [PubMed: 16371952]

Naldini L, Blomer U, Gage FH, et al. (1996). Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Proc Natl Acad Sci U S A 93: 11382–11388. [PubMed: 8876144]

Nelson NJ (1999). Scientific interest in Newcastle disease virus is reviving. J Natl Cancer Inst 91: 1708–1710. [PubMed: 10528013]

Nunez FJ, Mendez FM, Kadiyala P, et al. (2019). IDH1-R132H acts as a tumor suppressor in glioma via epigenetic up-regulation of the DNA damage response. Science translational medicine 11.

O'Rourke DM, Nasrallah MP, Desai A, et al. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. Sci Transl Med 9.

Ostertag D, Amundson KK, Lopez Espinoza F, et al. (2012). Brain tumor eradication and prolonged survival from intratumoral conversion of 5-fluorocytosine to 5-fluorouracil using a nonlytic retroviral replicating vector. Neuro Oncol 14: 145–159. [PubMed: 22070930]

Ostrom QT, Patil N, Cioffi G, et al. (2020). CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2013–2017. Neuro-oncology 22: iv1–iv96. [PubMed: 33123732]

Otani Y, Yoo JY, Lewis CT, et al. (2022). NOTCH-Induced MDSC Recruitment after oHSV Virotherapy in CNS Cancer Models Modulates Antitumor Immunotherapy. Clin Cancer Res 28: 1460–1473. [PubMed: 35022322]

Parolin C, Dorfman T, Palu G, et al. (1994). Analysis in human immunodeficiency virus type 1 vectors of cis-acting sequences that affect gene transfer into human lymphocytes. J Virol 68: 3888–3895. [PubMed: 7910642]

Patel DH, Misra A (2011). Gene delivery using viral vectors. Challenges in delivery of therapeutic genomics and proteomics. Elsevier Inc

Phuong LK, Allen C, Peng KW, et al. (2003). Use of a vaccine strain of measles virus genetically engineered to produce carcinoembryonic antigen as a novel therapeutic agent against glioblastoma multiforme. Cancer Res 63: 2462–2469. [PubMed: 12750267]

Pirollo KF, Nemunaitis J, Leung PK, et al. (2016). Safety and Efficacy in Advanced Solid Tumors of a Targeted Nanocomplex Carrying the p53 Gene Used in Combination with Docetaxel: A Phase 1b Study. Mol Ther 24: 1697–1706. [PubMed: 27357628]

Poeschla EM (2003). Non-primate lentiviral vectors. Curr Opin Mol Ther 5: 529–540. [PubMed: 14601523]

Pol JG, Levesque S, Workenhe ST, et al. (2018). Trial Watch: Oncolytic viro-immunotherapy of hematologic and solid tumors. Oncoimmunology 7: e1503032. [PubMed: 30524901]

Portnow J, Synold TW, Badie B, et al. (2017). Neural Stem Cell–Based Anticancer Gene Therapy: A First-in-Human Study in Recurrent High-Grade Glioma Patients. Clinical Cancer Research 23: 2951–2960. [PubMed: 27979915]

Prentice DA (2019). Adult Stem Cells. Circulation Research 124: 837–839. [PubMed: 30870122]

Puntel M, Muhammad AK, Candolfi M, et al. (2010). A novel bicistronic high-capacity gutless adenovirus vector that drives constitutive expression of herpes simplex virus type 1 thymidine kinase and tet-inducible expression of Flt3L for glioma therapeutics. Journal of virology 84: 6007–6017. [PubMed: 20375153]

Rainov NG (2000). A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 11: 2389–2401. [PubMed: 11096443]

Ram Z, Culver KW, Oshiro EM, et al. (1997). Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. Nat Med 3: 1354–1361. [PubMed: 9396605]

Rattigan Y, Hsu J-M, Mishra PJ, et al. (2010). Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu. Experimental Cell Research 316: 3417–3424. [PubMed: 20633553]

Riyad JM & Weber T (2021). Intracellular trafficking of adeno-associated virus (AAV) vectors: challenges and future directions. Gene Ther 28: 683–696. [PubMed: 33658649]

Sanchez-Hernandez L, Hernandez-Soto J, Vergara P, et al. (2018). Additive effects of the combined expression of soluble forms of GAS1 and PTEN inhibiting glioblastoma growth. Gene Ther 25: 439–449. [PubMed: 29941984]

Santiago-Ortiz JL & Schaffer DV (2016). Adeno-associated virus (AAV) vectors in cancer gene therapy. J Control Release 240: 287–301. [PubMed: 26796040]

Sarkaria JN, Hu LS, Parney IF, et al. (2017). Is the blood–brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. Neuro-Oncology 20: 184–191.

Sasportas LS, Kasmieh R, Wakimoto H, et al. (2009). Assessment of therapeutic efficacy and fate of engineered human mesenchymal stem cells for cancer therapy. Proceedings of the National Academy of Sciences 106: 4822–4827.

Scherr M, Venturini L, Battmer K, et al. (2007). Lentivirus-mediated antagomir expression for specific inhibition of miRNA function. Nucleic Acids Res 35: e149. [PubMed: 18025036]

Scherr M, Venturini L & Eder M (2010). Lentiviral vector-mediated expression of pre-miRNAs and antagomiRs. Methods Mol Biol 614: 175–185. [PubMed: 20225044]

Schirrmacher V, van Gool S & Stuecker W (2019). Breaking Therapy Resistance: An Update on Oncolytic Newcastle Disease Virus for Improvements of Cancer Therapy. Biomedicines 7.

Seita J & Weissman IL (2010). Hematopoietic stem cell: self-renewal versus differentiation. Wiley Interdisciplinary Reviews: Systems Biology and Medicine 2: 640–653. [PubMed: 20890962]

Senzer N, Nemunaitis J, Nemunaitis D, et al. (2013). Phase I study of a systemically delivered p53 nanoparticle in advanced solid tumors. Mol Ther 21: 1096–1103. [PubMed: 23609015]

Shimotohno K (1983). [Introduction of foreign genes into eukaryotic cells using retrovirus as a vector]. Tanpakushitsu Kakusan Koso 28: 1582–1598. [PubMed: 6324284]

Sonabend AM, Ulasov IV, Tyler MA, et al. (2008). Mesenchymal Stem Cells Effectively Deliver an Oncolytic Adenovirus to Intracranial Glioma. Stem Cells 26: 831–841. [PubMed: 18192232]

Song JL, Zheng W, Chen W, et al. (2017). Lentivirus-mediated microRNA-124 gene-modified bone marrow mesenchymal stem cell transplantation promotes the repair of spinal cord injury in rats. Exp Mol Med 49: e332. [PubMed: 28524176]

Spaeth E, Klopp A, Dembinski J, et al. (2008). Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. Gene Therapy 15: 730–738. [PubMed: 18401438]

Stilwell JL & Samulski RJ (2003). Adeno-associated virus vectors for therapeutic gene transfer. Biotechniques 34: 148–150, 152, 154 passim. [PubMed: 12545552]

Stuckey DW & Shah K (2014). Stem cell-based therapies for cancer treatment: separating hope from hype. Nature Reviews Cancer 14: 683–691. [PubMed: 25176333]

Stupp R, Mason WP, van den Bent MJ, et al. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352: 987–996. [PubMed: 15758009]

Sun BS, Dong QZ, Ye QH, et al. (2008). Lentiviral-mediated miRNA against osteopontin suppresses tumor growth and metastasis of human hepatocellular carcinoma. Hepatology 48: 1834–1842. [PubMed: 18972404]

Szabo E, Schneider H, Seystahl K, et al. (2016). Autocrine VEGFR1 and VEGFR2 signaling promotes survival in human glioblastoma models in vitro and in vivo. Neuro Oncol 18: 1242–1252. [PubMed: 27009237]

Tai CK & Kasahara N (2008). Replication-competent retrovirus vectors for cancer gene therapy. Front Biosci 13: 3083–3095. [PubMed: 17981778]

Tai CK, Wang WJ, Chen TC, et al. (2005). Single-shot, multicycle suicide gene therapy by replication-competent retrovirus vectors achieves long-term survival benefit in experimental glioma. Mol Ther 12: 842–851. [PubMed: 16257382]

Takahashi K & Yamanaka S (2006). Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell 126: 663–676. [PubMed: 16904174]

Takahashi M, Valdes G, Hiraoka K, et al. (2014). Radiosensitization of gliomas by intracellular generation of 5-fluorouracil potentiates prodrug activator gene therapy with a retroviral replicating vector. Cancer Gene Ther 21: 405–410. [PubMed: 25301172]

Tan AC, Ashley DM, López GY, et al. (2020). Management of glioblastoma: State of the art and future directions. CA Cancer J Clin 70: 299–312. [PubMed: 32478924]

Todo T, Ito H, Ino Y, et al. (2022). Intratumoral oncolytic herpes virus G47Δ for residual or recurrent glioblastoma: a phase 2 trial. Nature Medicine 28: 1630–1639.

Todo T, Martuza RL, Rabkin SD, et al. (2001). Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. Proc Natl Acad Sci U S A 98: 6396–6401. [PubMed: 11353831]

Tome-Garcia J, Erfani P, Nudelman G, et al. (2018). Analysis of chromatin accessibility uncovers TEAD1 as a regulator of migration in human glioblastoma. Nat Commun 9: 4020. [PubMed: 30275445]

Ulasov IV, Tyler MA, Zheng S, et al. (2006). CD46 represents a target for adenoviral gene therapy of malignant glioma. Hum Gene Ther 17: 556–564. [PubMed: 16716112]

van den Bossche WBL, Kleijn A, Teunissen CE, et al. (2018). Oncolytic virotherapy in glioblastoma patients induces a tumor macrophage phenotypic shift leading to an altered glioblastoma microenvironment. Neuro Oncol 20: 1494–1504. [PubMed: 29796615]

van Oers MM, Pijlman GP & Vlak JM (2015). Thirty years of baculovirus-insect cell protein expression: from dark horse to mainstream technology. J Gen Virol 96: 6–23. [PubMed: 25246703]

van Putten EH, Dirven CM, van den Bent MJ, et al. (2010). Sitimagene ceradenovec: a gene-based drug for the treatment of operable high-grade glioma. Future Oncol 6: 1691–1710. [PubMed: 21142657]

VanderVeen N, Paran C, Krasinkiewicz J, et al. (2013). Effectiveness and preclinical safety profile of doxycycline to be used “off-label” to induce therapeutic transgene expression in a phase I clinical trial for glioma. Hum Gene Ther Clin Dev 24: 116–126. [PubMed: 24007469]

VanderVeen N, Raja N, Yi E, et al. (2016). Preclinical Efficacy and Safety Profile of Allometrically Scaled Doses of Doxycycline Used to Turn “On” Therapeutic Transgene Expression from High-Capacity Adenoviral Vectors in a Glioma Model. Hum Gene Ther Methods 27: 98–111. [PubMed: 27056322]

Vigil A, Park MS, Martinez O, et al. (2007). Use of reverse genetics to enhance the oncolytic properties of Newcastle disease virus. Cancer Res 67: 8285–8292. [PubMed: 17804743]

Wang CY, Li F, Yang Y, et al. (2006). Recombinant baculovirus containing the diphtheria toxin A gene for malignant glioma therapy. Cancer Res 66: 5798–5806. [PubMed: 16740719]

Weiss RA (1987). Retroviruses and human disease. J Clin Pathol 40: 1064–1069. [PubMed: 2889752]

Wheeler LA, Manzanera AG, Bell SD, et al. (2016). Phase II multicenter study of gene-mediated cytotoxic immunotherapy as adjuvant to surgical resection for newly diagnosed malignant glioma. Neuro Oncol 18: 1137–1145. [PubMed: 26843484]

Wilcox DR & Longnecker R (2016). The Herpes Simplex Virus Neurovirulence Factor γ34.5: Revealing Virus-Host Interactions. PLoS Pathog 12: e1005449. [PubMed: 26964062]

Wiwatchaitawee K, Quarterman JC, Geary SM, et al. (2021). Enhancement of Therapies for Glioblastoma (GBM) Using Nanoparticle-based Delivery Systems. AAPS PharmSciTech 22: 71. [PubMed: 33575970]

Xiong W, Goverdhana S, Sciascia SA, et al. (2006). Regulatable gutless adenovirus vectors sustain inducible transgene expression in the brain in the presence of an immune response against adenoviruses. J Virol 80: 27–37. [PubMed: 16352528]

Xu J, Wang D, Liu D, et al. (2012). Allogeneic mesenchymal stem cell treatment alleviates experimental and clinical Sjögren syndrome. Blood 120: 3142–3151. [PubMed: 22927248]

Yanamandra N, Kondraganti S, Gondi CS, et al. (2005). Recombinant adeno-associated virus (rAAV) expressing TFPI-2 inhibits invasion, angiogenesis and tumor growth in a human glioblastoma cell line. Int J Cancer 115: 998–1005. [PubMed: 15723303]

Yoo JY, Yeh M, Kaur B, et al. (2021). Targeted delivery of small noncoding RNA for glioblastoma. Cancer Lett 500: 274–280. [PubMed: 33176185]

Yoshida J, Mizuno M, Nakahara N, et al. (2002). Antitumor effect of an adeno-associated virus vector containing the human interferon-beta gene on experimental intracranial human glioma. Jpn J Cancer Res 93: 223–228. [PubMed: 11856487]

Yu S, Li A, Liu Q, et al. (2017). Chimeric antigen receptor T cells: a novel therapy for solid tumors. J Hematol Oncol 10: 78. [PubMed: 28356156]

Zamarin D & Palese P (2012). Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions. Future Microbiol 7: 347–367. [PubMed: 22393889]

Zeng LL, He XS, Liu JR, et al. (2016). Lentivirus-Mediated Overexpression of MicroRNA-210 Improves Long-Term Outcomes after Focal Cerebral Ischemia in Mice. CNS Neurosci Ther 22: 961–969. [PubMed: 27390218]

Zitvogel L, Apetoh L, Ghiringhelli F, et al. (2008). Immunological aspects of cancer chemotherapy. Nat Rev Immunol 8: 59–73. [PubMed: 18097448]

Zolotukhin I, Luo D, Gorbatyuk O, et al. (2013). Improved Adeno-associated Viral Gene Transfer to Murine Glioma. J Genet Syndr Gene Ther 4.

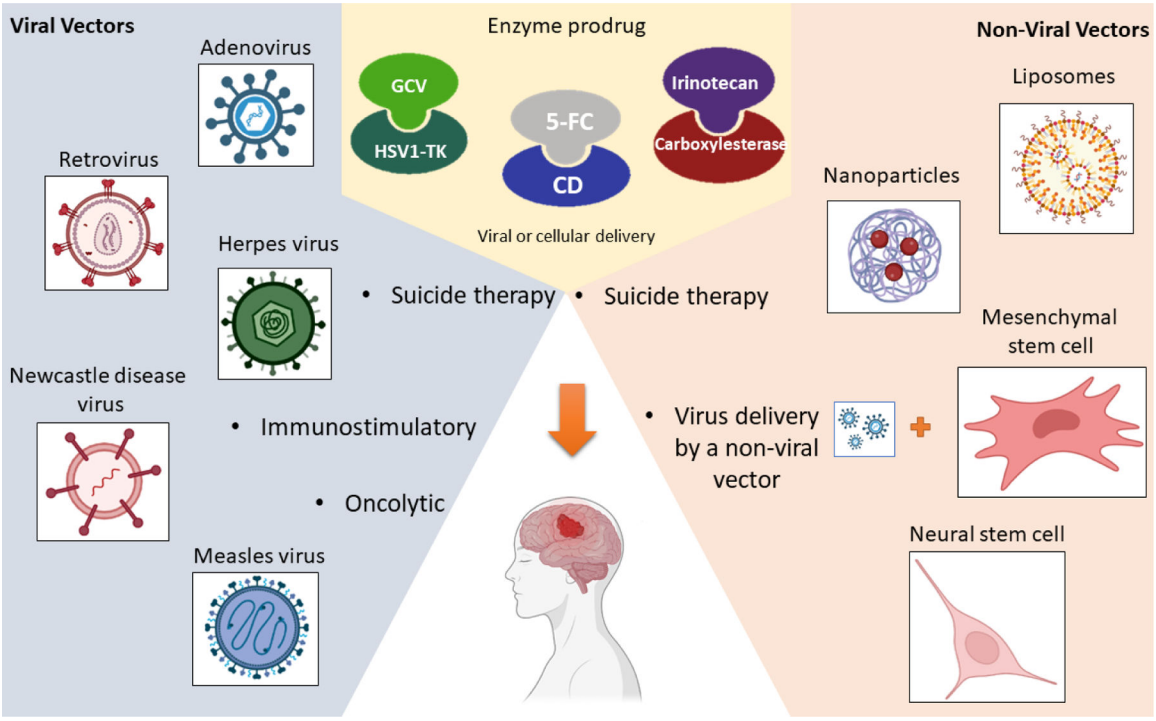

Figure 1. Clinical approaches to treat gliomas using gene therapy vectors and stem cells.

Viral vectors are the most common tool for gene therapy. Viral vectors include vectors derived from Adenovirus (AdV), Herpes virus (HSV), Retrovirus, Newcastle diseases virus (NDV) and Measles virus(MV). Non viral vectors include nanoparticles, liposome and stem cells. The stem cells employed in glioma therapy are neural stem cells (NSC) or mesenchymal stem cells (MSC). One of the most common therapeutic techniques employed in gene therapy is suicide therapy. This involves a system of enzyme plus prodrug: (1) Expression of herpes simplex virus-thymidine kinase (HSV-TK), plus ganciclovir (GCV) treatment, (2) Expression of cytosine deaminase (CD), plus 5-flucytosine (5-FC), (3) Expression of rabbit carboxylesterase, plus irinotecan. Suicide therapy has been explored with both viral and non viral vectors. Other therapeutic transgene used are immune modulators such as Flt3L and IL-12; tumor suppressor target, like wild type p53, or chimeric antigen receptors to be expressed in CAR-T cells, including EGFRvIII, CD1322, Her-2. There is also oncolytic viral therapy which used oncolytic virus (AdV, HSV-1, NM and NDV) can identify, infect, and lyse different cells. Also, MSC and NSC have been used to deliver oncolytic virus.

Table1.

Viral vectors employed in clinical trials for gliomas

Vector type	Treatment	Phase	Study State	Age	Pathology	NCT #
Adenovirus	AdV-TK + GCV	2	Completed	Adult	High-grade glioma	NCT00870181
Adenovirus	AdV-TK + Valacyclovir	2a	Completed	Adult	Malignant glioma	NCT00589875
Adenovirus	AdV-TK + Valacyclovir	1	Completed	Child, Adult	Malignant glioma, Recurrent ependymoma	NCT00634231
Adenovirus	Ad-hCMV-TK + Ad-hCMV-Flt3L + Valacyclovir	1	Completed	Adult	Malignant glioma	NCT01811992
Adenovirus	AdV-(HSV-TK) + Valacyclovir	1/2	Recruiting	Adult	GBM, Astrocytoma Grade III	NCT03596086
Adenovirus	AdV-(HSV-TK) + Valacyclovir	1/2	Recruiting	Adult	GBM, Anaplastic Astrocytoma	NCT03603405
Adenovirus	AdV-TK + Valacyclovir + Nivolumab	1	Active, not recruiting	Adult	Malignant glioma	NCT03576612
Adenovirus	Ad-RTS-hIL-12 + Veledimex	1	Completed	Adult	Malignant glioma	NCT02026271
Adenovirus	Ad-RTS-hIL-12 + Veledimex	1	Completed	Adult	Glioblastoma	NCT03679754
Adenovirus	Ad-RTS-hIL-12 + Veledimex + Nivolumab	1	Completed	Adult	Glioblastoma	NCT03636477
Adenovirus	Ad-RTS-hIL-12 + Veledimex + Cemiplimab-Rwlc		Completed	Adult	Glioblastoma	NCT04006119
Adenovirus	Ad-RTS-hIL-12 + Veledimex	1/2	Terminated	Child, Adult (up to 21)	Pediatric Brain Tumor, DIPG	NCT03330197
Oncolytic Adenovirus	DNX-2401	1	Completed	Adult	Recurrent malignant gliomas	NCT00805376
Oncolytic Adenovirus	DNX-2401 + Interferon gamma	1	Completed	Adult	Glioblastoma, gliosarcoma	NCT02197169
Oncolytic Adenovirus	DNX-2401	1/2	Completed	Adult	Recurrent glioblastoma	NCT01582516
Oncolytic Adenovirus	DNX-2401 + Pembrolizumab	2	Completed	Adult	Malignant glioma	NCT02798406
Oncolytic Adenovirus	DNX-2401 + Temozolomide	1	Completed	Adult	Recurrent glioma	NCT01956734
Oncolytic Adenovirus	DNX-2401	1	Recruiting	Adult	Recurrent glioma	NCT03714334
Oncolytic Adenovirus	DNX-2401	1	Active, not recruiting	Child	DIPG	NCT03178032
Oncolytic HSV-1	G207	1	Completed	Adult	Malignant glioma	NCT00157703
Oncolytic HSV-1	G207	1/2	Completed	Adult	Recurrent glioma	NCT00028158
Oncolytic HSV-1	G207	1	Active, not recruiting	Child, Adult	Progressive or Recurrent Supratentorial Brain Tumors	NCT02457845
Oncolytic HSV-1	G207	2	Not yet recruiting	Child, Adult (up to 21)	Recurrent High-grade Glioma	NCT04482933
Oncolytic HSV-1	G207	1	Recruiting	Child	Recurrent brain tumors	NCT03911388

Vector type	Treatment	Phase	Study State	Age	Pathology	NCT #
Oncolytic HSV-1	M032	1	Active, not recruiting	Adult	Recurrent glioma	NCT02062827
Oncolytic HSV-1	rQNestin34.5v.2 + Cyclophosphamide	1	Recruiting	Adult	Recurrent glioma	NCT03152318
Cell line producing Retrovirus	G1TKSVNa.53 Producer Cell Line + Cytovene	1	Completed	Adult	Brain tumors	NCT00001328
Retrovirus	Toca 511 vector + Toca FC	1	Completed	Adult	Recurrent glioma	NCT01156584
Retrovirus	Toca 511 vector + Toca FC	1	Completed		Recurrent glioma	NCT01985256
Retrovirus	Toca 511 vector + Toca FC	3/2	Terminated	Adult	Glioblastamo, Anaplastic astrocytoma	NCT02414165
Chimeric antigen receptor T cells	Autologous T cells transduced with a lentiviral vector expressing chimeric antigens for : EGFRVIII, IL13Rα2, Her-2, EphA2, CD133, GD2	1	Recruiting	Adult	Recurrent glioma	NCT03423992
Chimeric antigen receptor T cells	Autologous T cells transduced with a lentiviral vector expressig chimeric antigen receptor specific for EGFRvIII	1	Terminated	Adult	Recurrent EGFRvIII+ glioma	NCT02209376
Oncolytic-NDV	New Castle Disease Virus	1/2	Withdrawn	Adult	Glioblastoma, Sacroma, Neuroblastoma	NCT01174537
Oncolytic-MV	MV-CEA	1	Completed	Adult	Recurrent glioma	NCT00390299
Oncolytic-MV	MV-NIS	1	Recruiting	Child, Adult	Recurrent medulloblastoma, recurrent atypical teratoid rhabdoid tumor	NCT02962167

Table2.

Non viral vectors employed in clinical trials for gliomas

Type of vector	Type of therapy	Treatment	Phase	Study State	Age	Pathology	NCT #
Liposome	Encapsulating plasmid DNA	SGT-53 + Temozolomide	2	Terminated	Adult	Recurrent glioma	NCT02340156
Liposome	Encapsulating drug	2B3–101	1/2	Completed	Adult	Patients With Solid Tumors and Brain Metastases or Recurrent Malignant Glioma	NCT01386580
Nanoparticle	Encapsulating siRNA	NU-0129	1	Completed	Adult	Glioma, gliosarcoma	NCT03020017
NSC	Oncolytic adenovirus	NSC-CRAd-S-pk7	1	Completed	Adult	Newly diagnosed Malignant Glioma	NCT03072134
NSC	Oncolytic adenovirus	NSC-CRAd-S-pk7	1	Not yet recruiting	Adult	Recurrent High grade glioma	NCT05139056
NSC	Enzyme-prodrug	E. coli CD-expressing NSC + 5-fluorocytosine (5-FC)	1	Completed	13 years and older	Recurrent High grade glioma	NCT01172964
NSC	Enzyme-prodrug	E. coli CD-expressing NSC + 5-fluorocytosine (5-FC) + Leucovorin	1	Completed	Pediatric and adult	Recurrent High grade glioma	NCT02015819
NSC	Enzyme-prodrug	Carboxylesterase-expressing Allogeneic Neural Stem Cells + Irinotecan	1	Active, not recruiting	Adult	Recurrent High grade glioma	NCT02192359
MSC	Oncolytic adenovirus	AloCELYVIR + Radiotherapy	1/2	Recruiting	Child, adult	DIPG	NCT04758533
MSC	Oncolytic adenovirus	MSC loaded with Ad5-DNX-2401	1	Recruiting	Adult	Recurrent High grade glioma	NCT03896568