Chapter 14

GENE THERAPY FOR MALIGNANT GLIOMAS

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High-grade glial tumors, in particular anaplastic astrocytoma and glioblastoma multiforme (GBM), are the most common primary brain tumors in adults. Despite optimal current therapy, high-grade gliomas are associated with a poor prognosis. There is increasing hope that understanding the molecular derangements that give rise to these aggressive glial neoplasms may lead to targeted therapies. In particular, the identification of overlapping but recurring genetic alterations within gliomas has led to the idea of gene therapy to reverse or “treat” these alterations. In this regard, a variety of promising preclinical findings in animal models have encouraged the development of gene therapy for gliomas in early human clinical trials.

The term gene therapy comprises techniques aimed at delivering and expressing selected genetic material in cells and tissues of interest for therapeutic application. There are three basic components of this therapeutic strategy: (1) expression of the gene or genetic material in the desired cell population (vector strategy); (2) selection of the gene or genetic material to be expressed (transgene strategy); and (3) delivery of the transgene-containing vector to the therapeutic target (delivery strategy). In this review, I discuss these three components and summarize current strategies in preclinical and clinical development for gene therapy of human gliomas.

VECTOR STRATEGIES

Viral vector gene therapy has been divided into two general strategies. The first uses replication-defective viruses in which the viral genome has been altered to delete key viral genes involved in viral replication. In this approach, the virus cannot grow in cells but is used to deliver an anticancer transgene to effect tumor toxicity. Examples of this approach include retrovirus, adenovirus, adeno-associated virus (AAV), and herpes simplex virus type 1 (HSV-1). Advantages of this strategy include low toxicity and versatility of genetic construction. The primary potential disadvantage is a low volume of distribution in the tumor because of the viral inability to replicate.

An alternative strategy is the use of replication-competent viruses that are designed to selectively replicate in tumor cells but not in normal cells; these are also called replication-selective or oncolytic viruses. Examples include adenovirus, HSV-1, and reovirus. The primary advantage is improved volume of distribution in tumor. Disadvantages include potential toxicity, which may result from loss of selective replication, and higher immunogenicity.

Retroviral Vectors

Retroviruses are enveloped RNA viruses that possess the ability to integrate into the host cell genome by transcribing DNA from their RNA template via viral reverse transcriptase. The transcribed viral DNA is then integrated into the host cell genome nonspecifically. There is relative specificity for tumors because, except for lentiviruses, retroviral DNA preferentially integrates into the genome of dividing cells. Retroviral vectors have been derived from the Moloney murine leukemia virus (M-MuLV) established by Baltimore and colleagues. The replication-defective retroviral vector is constructed as follows.

DNA plasmid constructs containing long-terminal repeats (LTRs) and the packaging signal together with the transgene of interest are transfected into modified cultured cells termed vector-producer cells (VPCs). These VPCs, which are usually derived from the murine fibroblast 3T3 cell line, have been stably transfected with a plasmid with the entire retroviral genome, save the packaging signal. Thus the VPCs are able to transcribe and translate viral RNA but are unable to package viral genomic RNA into virions. Nevertheless, they efficiently complement the plasmid construct (containing the packaging signal), leading to packaging of the transgene of interest into virions, which can then be harvested from the medium of VPCs.

Advantages of retroviral vectors include (1) integration into dividing cells, which is particularly advantageous for tumor therapy, and (2) low toxicity because of replication deficiency. Disadvantages include (1) low transgene capacity, (2) the necessity of implanting transfected VPCs, which may not survive long in the host, and (3) a risk of insertional mutagenesis in host cells (e.g., by insertion at a proto-oncogene locus).

In the attempt to improve transduction efficiency, replication-competent retroviruses (RCR) have recently been developed. RCR are able to transduce human and rat glioma cell lines in vitro much more effectively than replication-defective retroviral vectors at the same dose. In addition, RCR capably and selectively transduce established U-87 gliomas in vivo. To date, RCR have not been used in clinical trials.
Adenoviral Vectors

Adenoviruses are nonenveloped DNA viruses associated with upper respiratory tract infections. Subgroup C adenovirus is used for the construction of adenoviral vectors and usually causes a mild upper respiratory infection in immunocompetent hosts. Generation of adenoviral vectors is accomplished as follows. The transgene of interest is cloned into a plasmid and flanked by DNA homologous to adenovirus DNA sequences. Adenovirus DNA with E1 deleted—a gene needed for adenoviral growth in cells—is co-transfected with the transgene-containing plasmid into a cell line (e.g., 293 cells) engineered to express the E1 gene. Homologous recombination between the E1-deleted adenoviral DNA and the transgene-containing plasmid DNA creates a new replication-defective (because E1 has been deleted) adenoviral vector which is complemented by (i.e., can grow in) 293 cells expressing E1. The E1-deleted vector can then be harvested from cells and used to infect tumor cells in which it will express the transgene of interest but be unable to replicate.

Whereas some adenoviral vectors are replication-defective, others are replication-selective or “oncolytic.” A potential advantage of replicative oncolytic viruses is that viral replication within infected tumor cells produces new viral progeny to infect additional cells within the tumor mass. For example, one strategy is to produce an adenoviral vector with a deletion in the E1B region; because E1B inactivation of the tumor-suppressor gene p53 is necessary for viral replication, this restricts replication in this mutant to cells lacking p53. This provides some tumor selectivity to viral replication, and could be useful in the subset of gliomas with p53 mutations. Another strategy involves oncolytic adenoviruses that replicate selectively in cells with mutations in the p16 tumor-suppressor gene pathway.

An important trend in the development of adenoviral vectors has been deletion of a portion of wild-type viral DNA (total 36 kb) to accommodate large amounts of foreign DNA (up to 10 kb). Other than this high cloning capacity, important advantages to adenoviral vectors include (1) high titers, (2) high virion stability, unlike retroviruses, (3) broad host cell range, and (4) ability to infect both proliferating and quiescent cells.

Disadvantages of adenoviral vectors include (1) virulence of wild-type virus and (2) immunogenicity. “First-generation” adenoviral vectors have induced strong immune responses in the host as well as acute and chronic toxicity from the vector itself. Aside from toxicity, one deleterious result of a robust host adaptive response is loss of vector genomes locally in the inflamed tissue. Progressive attenuation of the vectors and deletion of specific viral coding sequences will presumably mitigate these responses in future trials.

Adeno-Associated Virus Vectors

AAV is a parvovirus that is nonpathogenic to human cells and incapable of autonomous replication without the presence of helper virus, usually adenovirus. Recombinant adeno-associated virus (rAAV) vectors are replication-defective and can infect a broad host range of cells and tissues.

Advantages of rAAV vectors include (1) infection of both quiescent and proliferating cells, which is particularly important for gene therapy directed at postmitotic neurons, (2) lack of pathogenicity and immunogenicity, and (3) site-specific integration. Site-specific integration on human chromosome 19q is a unique feature of rAAV vectors and confers the ability of rAAV vectors to mediate long-term transgene expression in a variety of tissues. Disadvantages of rAAV vectors include (1) low transgene capacity (4.7 kb) and (2) low titers.

Herpes Simplex Virus Type 1 Vectors

HSV-1 is an enveloped double-strand DNA virus with a large genome (152 kb). Wild-type HSV-1 is neurotropic and can invade and replicate in both neurons and glial cells, resulting in a hemorrhagic necrotizing encephalitis (HSV-1 encephalitis). As a gene therapy vector, advantages of HSV-1 include (1) high transgene capacity (30 kb, compared with approximately 8 kb in retroviral vectors, 10 kb in adenovirus vectors, and 4.7 kb in AAV vectors), (2) high titer, (3) high virion stability, (4) neurotropism, and (5) availability of a specific antiviral agent (ganciclovir). In addition, unlike retroviral vectors, there is no risk of insertional mutagenesis because the HSV-1 genome does not integrate but instead persists as an episome in the host cell cytoplasm. Disadvantages include (1) difficulty of genetic manipulation given large viral genome, (2) preexisting immunity in the majority of humans (60% to 90%), which could stimulate host immune response and limit transgene delivery, and (3) potential toxicity caused by virulence of wild-type virus.

For glioma therapy, replication-selective (oncolytic) HSV-1 viruses have been constructed by deletion of various viral genes. These include viral thymidine kinase, DNA polymerase, uracil DNA glycosylase, ribonucleotide reductase, and γ34.5. For example, the G207 virus carries deletions in both copies of γ34.5 (encoding a protein essential for viral replication in neurons) and a lacZ insertion in the U139 gene (viral ribonucleotide reductase). This virus has been tested in a phase I clinical trial (see later discussion).

Advances in Vector Design

Developments in vector design offer new avenues for exploration. For example, reovirus, a nonenveloped DNA virus that replicates selectively in cells with an activated Ras pathway, has shown promise in preclinical animal models and is being developed for human clinical trials. In addition, advances in vector targeting include capsid modification for individual cell type targeting and differential transduction by use of serotypes.

TRANSGENE STRATEGIES

Transgene strategies have included (1) prodrug activation (2) correction of genetic defects and (3) provision of immune response-modifying genes.

Prodrug Activation

The most common transgene strategy to date used in clinical trials of gene therapy for malignant gliomas is the herpes simplex virus–thymidine kinase (HSV-TK)/ganciclovir system. Ganciclovir is an acyclic nucleoside analog that is clinically useful as an antitherapeutic drug, because it has speci-
ficity for viral TK over human nucleoside kinase. Thus viral TK monophosphorylates ganciclovir, which is then dually phosphorylated to ganciclovir triphosphate by cellular kinases. Ganciclovir triphosphate binds viral better than human DNA polymerase and serves as a false substrate, leading to DNA chain termination and cellular toxicity.

For gene therapy, the most important observation is that transduction of glioma cells with HSV-TK increases their ganciclovir sensitivity 5000-fold. Because it targets DNA, ganciclovir affects rapidly dividing cells such as tumor cells. In vivo efficacy in animal models was initially demonstrated by a dramatic increase in survival following intratumoral implantation of HSV-TK retroviral vectors and intraperitoneal ganciclovir in a rat gliosarcoma model. This was subsequently confirmed by other investigators.

The bystander effect of HSV-TK/ganciclovir has been crucial to its widespread application in cancer gene therapy. The bystander effect refers to the ability of ganciclovir to eradicate an entire population of tumor cells despite the expression of HSV-TK in only a fraction, often a minority, of cells. This effect has been shown to require cell-cell contact. Because HSV-TK cells contain mostly ganciclovir monophosphate and "bystander" HSV-TK cells contain mostly ganciclovir triphosphate, the bystander effect presumably involves transfer of ganciclovir triphosphate from HSV-TK cells to HSV-TK cells. The transfer is thought to occur across astrocyte-astrocyte gap junctions in gliomas, and indeed the magnitude of the bystander effect correlates with the extent of gap junctional coupling.

Other prodrug activation models are now in development, such as the cytosine deaminase/5-fluorocytosine (5-FC) system. 5-FC is a prodrug that is converted in vivo into 5-fluorouracil (5-FU) by cytosine deaminase (CD). 5-FU is a toxic chemotherapeutic agent that works primarily via chain termination and perturbation of DNA synthesis. Adenovirus-mediated transfer of CD followed by systemic administration of 5-FC has led to improved survival in rodent glioma models.

Correction of Genetic Defects

A distinct transgene approach has involved replacement of genes mutated in gliomas. P53 gene mutations are associated with a subset of glioblastomas, so-called secondary glioblastomas. In preclinical studies, reintroduction of wild-type p53 has been associated with glioma growth inhibition in vitro and in vivo. This result led to a phase I trial of adenovirus-mediated p53 gene therapy (see later discussion).

Immune Response Modification

Another approach has involved transducing tumor cells with genes that will increase immunogenicity. This has commonly been accomplished with cytokine genes. Cytokines used for this purpose have included interleukin (IL)-2, IL-4, IL-12, interferon (IFN)-β, IFN-γ. A significant potential problem with this approach is that significant toxicity as a consequence of cerebral edema has been associated with IL-2 and IFN-γ secretion.

CLINICAL TRIALS OF GENE THERAPY FOR MALIGNANT GLIOMA

Based on the strategies and preclinical work just outlined, several clinical trials of gene therapy for malignant gliomas are underway or completed.

Retroviral HSV-TK Trials

The first study of gene therapy for brain tumors involved implantation of murine cells modified to produce a retroviral vector with an HSV-TK transgene into recurrent brain tumors. Fifteen patients with brain tumors (12 with malignant gliomas, 3 with metastases) were studied. The results demonstrated survival of vector-producing cells (VPCs) at 7 days, but limited gene transfer into tumors; TK transcripts were identified in surviving VPCs but in less than 0.2% of neighboring tumor cells by in situ hybridization. Antitumor activity was observed in only five of the smaller tumors (1.4 ± 0.5 ml). In France a phase I and II study of gene therapy for recurrent glioblastoma was performed. This trial involved 12 patients in whom HSV-TK VPCs were injected into the cavity margins following tumor resection. Seven days postoperatively, ganciclovir was administered for 14 days. An important result was that no treatment-related adverse events were reported. Median survival was 206 days, and 25% of the patients survived longer than 12 months. One patient survived for 3 years after gene therapy and ultimately died of disseminated breast cancer with no evidence of glioblastoma recurrence on postmortem analysis.

Another phase I trial involved 12 children (ages 2 to 15) with recurrent tumors treated with VPCs carrying HSV-TK retroviral vectors, followed by ganciclovir treatment. Again, no treatment-related adverse events were reported. Median time to disease progression was 3 months, with the three longest times to progression being 5, 10, and 24 months.

An international phase II trial conducted between 1997 and 1998 involved 48 patients with recurrent glioblastoma. In this trial, retroviral VPCs were administered intracerebrally following tumor resection, and ganciclovir was infused intravenously 14 to 27 days after surgery. Median survival was 8.6 months, and the 12-month survival rate was 13/48 (27%). There was no evidence of tumor recurrence on magnetic resonance imaging (MRI) in seven of the patients for at least 6 months and in two patients for at least 12 months.

To further evaluate the rate of tumor cell transduction, immune response, and degree of antitumor effect, another study combined gene marking and a therapeutic trial in five patients. In this study, two trials of intratumoral VPC implantation were separated by intermediate tumor harvest to assay TK protein, enzymatic activity, and immune response. Stereotactic biopsy sampling and intratumoral implantation with VPCs was performed; after 5 days, the tumor was resected, the cavity was reimplemented with VPCs, and ganciclovir was given. Four patients tolerated the treatment well but had tumor progression. One patient developed a lethal brain abscess after the second operation. Increased HSV-TK enzymatic activity was demonstrated in one tumor specimen, but immunohistochemical evidence of TK gene expression was limited to VPCs with no obvious tumor cell transduction. In addition, minimal immune response was seen.
These uncontrolled trials indicated that HSV-TK gene therapy with retroviral VPCs was largely safe but was quite limited by poor viral transduction to tumor cells. Of course, efficacy could be established only by a phase III randomized clinical trial, which occurred between 1996 and 1998. In that study—the only phase III study of gene therapy for brain tumors published to date—patients with newly diagnosed and previously untreated GBM were divided into two groups of 124 patients each. The control group received standard therapy (surgery and radiation therapy), and the gene therapy group received standard therapy plus adjuvant gene therapy during surgery. Following tumor resection, HSV-TK VPCs were manually implanted into the tumor bed via multiple injections, and ganciclovir was given intravenously from days 14 to 27. In the gene therapy group versus the control group, progression-free median survival was 180 days versus 183 days; median survival was 365 versus 354 days; and 12-month survival was 50% versus 55%. This trial demonstrated feasibility and safety, but lack of efficacy—again presumably because of poor tumor transduction. A separate immunophenotyping study of a subset of 13 patients from this study demonstrated a mild systemic immune response but no difference in numbers of tumor-infiltrating lymphocytes. A distinct retroviral vector in development is the pLIL-2-TK, which co-expresses the suicide gene HSV-TK and the immunomodulatory gene IL-2 in an attempt to amplify the antitumor effect. A pilot study has been reported of four patients with recurrent GBM who received stereotactic injection of retroviral VPCs. No treatment-related adverse effects were reported, and evidence of transgene expression in treated tumors is promising. Adenoviral HSV-TK Trials

Adenoviral HSV-TK vectors have also been studied in early clinical trials. Between 1996 and 1998, 13 patients with recurrent malignant brain tumors (nine with GBM, one with gliosarcoma, and three with anaplastic astrocytoma) were treated with intratumoral injection of between $2 \times 10^9$ and $2 \times 10^{11}$ vector particles (VP) of a replication-defective adenoviral vector carrying HSV-TK followed by ganciclovir treatment. The highest dose ($2 \times 10^{11}$ VP), central nervous system (CNS) toxicity was manifested by confusion, hyponatremia, and seizures. Within 10 months after treatment, 10 of 13 patients died: 9 from tumor progression and 1 from sepsis. Two patients survived more than 25 months, and one was alive 29.2 months after treatment. Postmortem neuropathologic examination demonstrated variable inflammation within the residual tumors. In a similar trial from the Netherlands, performed between 1998 and 2001, 14 patients with recurrent high-grade gliomas were treated with a replication-defective adenoviral vector carrying HSV-TK followed by ganciclovir treatment. The vector was injected intraoperatively into the tumor bed following resection. Although there were no treatment-related adverse effects in this study, all patients had recurrence or progression, with an overall median survival of 4 months following treatment.

A phase I and II trial conducted in Finland between 1998 and 1999 directly compared retroviral with adenoviral vector-mediated delivery of the HSV-TK transgene. This study involved two experimental groups—retroviral vector treatment in seven patients and adenoviral vector treatment in seven patients—and a control group of seven patients receiving *Escherichia coli* β-galactosidase marker vector treatment. Adverse effects included fever in two patients with adenoviral vector therapy and an increase in seizure frequency in two patients. In the group treated with retroviral vector, all patients showed progression on MRI by 3 months, whereas three of the seven patients treated with adenoviral vectors did not. Mean survival times for retroviral, adenoviral, and control groups were 7.4, 15, and 8.3 months, respectively. On the basis of this comparison, the authors speculated that adenoviral vectors may be therapeutically advantageous, considering their greater titer and ability to infect nonreplicating cells.

**Oncolytic HSV-1 Trials**

The third virus used in clinical trials for glioma is oncolytic (replication-selective) HSV-1. Two phase I trials have been reported. In a Scottish study, nine patients with recurrent high-grade gliomas (eight with GBM, one with anaplastic astrocytoma) were treated with intratumoral inoculation of 1716, a γ34.5 mutant oncolytic HSV-1. No treatment-related adverse effects were documented. Of the nine patients, four were alive and well 14 to 24 months after 1716 administration. In a U.S. trial, 21 patients with recurrent malignant glioma were treated with intratumoral inoculation of G207, an HSV-1 double mutant for γ34.5 and ribonucleotide reductase (see previous discussion). No toxicity or serious adverse effects could be unequivocally ascribed to viral inoculation, and no patient developed HSV encephalitis. Forty of twenty-one patients were alive at the time the results were published. The most encouraging result was that examination of tissue from re-resections demonstrated expression of HSV-1 and the lacZ reporter gene in two patients at 56 and 157 days after inoculation.

**Other Trials**

Many new clinical trials of novel vectors and transgenes for glioma therapy are open and in various stages of development. For example, two new transgene strategies focus on the introduction of immunomodulatory genes or genetic correction with *p53*. A phase I trial using a recombinant adenovirus that expresses human IFN-β is underway at the University of Pennsylvania. The primary goal of that study is to examine toxicity of intratumoral injection of the vector in patients with recurrent or progressive malignant glioma; other goals include obtaining evidence of gene transfer in resected tissue specimens and clinical or biologic response. A phase I trial of adenovirus-mediated *p53* gene therapy has recently been reported. In this multicenter study, 15 patients with recurrent malignant glioma were enrolled for a two-stage treatment. In the first stage, a replication-defective E1-deleted adenoviral vector carrying *p53* (Ad-p53) was stereotactically injected as a single bolus into the tumor by using an implanted catheter. In the second stage, the tumor and catheter were resected en bloc 3 days later, and the resection cavity treated again with Ad-p53. The intratumoral injection of Ad-p53 led to minimal toxicity and resulted in *p53* gene transfer and *p53* protein expression. However, this study also demonstrated that Ad-p53 did not penetrate far from the injection site.
DEVELOPMENT STRATEGIES

One point made clear by preclinical and early clinical trials of gene therapy for gliomas is the importance of the delivery strategy. The goal of optimal delivery of the transgene-containing vector to as many tumor cells as possible. So far, as described above, clinical studies of gene therapy for glioma have used direct injection of the vector into the tumor or tumor bed. One possibility is to optimize infusion into the tumor or tumor bed. Freehand injection of VPCs is inaccurate and offers little control of injection parameters. Other problems include tissue disruption from forceful injection and injectate reflux along the needle tract. Novel strategies involving stereotactic needle guidance and mechanical control over VPC infusion and needle withdrawal may improve tumor saturation.

Convection-enhanced drug delivery (CEDD) may optimize the delivery of infusate into a defined tissue volume. Developed by Oldfield and colleagues, CEDD overcomes the limitations of simple diffusion by adjusting the infusion parameters to induce bulk flow in the interstitial space, thereby distributing macromolecules into brain interstitium centimeters instead of millimeters from the infusion site. Drug distribution can be controlled by varying infusion volume or rate. In recent important work, Bankiewicz and colleagues have demonstrated the efficacy of CEDD to deliver rAAV vectors to CNS parenchyma in rats and monkeys.

Two clinical trials using CEDD have been reported. First, Oldfield and colleagues used CEDD to administer transferrin-CRM107, a conjugate of transferrin and a mutant diphtheria toxin, to 15 patients with malignant glioma. At least 50% reduction in tumor volume occurred in 9 of 15 patients. Second, a clinical trial using CEDD for intratumoral administration of IL-4 Pseudomonas exotoxin (NBI-3001) for patients with recurrent malignant glioma has recently been reported. In that study, 31 patients received various doses of NBI-3001 administered via CEDD; safety and toxicity evaluations were performed, but the volume of drug distribution is unclear.

Another delivery approach is intra-arterial vector infusion combined with blood-brain barrier disruption. One study in a 9L gliosarcoma rat model used intra-arterial delivery of HSV-1 vector and bradykinin-induced blood-brain barrier disruption. Delivery of HSV-1 into 9L gliosarcoma cells was enhanced by intracarotid bradykinin. A follow-up study demonstrated that intra-arterial infusion of an attenuated HSV-1 vector followed by blood-brain barrier disruption and systemic ganciclovir administration led to regression of established tumors. Adenoviruses have also demonstrated promise when administered using this approach.

A distinct delivery strategy is nonviral DNA delivery via liposome-gene complexes. Potential advantages of cationic liposomes include (1) uncomplicated preparation, (2) minimal safety requirements, (3) ability to complex a large amount of DNA, (4) lack of immunogenicity, and (5) greater stability. Liposome systems deliver DNA to the cytoplasm of cells by plasma membrane fusion and endocytosis. Liposome-mediated HSV-TK gene transfer led to decrease in tumor volume and tumor regression in an experimental F98 glioma model. In another study, cationic liposome-plasmid DNA complexes showed increased efficacy but reduced specificity of gene transfer following intra-arterial delivery compared with adenovirus vectors. A phase I and II trial of cationic liposome-HSV-TK treatment in patients with recurrent malignant gliomas is underway.

Yet another possibility is the use of lipophilic prodrugs. Prodrugs, such as ganciclovir or 5-FC, must be efficient and selective substrates for the activating enzyme and must be metabolized to potent cytotoxins. Lipophilicity of the prodrug determines blood-brain barrier penetration, and furthermore the lipophilicity of the activated prodrug is pivotal in determining bystander effects. For example, ganciclovir triphosphate is highly polar, cannot diffuse across cell membranes, and therefore requires intercellular gap junctions for its bystander toxicity (see previous discussion). In principle, lipophilic prodrugs could better penetrate the blood-brain barrier and in their activated forms could cause a tumor-killing bystander effect without requiring cell-cell contact.

Whereas a full discussion of the advantages and disadvantages of these macroscopic delivery methods is beyond the scope of this chapter, refinement of vector delivery is clearly critical to optimizing vector-target interaction.

THE FUTURE

Despite a great deal of development, gene therapy for brain tumors is still at an early stage. Completed clinical trials demonstrate the overall safety of the approach, but also show limited efficacy due largely to poor tumor transduction. A combination of advances in vector design and creative transgene approaches, together with optimized macroscopic delivery systems, should improve therapeutic efficacy and minimize toxicity. Molecular imaging techniques using marker substrates will also be important to track transgene expression in tissue noninvasively. Combination therapies may help to target the many distinct genetic alterations known to exist within glial tumors, and could in principle be tailored to the molecular signature of individual lesions. Significant parallel progress in all of these areas should foster ongoing interest in further translational clinical trials of novel gene therapy for gliomas.

References


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