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Review

Gene therapy for brain tumors: Basic developments and clinical implementation

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HIGHLIGHTS

- ▶ GBM models that reproduce the salient features of the human disease still need to be developed.
- ▶ TLR2 activation is critical for initiating anti-brain tumor specific adaptive immune responses.
- ▶ Combined cytotoxic and immune-stimulatory approaches are most effective against brain tumors.
- ▶ Viral vectors are safe to administer to human patients diagnosed with GBM.

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ABSTRACT

Glioblastoma multiforme (GBM) is the most common and deadliest of adult primary brain tumors. Due to its invasive nature and sensitive location, complete resection remains virtually impossible. The resistance of GBM against chemotherapy and radiotherapy necessitate the development of novel therapies. Gene therapy is proposed for the treatment of brain tumors and has demonstrated pre-clinical efficacy in animal models. Here we review the various experimental therapies that have been developed for GBM including both cytotoxic and immune stimulatory approaches. We also review the combined conditional cytotoxic immune stimulatory therapy that our lab has developed which is dependent on the adenovirus mediated expression of the conditional cytotoxic gene, Herpes Simplex Type 1 Thymidine Kinase (TK) and the powerful DC growth factor Fms-like tyrosine kinase 3 ligand (Flt3L). Combined delivery of these vectors elicits tumor cell death and an anti-tumor adaptive immune response that requires TLR2 activation. The implications of our studies indicate that the combined cytotoxic and immunotherapeutic strategies are effective strategies to combat deadly brain tumors and warrant their implementation in human Phase I clinical trials for GBM.

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Contents

1. Introduction	72
2. Experimental glioblastoma models	72
2.1. Transplantable murine GBM models	72
2.2. Genetically engineered murine GBM models	73
3. Cytotoxic gene therapy	73
4. Immune mediated gene therapy: stimulating anti-brain tumor immunity	74
5. The clinical scenario	75
Funding	76
References	76

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1. Introduction

Glioblastoma multiforme (GBM) is a highly malignant brain tumor of astrocytic origin. In the United States, GBM accounts for over 50% of all gliomas and carries an annual incidence rate of 3.2 cases per 100,000 persons, making it both the most common and lethal primary brain tumor in adults [14]. The World Health Organization classifies GBM as a grade IV malignant neuroepithelial cancer of the central nervous system with two distinct variants: giant cell glioblastoma and gliosarcoma [47]. Although histological differences exist between its various forms, GBM is by definition a highly mitotic and diffusely infiltrating glial anaplasia that demonstrates marked nuclear and cytoplasmic pleomorphism. Other defining histological features of GBM include prominent glomeruloid microvascular hyperplasia and central areas of tumor necrosis that are often, but not necessarily, associated with perinecrotic nuclear pseudopalisading [71]. Genetically, GBM displays heterogeneous alterations in a number of key bio-molecular pathways implicated in processes of cellular proliferation, survival, invasion and angiogenesis [28]. The current gold standard of therapy for GBM consists of surgical resection when feasible followed by chemo and radiotherapy [74]. The oral alkylating agent temozolomide, introduced in 2005, is now the cytotoxic drug of choice for patients with newly diagnosed GBM due to its additional role as a radio-sensitizer when administered concurrently with radiotherapy [70].

GBM presents a number of significant drawbacks to therapy. Widespread tumor cell migration occurring predominantly in association with white matter tracts and the basement membrane of brain microvasculature preclude complete surgical resection and invariably leads to tumor recurrence, frequently within 2–3 cm of the resection cavity [31]. Another significant drawback to therapy is the inherent resistance of GBM cells to cytotoxic therapies. Constitutive up-regulation of DNA repair enzymes such as O6-methylguanine-DNA methyltransferase (MGMT) and activation of anti-apoptotic regulators such as Bcl2-like 12 (Bcl2L12) allow resistant tumor cells to persist in the face of chemo and radiotherapy [34,69]. Clinically, it has been determined that GBM patients with high levels of MGMT promoter methylation survive significantly longer than those without evidence of MGMT promoter methylation [44].

The rampant vascular endothelial proliferation seen in high-grade gliomas coupled with the phenomenon of tumor dormancy, the inability of solid tumors to grow larger than a few mm³ in the absence of sprouting angiogenesis [26,27], served as the impetus for the use of anti-angiogenic agents in the treatment of GBM. However, clinical studies show that its use in the adjuvant setting along with current first-line chemotherapies only modestly increases progression-free survival and provides no additional survival benefit in patients with GBM [32,46,56,57]. Furthermore, the FDA has recently announced that it has removed bevacizumab's indication for HER2-negative metastatic breast cancer, stating that a detailed analysis of the clinical data revealed a lack of therapeutic efficacy. The effects of anti-angiogenic therapy are likely attributed to those of vascular normalization, a phenomenon that temporarily reduces peri-tumoral vasogenic edema and leads to improved patient symptoms and quality of life [40]. It has also been suggested that vascular normalization acts by allowing for better penetration of systemically administered chemotherapeutic agents into the tumor bed, but only over a relatively narrow window of time [40].

Despite recent advances made in the field of neuro-oncology, GBM remains a uniformly lethal disease with a dismal prognosis. Even with optimal therapeutic intervention, median patient survival continues to be 12–15 months post-diagnosis [74]. GBMs aggressive growth and invasion, coupled with its inherent

resistance to cytotoxic therapy, make it a formidable opponent even for today's optimal therapeutic modalities. Thus the field is in great need for new and novel tumor-specific therapies.

2. Experimental glioblastoma models

One of the most important tools in the development of translational therapeutics is the availability of adequate animal models to preclinically test the efficacy and toxicity of novel therapies. The ideal animal model to test anti-glioma therapies comprises the following features: (1) histopathological resemblance to human GBM, i.e. similar invasion pattern; (2) biochemical resemblance to human GBM, i.e. similar genetic lesions; (3) intact tumor–host biochemical and immunological interactions, i.e. the tumor needs to be non-immunogenic in a host with an intact immune system; (4) intracranial location, so that the tumor is surrounded by normal brain parenchyma; (5) accurate knowledge of tumor location; (6) predictable growth pattern; (7) high reproducibility. Unfortunately, a GBM animal model that comprises all these features does not exist and, thus, experimentation in different tumor models is advised in order to better predict the clinical outcome of translational therapies.

2.1. Transplantable murine GBM models

The first brain tumor models were generated by systemic administration of chemical carcinogens to rodents [43]. Although this strategy is no longer utilized, glioma cell lines obtained by this method [6,7] are still extensively used for in vivo tumor models, developed by intracranial or subcutaneous (s.c.) implantation in rodents. Although s.c. GBM models allow to follow tumor growth by daily measurement using a caliper and are a faster and easier alternative to intracranial tumor implantation, the lack of surrounding non-neoplastic brain parenchyma, the absence of a blood–brain barrier and the immune-privilege present in the brain make s.c. models unsuitable to assess the efficacy or the neurotoxicity of anti-glioma therapeutic approaches.

The advantages of intracranially implanted tumor models are their predictable and highly reproducible tumor growth rates, the accurate knowledge of the site of the tumor, the possibility of testing a large cohort of animals, and the relatively fast progression from tumor implantation to death [9], which make *them an invaluable* tool for the preclinical assessment of novel therapies. Syngeneic GBM models are generated by implantation of murine GBM cell lines that are not immunogenic when implanted in animals with an intact immune system [9]. Syngeneic mouse models of GBM are not abundant, and they are constituted by the following cell lines implanted in their corresponding mouse host: GL26 and GL261 GBM cells in C57BL6 mice [9,19], SMA-560 cells in VMDK mice [10] and VM-M3 in VM mice [65]. Amongst the syngeneic rat GBM models, the most extensively used are CNS-1 cells in Lewis rats [9], F98, 9L and RG-2 cells in Fisher rats [5]. The integrity of the immunological interaction between host and GBM makes these models an excellent tool to study antitumor immunity, as well as the efficacy and toxicological profile of immunotherapeutic approaches for GBM [19].

Xenograft models allow assessing the response of human GBM cells in the context of the normal brain, and have been extensively used as preclinical in vivo models. Although the hosts are immune-compromised mice and rats, human GBM xenografts require the injection of much larger number of cells than syngeneic models to growth with reproducible rates [9]. Besides the obvious limitation of xenograft models, which is the lack of an intact immune system, there is an additional question that needs to be addressed when choosing a human GBM xenograft: some of the main genetic

lesions detected in the original GBM specimens, such as EGFR amplification and hypermethylation of the DNA O6-methylguanine methyltransferase (MGMT) promoter, can be lost after prolonged cell culture [13]. This constitutes a concern when using human GBM cell lines that have been maintained in culture for years. In order to address this limitation patient tumor specimens have been implanted directly in the flank of nude mice and maintained by serial transplantation in vivo [13]. These tumors can be cryopreserved or cultured for short periods before injecting the cells into the brain of immune-compromised mice [30]. We have recently employed one of these transplantable human tumors, GBM12, which retains EGFR amplification [30], p53 mutation [30], and expression of IL13R α 2 [3] from the original GBM specimen, to address the efficacy of a targeted toxin delivered using a regulated adenoviral vector [11].

The main limitation of implantation tumor models is that although they resemble the histopathological features of human GBM, they do not replicate exactly their invasive pattern, being less diffuse than their human counterpart [9]. Also, glioma-genesis is artificially achieved and does not resemble the pathogenesis of the human disease. In spite of these shortcomings, implantation models serve as a reliable tool in translational neuro-oncology that allows the preclinical assessment of novel therapies.

2.2. Genetically engineered murine GBM models

Genetically engineered murine GBM models mimic gliomagenesis more accurately and exhibit the histological and molecular hallmarks of human GBM. Transgenic mouse models have been constructed by introducing genetic alterations known to be present in human gliomas. Although the alteration of a single tumor suppressor gene or overexpression of an oncogene is insufficient to induce high-grade gliomas with good penetrance, the introduction of more than one genetic lesion found in human GBM leads to murine glioma models that resemble the main histological features of their human counterparts.

Amplification of growth factors and their receptors, i.e. PDGF and EGFR, or silencing of tumor suppressor genes, i.e. p53 and PTEN, are detected in the majority of human GBM specimens [52,62,64,77]. The intracranial delivery of these genes into the brain of pre-natal or adult rodents using gene therapy vectors that integrate into the host genome has been used to generate endogenous brain tumors with variable success. Delivery of retroviral vectors that encode for PDGF into the rat adult brain or in the newborn mouse brain leads to the formation of GBM in less than 20 days [4] or 4–12 weeks [66], respectively. Generation of models that harbor combined genetic lesions mimics more closely the clinical scenario [58]. These models have been generated by delivering retroviral vectors encoding for growth factors (bFGF) or their constitutively activated receptors (EGFRvIII) and cycline dependent kinases (cdk4) in the brain of p53 deficient mice [36]. While mice that harbor single genetic lesions did not develop brain tumors, combination of genetic lesions led to 50% of the mice [36].

Since Ras activation has been involved in gliomagenesis [37], this molecule has been targeted in order to develop novel endogenous mouse models [50,51]. Constitutive Ras activation in neural stem cells leads to the generation of Grade III astrocytomas in mice, which evolve to Grade IV astrocytomas when p53 and p16/p19 are suppressed [51]. Delivery of Ras and AKT to specific areas of the brain has also been achieved using lentiviral vectors [50]. Although administration of single oncogenes did generate tumor in some of the animals, combination of Ras and AKT in p53 KO mice led to tumor formation in 75–100% of the mice injected, depending on the area injected [50], supporting the notion that gliomagenesis requires several genetic abnormalities occurring in definite areas of the brain.

The use of the *Sleeping Beauty* (SB) transposable element also allows integration of known oncogenes in the genome of brain cells [75]. SB is a synthetic transposable element constituted by a transposon DNA substrate and a transposase enzyme. SB transposase mediates the excision and insertion of transposon DNA into the host genome [53]. Delivery of SB-dependent plasmids encoding for AKT, Ras, EGFRvIII, and a p53 shRNA into the brain of neo-natal mice led to brain tumor formation [75]. The combination of Ras, EGFRvIII and p53 shRNA generated tumors in 100% of the mice that had a median survival of 83 days. An advantage of the SB system is that allows integration of large transposons (<10 kb) into the genome of many different strains of mice [75].

The main limitations of genetically engineered GBM models that restrict their use in translational neuro-oncology are: their variable reproducibility, their long tumor latency and the lack of an accurate knowledge of tumor location. Nevertheless, the fast development of novel imaging techniques [22] allows following tumor growth and assessing its exact location, facilitate the use of genetically engineered models in the preclinical testing of translational therapeutics.

3. Cytotoxic gene therapy

Cytotoxic agents have been traditionally used to treat cancer [17,63]. However, one of the therapeutic challenges of treating GBM is that pro-apoptotic agents may be cytotoxic to the surrounding brain parenchyma. The neurotoxicity of pro-apoptotic agents can be reduced by targeting the cytotoxic molecule to GBM cells, which can be achieved using different strategies. The use of gene therapy vectors encoding the therapeutic transgene under the control of a tumor-specific promoter allows restricting the expression of cytotoxic agents to tumor cells. Likewise, p53-upregulated modulator of apoptosis (PUMA) induces GBM-cell death when its expression is controlled by the hTERT promoter, without affecting surrounding non-neoplastic tissue [39]. Vectors encoding pro-apoptotic molecules, such as Bax, under the control of hypoxia responsive elements are also useful to target cytotoxic gene expression to GBM cells within the hypoxic tumor microenvironment [55]. Radio-inducible promoters allow temporal and spatial control of cytotoxic transgene expression. Overexpression of caspase 8 or TRAIL under the control of a radiation-inducible promoter (early growth response gene-1, EGR-1) induces GBM cell death and tumor regression only when combined with fractionated radiotherapy [72]. Restriction of transgene expression to GBM cells becomes crucial when delivering powerful pro-apoptotic molecules into the brain, such as FasL or TRAIL which are highly toxic to the non-neoplastic brain parenchyma [12].

Another strategy to restrict the cytotoxicity of pro-apoptotic molecules to GBM cells is to target receptors that are exclusively expressed on tumor cells and are absent in the normal brain. Chimeric proteins conformed by ligands of these receptors, i.e. IL-13R α 2, transferrin receptor, EGFR, fused to highly cytotoxic proteins, such as *Pseudomonas* exotoxin. We constructed a doxycycline-dependent regulatable adenoviral vector (Ad.mhIL-4.TRE.mhIL-13-PE) that encodes a mutated human IL-13 fused to *Pseudomonas* exotoxin (mhIL-13-PE) that specifically binds to IL13R α 2 [11,20], an IL13 receptor that is overexpressed in GBM in most human patients [41,54,76]. Ad.mhIL-4.TRE.mhIL-13-PE also encodes a mutated human IL-4 that binds to the physiological receptor IL4R/IL13R without interacting with IL13R α 2 [20,49], to block any potential binding of mhIL-13-PE to normal brain cells. This therapeutic vector exhibited higher efficacy and negligible neurotoxicity when compared to the protein formulation, i.e. Cintredekin Besudotox [11], which when tested in clinical trials failed to achieve clinical endpoints and revealed severe neurotoxicity

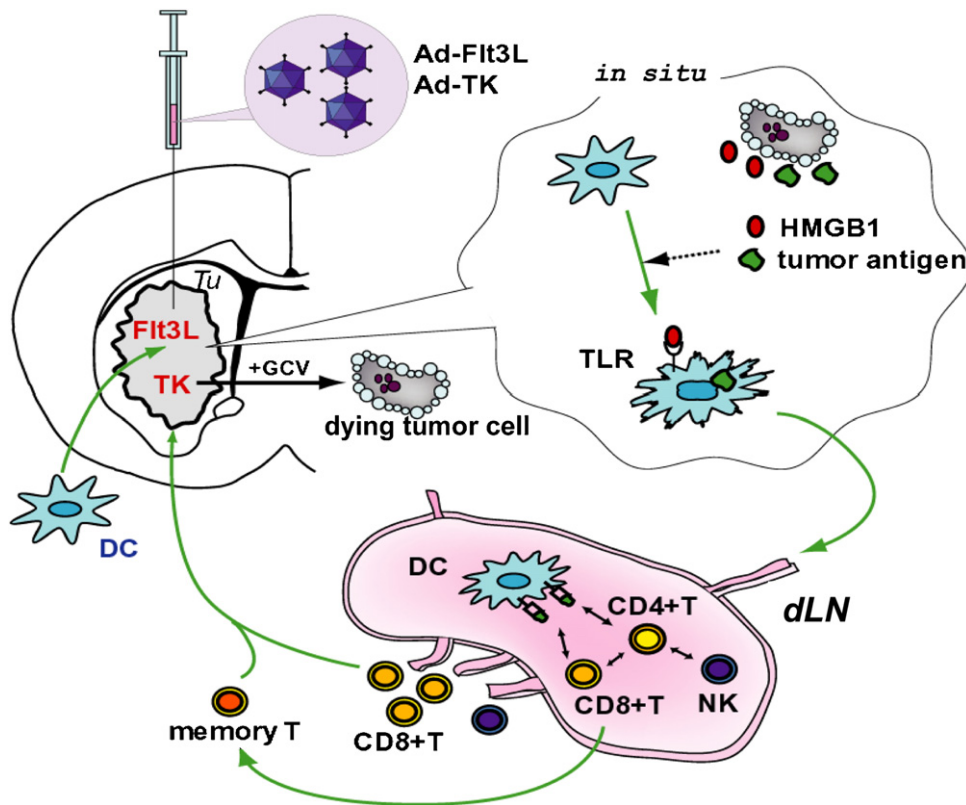


Fig. 1. Diagram illustrating the effects of adenoviral mediated TK/Flt3L gene therapy. Intra-tumoral injections of adenovirus expressing TK in combination with adenovirus expressing Flt3L into a brain tumor induce tumor cell death, release of intracellular inflammatory molecules, such as HMGB1 and tumor antigen. Flt3L recruits DCs to the tumor site, where they phagocytose tumor cell remnants and migrate to the dLN followed by priming of a T cell mediated cytotoxic anti-tumor immune response. *Abbreviations:* Tu, tumor; NK, natural killer cell; dLN, draining lymph node; TLR, toll like receptor; GCV, Ganciclovir; TK, thymidine kinase; Flt3L, fms-like tyrosine kinase 3 ligand; HMGB1, high-mobility group box-1.

[45]. Thus, gene therapy vectors emerge as a useful alternative to deliver these cytotoxins into the GBM microenvironment.

4. Immune mediated gene therapy: stimulating anti-brain tumor immunity

To protect itself against damage the mammalian CNS has evolved various mechanisms to restrict the function of the immune system. The brain occupies a niche in the body where the immune system has limited capacity to detect and eliminate foreign antigens. This is referred commonly to as “immune privilege”. Experimentally, it has been significantly more difficult to elicit immune responses towards CNS antigens in comparison to peripheral antigens. A number of key physiological processes that contribute to the suppression of CNS immunity have been identified, i.e. paucity of dendritic cells (DCs) in the brain, lack of lymphatic drainage, production of anti-inflammatory mediators such as TGF- β and NO by cells in CNS including low major-histocompatibility (MHC-II) expression on infiltrating microglia. These mechanisms are ways of protecting vital organs from immune-mediated attack [24].

The immune system is a key determinant of tumor rejection and escape. Therefore one of our research goals was to try and overcome this so-called immune privilege and in the process discover novel immune mediated gene therapeutic approaches for the treatment of GBM. As the brain is virtually absent of any dendritic cells, it is difficult to prime an adaptive immune response against antigens that are exclusive to the brain. Our goal was to generate a tumor micro-environment that is conducive to dendritic cell migration and maturation in the hopes of rescuing animals from lethal models

of GBM. We have succeeded in developing an adenoviral mediated immunotherapy for brain tumors that is dependent on the expression of two genes: *Thymidine Kinase* (TK), phosphorylates the prodrug Ganciclovir which induces DNA crosslinking followed by cell death [2] and *fms-like tyrosine kinase-3 ligand* (Flt3L), a potent DC growth factor that serves to increase the number of infiltrating DCs within the tumor microenvironment [1]. Tumor cell death induced by TK causes the release of tumor antigens, which are phagocytosed by surveying DCs and transported to the draining lymph nodes. Here T cells are primed to elicit an antigen specific cytotoxic anti-tumor immune response (Fig. 1). Combination therapy using the two adenoviruses induces tumor regression, long term survival and immunological memory in several mouse and rat GBM models [2,19].

Toll-like receptor (TLR) signaling; specifically TLR2 in DCs is essential for priming an effective immune response [19]. Using TLR2 KO mice, we demonstrated a defect of DCs to migrate to the tumor microenvironment when treated with adenovirus expressing TK/Flt3L. In addition, DCs lacking TLR2 failed to stimulate the proliferation and activation of tumor antigen specific T cells. The loss of TLR2 signaling abolished the CD8+ cytotoxic T cell mediated response seen in wild type mice [19]. These data highlight the importance of a receptor thought to be involved in innate immunity orchestrating an adaptive anti-brain tumor immune response.

But if this classical danger sensing receptor is required for initiating the immune response then surely there must be a tumor derived ligand. We identified high-mobility-group box 1 (HMGB1), a protein thought to be involved in controlling inflammation and sepsis as the TLR ligand in our model [19]. In viable cells HMGB1 is part of the chromatin structure but in response to

pro-apoptotic stimuli, including TK (plus GCV) mediated gene therapy, radiotherapy and TMZ, GBM cells shed nuclear HMGB1 [19], suggesting that cytotoxic agents may also be suitable adjuvants for immunotherapeutic strategies. HMGB1 binds TLR2 on bone marrow derived DCs stimulating their maturation. Neutralizing HMGB1 through small molecule inhibitors or antibodies leads to a loss of TLR2 stimulation, subsequent DC anergy and loss of therapeutic efficacy [19]. Our data suggest that HMGB1 released from a dying tumor is a critical TLR2 ligand that initiates the anti-tumor immune response [12,19]. Understanding how the TLR2 pathway in DCs is activated might potentially yield new targets for eliciting clinically effective anti-brain tumor immune responses.

An important facet to any adaptive immune response is the proper activation of cytotoxic T lymphocytes (CTLs). As such, several other groups have attempted to develop treatments based on T cell augmentation. In a rat glioma model, recombinant vaccinia virus expressing the cytokines IL-2 or IL-12 resulted in inhibition of tumor growth when injected intra-tumorally [16]. These soluble mitogenic factors mediate the growth survival and differentiation of antigenic T cells. As such combination therapies of IL-2 and IL-12 proved to be more effective in increasing the levels natural killer, Mac-1⁺, and NKT cells in blood as well as increased interferon-gamma, and tumor necrosis factor-alpha expression in tumors. Similar results were obtained with intra-tumoral injection of adenovirus expressing IL-12 or IL-2 in a breast cancer model [23].

Tumor necrosis factor-alpha (TNF α) and interferon-gamma (IFN γ) are potent immune-stimulatory cytokines capable of inducing tumor cell death that have been used in experimental GBM models. IFN γ is a key cytokine that promotes Th-1 polarization [25] and is thought to have anti-angiogenic properties [67]. Aside from facilitating immune cell migration, TNF α has been shown to regulate multiple functions of immune cells including cell growth, inflammation, and autoimmunity [15,73]. TNF α has also been shown to cause direct necrosis in neoplastic cells [8]. In a mouse glioma model the use of adenoviral vectors expressing TNF α or IFN γ delivered intra-tumorally induced infiltration of CD4⁺ and CD8⁺ cells in addition to increasing expression of MHC-I and -II on the tumor cells. Intracranial administration of both vectors led to a statistically significant increase in survival of tumor bearing mice [21]. These studies highlight the various ways the immune system has been harnessed to elicit effective albeit experimental treatments for brain tumors. Combination therapies that elicit immunogenic cell death and are immune stimulatory are predicted to be of most clinical benefit.

5. The clinical scenario

The relatively high efficiency of preclinical trials of various different therapeutic modalities gave confidence to clinicians and scientists to move ahead into early clinical trials in human patients. The high experimental therapeutic efficacy of TK+GCV in *in vitro* and *in vivo* models of GBM prompted the use of this conditional cytotoxic gene therapy strategy in human patients. The first clinical studies using suicide gene therapy in GBM patients consisted of intra-tumoral injections of the HSV1-TK vector producing cells (VPCs) alongside the standard treatment of surgery and radiotherapy [60]. These patients had increased numbers of IFN- γ -producing T cells and an increase in serum IL-12 and FasL levels compared to patients that received standard treatment alone, suggesting that TK (+GCV)-mediated gene therapy can stimulate a Th1 type immune response [60]. These potentially positive results prompted clinicians to test retroviral vectors encoding the suicide gene HSV1-TK in a large Phase III double blinded, controlled clinical trial in patients with glioblastoma. No statistically

significant efficacy benefits were obtained, and the use of retroviral vectors encoding HSV1-TK has now been abandoned. As an alternative, adenoviral vectors encoding the suicide gene HSV1-TK were also tested in small trials and their administration was deemed safe in patients diagnosed with GBM [29,61,68]. In one particular study a clinically and statistically significant increase in mean survival from 39.0 ± 19.7 (SD) to 70.6 ± 52.9 weeks was reported when patients received injections of AdvHSV-TK into the tumor bed following surgical resection [38]. This prompted progression towards a larger Phase III double blinded and controlled trial. Unfortunately, this Phase III trial failed to live up to early clinical success, and did not provide strong evidence of either clinical or statistically significant benefits. In 248 cases with newly diagnosed and previously untreated Glioblastoma multiforme patients received either standard therapy (surgery resection and radiotherapy) or standard therapy combined with adjuvant gene therapy. Twelve months survival rates were 50% vs. 55% in the gene therapy and control groups respectively [59]. Median survival was also similar across the two groups indicating a low therapeutic effect of TK.

These results demonstrate the challenges of translational medicine, and the poor predictability of preclinical science when tested in the human diseases. Secondly, these challenges possibly will work to highlight the need of using multiple agents simultaneously to elicit tumor cell killing and activation of the immune system. One such approach is the combination of immune-stimulatory strategies, coupled to cytotoxic strategies [33,35].

The pleiotropic cytokine IFN β has also been tested in a Phase I trial. In a dose escalation study, patients diagnosed with grade 3 or grade 4 brain tumors received doses of adenovirus expressing IFN β up to 2×10^{11} viral particles (VPs) alongside standard resection [18]. Doses up to 5×10^{10} VP were deemed safe and were associated with a dose-dependent induction of apoptosis within the tumors. With the exception of one case in which a patient exhibited post-surgical confusion in the high dose cohort, no other adverse events were reported. The therapeutic benefit of IFN β gene therapy remains to be seen, and will require multi-center trials in a large number of patients.

The combined Ad-Flt3L+Ad-TK (GCV) gene therapy strategy for the treatment of GBM has received approval from the FDA, for a Phase I dose escalation study in patients newly diagnosed with GBM. This preliminary clinical study will provide information on the safety and efficacy of adenovirus mediated delivery of cytotoxic TK and immune stimulatory Flt3L [42]. In conclusion, the pre-clinical and clinical findings highlight the importance and synergy of tumor cell killing to be used in combination with stimulation of an effective immune response. It is very likely that the combination of cytotoxic agents and immune stimulatory approaches with synchronous TLR2 activation will result in improved therapies for the treatment of brain tumors in humans. The lack of major clinical success highlights the limitations in developing new treatments for brain tumors [48]. Poor clinical responses are seen with most major new drugs being used, and although the contemporary standard of care has pushed median patient survival to 2 years post-tumor resection, it has been difficult to discover any agents that have made a much larger impact in prolonging patients' life. As we improve the preclinical models, their statistical analysis, and also aim to enhance the design, analysis, and interpretation of clinical trials for brain tumors, the challenge to improve patients' lives remains daunting. Though we trust that the combination of powerful immunotherapies and powerful cytotoxic approaches has the potential to improve patients' lives, it will only be clinically and statistically significant Phase III double blind randomized clinical trials that will give us assurance that real progress towards finding a cure has been made.

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