Gene Therapy and Virotherapy: Novel Therapeutic Approaches for Brain Tumors

Kurt M. Kroeger, M.S.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

A.K.M. Ghulam Muhammad, M.D., Ph.D.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Gregory J. Baker, Pharm.D.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Hikmat Assi, B.S.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Mia K. Wibowo, B.S.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Weidong Xiong, M.D., Ph.D.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Kader Yagiz, Ph.D.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Marianela Candolfi, D.V.M., Ph.D.,
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Pedro R. Lowenstein, M.D., Ph.D., and
Gene Therapeutics Research Institute, Departments of Biomedical Sciences and Medicine, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA

Maria G. Castro, Ph.D.
Gene Therapeutics Research Institute, Cedars-Sinai Medical Center, Los Angeles, California 90048, USA and Departments of Medicine and Molecular & Medical Pharmacology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California 90095, USA

Abstract
Glioblastoma multiforme (GBM) is a deadly primary brain tumor in adults, with a median survival of ~12–18 months post-diagnosis. Despite recent advances in conventional therapeutic approaches, only modest improvements in median survival have been achieved; GBM usually recurs within 12 months post-resection, with poor prognosis. Thus, novel therapeutic strategies to target and kill GBM cells are desperately needed. Our group and others are pursuing virotherapy and gene therapy strategies for the treatment of GBM. In this review, we will discuss various virotherapy and gene therapy approaches for GBM currently under preclinical and clinical evaluation including direct or conditional cytotoxic, and/or immunostimulatory approaches. We also discuss cutting-edge technologies for drug/gene delivery and targeting brain tumors, including the use of stem cells as delivery platforms, the use of targeted immunotoxins, and the therapeutic potential of using GBM microvesicles to deliver therapeutic siRNAs or virotherapies. Finally, various animal models available to test novel GBM therapies are discussed.

Introduction

Glioblastoma multiforme (GBM) is the most common type of brain tumor in adults, with a median survival of ~12–18 months post-diagnosis (Grossman et al., 2010; Wen and Kesari, 2008). The current standard of care for GBM patients consists of surgical resection, followed by radiotherapy and chemotherapy (Stupp et al., 2006; Wen and Kesari, 2008). Despite recent advances in conventional therapeutic approaches including the gamma knife (radiation) and temozolomide (chemotherapy) (Stupp et al., 2006; Stupp et al., 2005), GBM usually recurs within 12 months post-resection (Grossman et al., 2010; Wen and Kesari, 2008). Recurrent GBM tumors are usually comprised of tumor cells that are radiation and/or chemotherapy resistant and do not respond or do so poorly to further treatments. Interestingly, the susceptibility of tumor cells and the therapeutic benefit of temozolomide were shown to correlate with epigenetic silencing of the DNA repair enzyme O(6)-methylguanine-DNA-methyltransferase (MGMT) (Hegi et al., 2005); patients with a methylated MGMT promoter exhibit a more favorable therapeutic outcome when treated with temozolomide (Stupp et al., 2009). Nevertheless, novel therapeutic strategies to target residual tumor cells and prevent tumor recurrences are urgently needed (Curtin et al., 2005; Heimberger and Sampson, 2009; Wheeler and Black, 2009). Our group and others are pursuing virotherapy and gene therapy strategies for the treatment of GBM. In this review, we will discuss various virotherapy and gene therapy approaches for GBM that are currently under pre-clinical and clinical evaluation including cytotoxic, and/or immunostimulatory approaches. Also highlighted are cutting-edge technologies and approaches for the delivery of therapeutic agents and/or targeting of GBM cancer cells including using stem cells to target the vector at GBM cells and GBM microvesicles to deliver therapeutic siRNA. Various animal models available to test novel GBM therapies are also discussed.

Gene Therapy for Brain Tumors: Conditional Cytotoxic Approaches

Cytotoxic therapeutic approaches mainly consist of either delivering conditional or direct cytotoxic trans-genes (gene therapy) (Aboody et al., 2008; Aghi and Chiocca, 2006; Candolfi et al., 2009; Lawler et al., 2006), or delivery of conditionally replicating viral vectors that exclusively replicate in tumor cells and kill them (virotherapy) (Ferguson et al., 2010; Jiang et al., 2009; Markert et al., 2006). The most widely studied conditional cytotoxic transgene is Herpes Simplex Type 1 thymidine kinase (TK), which converts the prodrug ganciclovir (or valacyclovir) into the highly toxic deoxyguanosine triphosphate causing early chain termination of nascent DNA strands (Beltinger et al., 1999). The bystander effect of the TK approach relies on the passage of phosphorylated ganciclovir to neighboring cells through gap junctions, amplifying the cytotoxic effect of TK gene therapy (Mesnil and Yamakawa, 2000). This approach has been widely studied using either
adenoviral or retroviral vectors in numerous clinical trials in the U.S. and Europe and has demonstrated modest increases in median survival (Germano et al., 2003; Immonen et al., 2004; Sandmair et al., 2000; Smitt et al., 2003; Trask et al., 2000). In April 2009, the U.K. based Ark Therapeutics released an update on promising results from a multi-center Phase III clinical trial using a first-generation adenoviral vector encoding TK (Cerepro®) (Ark Therapeutics, 2009; Osborne, 2008). Unfortunately, the European Medicines Agency (EMEA) recently rejected Ark Therapeutics’s marketing application after deciding that the study was statistically underpowered and failed to show sufficient efficacy in terms of postponing death or re-intervention. The decision by the EMEA is currently under appeal by Ark Therapeutics (Mitchell, 2010).

Gene Therapy Strategies to Deliver Targeted Immunotoxins

An attractive cytotoxic gene therapy approach for GBM consists of using non-replicating, first generation adenoviral vectors to deliver transgenes encoding for highly toxic proteins such as *Pseudomonas* exotoxin (PE), which disrupts protein translation in the target cell leading to cell death. To restrict the cytotoxicity of this approach to brain tumor cells, a targeted toxin was formulated by linking the PE toxin to human IL-13. 50–80% of human GBM cells overexpress a variant of the IL-13 receptor, i.e., IL13Ra2 (Okada et al., 2008; Wykosky et al., 2008), that differs from its physiological counterpart IL4R/IL13R, expressed in normal tissues (Hershey, 2003). A protein formulation of this targeted toxin approach, Cintredekin Besudotox, was tested in Phase I–III human clinical trials but due to the short half life of the hIL-13-PE protein formulation (Kawakami et al., 2004; Vogelbaum et al., 2007) multiple injections or continued delivery was necessary to achieve therapeutic effects (Kawakami et al., 2004; Kunwar et al., 2007). As a result, the Phase III trial studying the efficacy of convection-enhanced delivery (CED) of Cintredekin Besudotox compared with Gliadel wafers (GW) in adult patients with glioblastoma multiforme did not demonstrate a significant survival difference between the two treatment groups. The average intraparenchymal distribution of Cintredekin Besudotox ranged from 10 to 15 mm radially from the tip of catheter. Therefore, poor drug distribution could have contributed to the lack of significant clinical responses (Kunwar et al., 2010). To overcome the short half life of the hIL-13-PE protein formulation, we have used regulatable first generation adenoviral vectors to deliver IL-13.E13K, a mutated variant of the hIL13 (Debinski et al., 1998) with a high binding affinity to the GBM-associated IL13Rα2 (Candolfi et al., in press, 2010). In encouraging pre-clinical experiments in human GBM xenografts, we demonstrated that adenoviral vector mediated delivery of mhIL-13-PE led to tumor regression and long term survival in ~70% of the animals without causing apparent neurotoxicity (Candolfi et al., in press, 2010).

Virotherapy for Brain Tumors: Conditionally Replicating HSV Vectors

Using virotherapy approaches, tumor cell death is achieved by oncolytic virus replication within the neo-plastic cells, ultimately leading to tumor cell lysis. To achieve safe and effective oncolytic activity, an oncolytic vector must conditionally replicate within the target tumor cells with minimal toxicity to the surrounding normal brain. Oncolytic vectors are classified as either mesogenic (moderately pathogenic, capable of producing viable progeny and infecting adjacent cells) or lentogenic (attenuated non-pathogenic, produces defective progeny and is incapable of spreading between tissues) (Dey et al., 2010). Replicating, oncolytic viruses have been developed from numerous species of viruses.

Conditionally replicating Herpes Simplex Virus (HSV) vectors have been tested for the treatment of malignant glioma (Grandi et al., 2009). The most widely studied oncolytic HSV vector is G207, a genetically engineered HSV-1. It has deletion of γ34.5 gene at both alleles.
Granelli-Piperno et al., 2000) and an insertion of the lacZ gene that prevents the expression of the UL 39 gene, which encodes for the large subunit of viral ribonucleotide reductase. As a result of these mutations, G207 can only replicate in rapidly dividing cells but not in quiescent cells. Furthermore, the HSV derived thymidine kinase gene was left intact in G207, thus delivery of G207 can be combined with produgs like ganciclovir to further increase the oncolytic effects of this approach. A Phase Ib clinical trial of G207 was recently published in which six patients with resectable, recurrent GBM were treated with two administrations of G207, the first administration was given via a direct injection into the tumor mass followed by surgical resection several days later. After tumor debulking, a second administration of G207 was given by multiple injections into the tumor cavity post surgical resection (Markert et al., 2009). Results from this trial demonstrated the high safety profile of multiple administrations of G207 with no evidence of encephalitis. Although the Phase Ib study was not designed to demonstrate therapeutic efficacy, viral replication was observed and limited evidence of anti-tumor activity was reported. Additional clinical trials of G207 are in the planning stages and second generation oncolytic HSV vectors are under pre-clinical development, such as vectors in which a single copy of the γ34.5 gene was re-introduced into the vector to enhance oncolytic replication (Kambara et al., 2005) and oncolytic HSV vectors genetically engineered to encode cytotoxic transgenes such as TNFα (Han et al., 2007), angiostatic transgenes such as Platelet Factor-4, extracellular fragment of brain-specific angiogenesis inhibitor 1, and shRNA specific for VEGF (Hardcastle et al., 2010; Liu et al., 2006; Yoo et al., 2007), or immuno-stimulatory transgenes such as IL-4 (Terada et al., 2006).

**Virotherapy for Brain Tumors: Conditionally Replicating Adenovirus Vectors**

Conditionally replicating adenoviruses can also be engineered to selectively replicate and lyse malignant cells. ONYX-015 and Ad5-Delta24 are two widely studied oncolytic adenoviruses (Chiocca et al., 2003; Fueyo et al., 2003; Jiang et al., 2009). ONYX-015 has a deletion in the E1B 55K gene that allows its replication in p53 defective tumor cells (Geoerger et al., 2002). Ad5-Delta24, can replicate and lyse cancer cells due to a 24-bp deletion in the E1 region responsible for binding the retinoblastoma (Rb) protein, which is often disrupted in human GBM (Fueyo et al., 2003; Fueyo et al., 2000). In a Phase I clinical study, ONYX-015 was safely administered into the tumor bed cavity post resection (Chiocca et al., 2004). Several groups are actively pursuing second generation oncolytic adenoviruses. One such example is Ad5-Delta24RGD, which has a genetically modified capsid that incorporates an Arg-Gly-Asp (RGD) motif into the HI loop of the viral fiber knob. The RGD motif enhances the virus’s affinity for αv integrins, which are abundant in glioma cells (Lamfers et al., 2002; Suzuki et al., 2001). Ad5-Delta24RGD has shown promise in pre-clinical studies using human GBM bearing xenograft nude mice in combination with low dose radiation (Lamfers et al., 2002). Other strategies include the use of tissue specific or glioma specific promoters including GFAP, nestin, human telomerase reverse transcriptase (hTERT), and survivin. One promising example of an oncolytic adenovirus incorporating tissue specific promoter technology is CRAd-Survivin-pk7, which contains a pk7 mutation in the adenovirus fiber and incorporates the survivin promoter driving E1A replication. Using human GBM xenografts, CRAd-Survivin-pk7 resulted in 67% long-term survival with evidence of enhanced adenovirus infectivity, decreased mitotic activity, and enhanced tumor apoptosis (Ulasov et al., 2007).
Virotherapy for Brain Tumors: Replication-competent Retrovirus (RCR) Vectors

Replication-competent retrovirus (RCR) vectors based on murine leukemia virus (MLV) exhibit unique characteristics. Due to its inability to infect quiescent cells, MLV based RCR exhibit high selectivity for tumor cells and has been shown to achieve highly selective and stable gene transfer throughout entire solid tumors in vivo. Most RCR vector genomes consist of an intact retrovirus genome including the transgene expression cassette containing an internal ribosome entry site (IRES) inserted immediately after the stop codon of the env gene (Jespersen et al., 1999; Logg et al., 2001). In contrast to oncolytic HSV and adenovirus, RCR are naturally non-cytolytic and their tumor killing power is derived from the incorporation of suicide transgene genes into the vector genome, which kill tumor cells in the presence of a prodrug. Examples of suicide genes used in RCR include the yeast cytosine deaminase (CD) gene, which converts the nontoxic prodrug 5-fluorocytosine (5FC) into the chemotoxin 5-fluorouracil (Hiraoka et al., 2007), and most recently Escherichia coli purine nucleoside phosphorylase (PNP), which results in potent cytotoxicity after administration of the prodrug fludarabine phosphate (F-araAMP) (Tai et al., 2010).

Virotherapy for Brain Tumors: Oncolytic Reovirus and Measles Virus Vectors

Finally, oncolytic reovirus and measles viral vectors are under development for GBM virotherapy. Reoviruses selectively replicate in glioma cells, where stimulation of RAS pathway by PDGFR or EGFR inhibits RNA-activated protein kinase activation, and thus viral proteins can be synthesized leading to tumor regression in preclinical studies using nude mice bearing intracranial human glioma xenografts in mice (Coffey et al., 1998). Delivery of live, replication competent, and genetically unmodified reovirus directly into the tumors of patients with malignant gliomas in a Phase I clinical trial demonstrated that oncolytic reoviruses are safe and well tolerated with no evidence of clinical encephalitis (Forsyth et al., 2008). Strains of the attenuated measles virus derived from the Edmonston vaccine lineage (MV-Edm) have been shown to preferentially infect and kill malignant cells while sparing the surrounding non-neoplastic tissues (Msaouel et al., 2009). The MV-Edm vector backbone has been engineered to express soluble marker peptides, such as the human carcinoembryonic antigen (CEA; MV-CEA) gene and the human thyroidal sodium iodide symporter (NIS; MV-NIS virus) gene to monitor the in vivo spread and elimination of the virus over time (Peng et al., 2002a; Peng et al., 2002b). In pre-clinical experiments, MV showed promising therapeutic efficacy in the U87, U118, and U251 glioma cell lines, with significant cytopathic effect being observed in all tumor cell lines tested (Phuong et al., 2003). A Phase I clinical trial of intratumoral and administration into the resection cavity of MV-CEA in patients with recurrent glioblastoma multiforme is currently recruiting patients (http://www.clinicaltrials.gov/ct2/show/NCT00390299).

Immune Challenges of the Brain Tumor Microenvironment

Immunotherapy is an alternative approach to direct cytotoxicity of GBM cells using gene therapy. The principle of immunotherapy approaches is the expectation that specific activated anti-tumor T cells would be able to eliminate any residual tumor cells that remain post-surgery, and thus inhibit tumor recurrence. In spite of the evidence that anti-brain tumor immunity can be induced through various immune-therapeutic approaches, i.e., vaccination (Broder et al., 2003; Choi et al., 2009; Heimberger et al., 2002; Liau et al., 2005; Yu et al., 2004), challenges to induce effective immune responses to eliminate GBM still remain. These include, a paucity of antigen presenting dendritic cells (DCs) within the brain.
parenchyma which may thus limit the capacity to stimulate an anti-tumor immune response, a lack of classic lymphatic outflow channels to allow activated DCs to exit the brain, the presence of immune suppressive regulatory T cells (Tregs), and immune suppressive cytokines, i.e., TGFβ (Gomez and Kruse, 2006; Learn et al., 2006; Yang et al., 2009). All these factors contribute to creating an immune suppressive tumor microenvironment. Further evidence has shown that a population of cells with immune suppressive activities, i.e., myeloid derived suppressor cells (MDSCs), play a major role in promoting immune suppression in several cancer models in rodents and also in human cancers (Gabrilovich and Nagaraj, 2009), thus contributing to poor priming of systemic immune responses against tumor antigens (Lowenstein, 2002). These immunosuppressive cells have also been recently described in human GBMs (Rodrigues et al., 2010).

Gene Therapy for Brain Tumors: Combining Immunotherapy and Cytotoxic Approaches

To prime an effective anti-tumor immune response from within the brain tumor microenvironment itself, our group has pioneered a novel gene therapy approach to recruit DCs to the brain parenchyma. The cytokine Flt3L increases the number of DCs in gastrointestinal lymphoid tissue, lymph nodes, lung, peripheral blood, spleen, thymus, and bone marrow (Brawand et al., 2002; Diao et al., 2004), and these increases are higher than those achieved by GM-CSF, or by GM-CSF and IL-4 (Lynch et al., 1997; Pulendran et al., 1997). We have demonstrated that intracerebral or intratumoral delivery of Flt3L increased the levels of DCs within the normal brain tissue, or intracranial GBM tumors (Curtin et al., 2006; Larocque et al., 2010). However, to prime an immune response, DCs must be exposed to tumor antigens, take them up, process them, and migrate to local lymph nodes (LN) to present antigenic epitopes on MHC-I and MHC-II to naïve T-cells. Using first generation adenoviral vectors, we delivered Flt3L to recruit DCs into the brain tumor microenvironment and expose them in situ to tumor-derived antigens within the tumor mass generated by using HSV1 thymidine kinase + ganciclovir to induce tumor cell death, thus making brain tumor antigens available to the tumor infiltrating DCs (Figure 1). Using this approach, we have demonstrated therapeutic efficacy, induction of anti-tumor immune responses, and immunological memory in several transplantable, orthotopic, syngeneic models of GBM (Ali et al., 2005; Candolfi et al., 2009; Curtin et al., 2008; Curtin et al., 2009; Ghulam Muhammad et al., 2009; King et al., 2008; King et al., 2008). By enabling the in vivo intra-tumoral antigen loading of DCs, we aim to direct the immune response against a large repertoire of tumor antigens (Ali et al., 2005; Curtin et al., 2006). This therapeutic approach is scheduled to commence a Phase I dose-escalation study in early 2011 in 18 patients with primary GBM.

Novel Therapeutic Delivery Platforms: Stem Cells and Exosomes

Exciting novel technologies are also emerging in the field of gene therapy and virotherapy for GBM. GBM tumor cells have been shown to release microvesicles (exosomes), which are endosomally derived 30–100 nm membranous vesicles (Al-Nedawi et al., 2009). Microvesicles containing mRNA, miRNA, and/or angiogenic proteins are taken up by surrounding normal cells, thus serving as a conduit of intercellular communication between cancer cells and their surrounding normal cells (Chen et al., 2010; Graner et al., 2009). The therapeutic potential of encoding anti-GBM specific siRNA and miRNA is being explored by several groups (Graner et al., 2009; Skog et al., 2008). Another exciting technology is the use of neural and mesenchymal stem cells to deliver oncolytic vectors to the tumor mass in the hope of improving therapeutic efficacy by overcoming the limited distribution of oncolytic vectors beyond the site of injection (Dembinski et al., 2010; Ferguson et al., 2010; Kranzler et al., 2009; Yong et al., 2009). Stem cells have been shown to selectively migrate

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to intracranial gliomas, invade tumor foci, and track single isolated tumor cells infiltrating into the surrounding normal brain parenchyma (Kendall et al., 2008; Zhao et al., 2008). Recently stem cells were shown to effectively deliver oncolytic adenovirus in mice bearing intracranial U87 brain tumors, even when administered at sites distant to the brain tumor mass (Sonabend et al., 2008).

**Animal Models for Testing Novel GBM Therapeutics**

To successfully test the therapeutic efficacy of novel GBM therapeutics, orthotopic animal models of GBM must be used that closely mimic the histopathological features and tumor microenvironment found in human GBMs. While transplantable syngeneic brain tumors share many histopathology characteristics with those of human GBMs, transplantable rodent tumors display low levels of normal brain tissue infiltration, a hallmark of human GBMs (Candolfi et al., 2007). Moreover, these tumors do not harbor the genetic lesions present in human tumors (i.e., PDGF and/or EGFR) and silencing of tumor suppressor genes (i.e., p53 and/or PTEN) (Ohgaki and Kleihues, 2007), but rather have a larger spectrum of heterogeneous alterations. Although orthotopic human GBM xenograft models in nude rodents display tissue infiltration, and contain genetic lesions of human GBM tumors, the human origin of these GBM cells requires their implantation into severely immune-compromised rodents (Giannini et al., 2005; Sarkaria et al., 2007; Xie et al., 2008). While these models are very useful for studying the molecular pathways involved in gliomagenesis (Dasgupta et al., 2009; Solomon et al., 2008a; Solomon et al., 2008b) and evaluating therapeutic efficacy of cytotoxic, anti-angiogenic, and other anti-GBM approaches (de Bouard et al., 2007; Dinca et al., 2008; Harding et al., 2006; Kitange et al., 2009; Kitange et al., 2008), it is impossible to assess the potential therapeutic efficacy of experimental immunotherapeutics because of the absence of an intact immune system. Within the past decade, several groups have used a variety of germline or virally encoded mutations to induce the development of endogenous brain models of gliomas (Gutmann, 2009; Huse and Holland, 2009; Reilly, 2009). Brain tumors of astrocytic and oligodendrocyte origins develop in transgenic mouse models with altered expression profiles of Ras, Ink4a/Arf, erbB, PTEN, and/or p53 (Charest et al., 2006; Kwon et al., 2008; Reilly et al., 2000; Uhrbom et al., 2002; Wei et al., 2006). RCAS vectors [Replication-Competent ASLV long terminal repeat (LTR) with a Splice acceptor] encoding Ras and AKT (Holland and Varmus, 1998) or PDGF-B (Dai et al., 2001) induced brain tumors of astrocytic origin in mice. Brain gliomas also develop when RCAS vectors are used to deliver Ras and or Akt into transgenic mice with PTEN or In4kaArf knockouts (Hu et al., 2005; Uhrbom et al., 1998). MoMuLV retroviral vectors encoding PDGF-B were also used to induce tumors of oligodendrocyte origin in mice (Uhrbom et al., 2002). A retroviral vector encoding EGFR was used to generate tumors of oligodendrocyte tumors in neonatal mice (Ivkovic et al., 2008), and a retroviral vector expressing PDG F-B was used to generate gliomas in adult rats (Assanah et al., 2006). Most recently lentiviral vectors encoding activated Akt and Ras in wildtype or in Tp53+/− knockout mice induced the development of brain tumors in adult mice (Marumoto et al., 2009). In all of these models, tumors appeared within 3–12 months post-tumor induction. Evaluation of therapeutic approaches using these endogenous brain tumor models has begun; in a recent study, the effects of radiotherapy and perifosine were assessed using three endogenous tumor models induced by RCAS delivery of proto-oncogenes (Hambardzumyan et al., 2008). Another recent approach to induce endogenous GBM uses the Sleeping Beauty (SB) transposable element to achieve integration of oncogenes in immune competent neonatal mice (Wiesner et al., 2009). Spontaneous brain tumors were induced by injecting an SB-tranposase encoding plasmid in combination with transposon DNA plasmids harboring several genetic lesions (AKT, N-RAS, EGRFvIII, and/or shRNA specific for p53) into the brain of neonatal mice (Wiesner et al., 2009). The histological
characteristics of the tumors resembled many of the features encountered in human astrocytoma or GBM (Wiesner et al., 2009).

Conclusions

Glioblastoma multiforme constitutes a formidable therapeutic challenge, due in part to the infiltrative and aggressive nature of the tumor, the presence of the blood brain barrier, which restricts entry of therapeutic entities to the tumor area, the recurrent nature of the tumor, the paucity of antigen presenting cells and lymphatic drainage within the brain, and the immune suppressive nature of the tumor microenvironment. All these factors contribute to the short survival post-diagnosis and the lack of treatments that substantially prolong median survival of GBM patients. Gene therapy, which includes virotherapy and the use of stem cells and exosomes as novel platforms for therapeutic gene delivery, presents powerful, novel opportunities for developing adjuvant therapies for this devastating cancer. They include the delivery of direct and conditional cytotoxic genes, immunotoxins, oncolytic viruses, and immune-modulatory molecules to overcome immune suppression and mount an effective and specific antitumor immune response. These novel strategies, some of which are currently being tested in Phase I clinical trials, provide new hope for improved therapeutic outcomes for this devastating cancer.

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Figure 1.
Schematic depiction of the mechanism underlying the induction of a specific anti-GBM immune response using Flt3L/TK gene therapy. First generation adenoviral vectors encoding Flt3L and HSV1-TK are injected into an intracranial brain tumor; ganciclovir is administered systemically. Expression of HSV1-TK in the presence of ganciclovir mediates tumor cell death, releasing endogenous brain tumor antigens and danger signals, including HMGB1. Intratumoral expression of Flt3L recruits dendritic cells (DCs) into the brain tumor milieu where they take up brain tumor antigens released from the dying GBM cells and present them on their MHC complexes. This phenomenon is dependent on the TLR2/RAGE agonist HMGB1, which is released from dying tumor cells. The DCs loaded with brain tumor antigens migrate to the cervical draining lymph nodes where they present brain tumor antigens to naïve T cells, mediating a clonal expansion of brain tumor specific anti-GBM effector T cells. The GBM specific effector T cells then migrate back into the brain and kill residual GBM cells. Therapeutic efficacy of the approach is not diminished when mice are also treated with the chemotherapy temozolomide (TMZ).