

# Future of gene therapies in high grade gliomas

## Research Article

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**Abbreviations:** 5-fluorocytosine, (5-FC); 5-fluorouracil, (5-FU); Adenovirus, (Ad); anaplastic astrocytoma, (AA); antisense phosphorothioate oligonucleotides, (S-ODN); blood-brain barrier, (BBB); carcinoembryonic antigen, (CEA); central nervous system, (CNS); Convection-enhanced delivery, (CED); cytosine deaminase, (CD); Cytotoxic T lymphocytes, (CTL); epidermal growth factor receptor, (EGFR); early growth response 1, (Egr1); ganciclovir, (GCV); glial fibrillary acidic protein gene, (GFAP); glioblastoma multiforme, (GBM); herpes simplex virus thymidine kinase, (HSV-tk); Hypoxia-inducible factor, (HIF); hypoxia-responsive elements, (HRE); interferon-alpha, (IFN- $\alpha$ ); interleukin 12, (IL-12); ionizing radiation, (IR); Lymphokine activated killer, (LAK); radiotherapy, (RT); retroviral, (RV); Semliki forest virus vector carrying the human interleukin 12 encapsulated in cationic liposomes, (LSFV-IL12); Semliki forest virus vector, (SFV); transferring receptors, (TfR); transferring, (Tf); Transforming growth factor beta, (TGF  $\beta$  2); Tumor infiltrating lymphocytes, (TIL); tumor necrosis factor-related apoptosis-inducing ligand, (TRAIL)

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## Summary

High-grade gliomas are relatively frequent in adults, and consist of the most malignant kind of primary brain tumor. Being resistant to standard treatment modalities such as surgery, radiation, and chemotherapy, it is fatal within 1 to 2 years of onset of symptoms. Although several gene therapy systems proved to be efficient in controlling or eradicating these tumors in animal models, the clinical studies performed so far are not equally successful. Most clinical studies showed that methodologies that increase tumor infection/transduction and, consequently confer more permanent activity against the tumor, would lead to enhanced therapeutic results. Most gliomas are incurable despite improvements in surgical techniques, radiotherapy, and chemotherapy. The therapeutic challenge is partially a result of diffuse tumor infiltration into surrounding normal brain tissue having an intact blood-brain barrier (BBB). Along with the development of novel antineoplastic therapies with improved tumor specificity, innovative ways of delivering these agents to the brain tumor are also under investigation. Although survival in patients with malignant gliomas remains limited, there is renewed optimism with the emergence of novel treatment strategies. Cytotoxic agents such as temozolomide and CPT-11 have shown promising clinical activity. Biological treatments for brain tumors, including antisense oligonucleotides, gene therapy, and angiogenesis inhibitors, are also being evaluated in clinical trials. Delivery strategies have been developed to overcome challenges presented by the blood-brain barrier. These noteworthy treatments, alone or in combination, may ultimately prolong survival and enhance quality of life in this group of patients.

## I. Introduction

Malignant gliomas (glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA)) comprise the most common types of primary central nervous system (CNS) tumors and have a combined incidence of 5-8/100,000 population. The median survival of patients with malignant gliomas treated conservatively is 14 weeks; by surgical resection alone, 20 weeks; by surgery and radiation, 36

weeks; and by the addition of chemotherapy, 40-50 weeks. In spite of the application of multimodal therapies, 94% of glioma patients still die within 24 months after initial diagnosis, this outcome has not improved considerably during the last two decades despite technical advances in neurosurgery, radiotherapy and the evaluation of novel anticancer chemotherapeutic agents (Davis et al, 1998). Numerous experimental therapies based on

augmentation of antitumor host immune response by local and/or systemic application of Lymphokine activated killer cells (LAK), Tumor infiltrating lymphocytes (TIL) and Cytotoxic T lymphocytes (CTL) and various immunostimulants (e.g. cytokines) did not prolong patient survival in the past (Mahaley et al, 1988). Although survival for GBM has not changed significantly over the past three decades, the emergence of novel treatment strategies for these tumors has led to heightened interest and optimism among oncologists. In adults, gliomas are devastating diseases and the best available treatments, such as surgical resection and radiotherapy, have been only temporarily successful. This happens because with the post-resection tumor residues, which are almost always present, it becomes fatal within 1 to 2 years of the first onset of symptoms. The two factors that promote the use of gene therapy for gliomas are the failure and toxicity of conventional therapies, as well as the identification of genetic abnormalities, which contribute to the malignancy

of gliomas. Uncontrolled cellular proliferation, lack of apoptosis, invasion, and angiogenesis are among the biological processes that make these tumors both aggressive and difficult to treat (Phuong et al, 2003).

### A. Genetic therapies in gliomas

During the malignant progression of gliomas, several tumor suppressor genes are inactivated, and numerous growth factors and oncogenes are overexpressed progressively. Consequently, gliomas' gene therapy may aim at molecular interference with 'gain of function' genes (oncogenes) or replacement of 'loss of function' genes (tumor suppressor genes). Such approaches require transgene expression in entire tumor cell populations (if other mechanisms do not come into play), which cannot be achieved with current vector systems. Hence, other strategies have been pursued that may be independent of the genes actually involved in tumor genesis (**Tables 1-4**).

**Table 1.** List of different molecular strategies and the related genes for treating gliomas

Anti-angiogenic factors
Angiostatin, Endostatin, IFN- $\gamma$ , Platelet factor 4 (sPF4), p16 Apoptosis-related genes
p-53, Rb, gas-i, TRAILFas, FADD, NF- $\kappa$ B, gas-i Bcl-2, Bcl-X(L), B. p73 . Bax, Apaf-1 caspase-3, caspase-8 and caspase-9
Prodrug Activation Systems
HSV-tk. Na <sup>+</sup> /I <sup>-</sup> symporter (hNIS) cytochrome P450 2B1 (Cyclophosphamide) Folylpolyglutamyl synthetase
Immunogenes
IL-12, IL-6, IL-4, IL-18, GM-CSF, IFN- $\gamma$ , TNF- $\alpha$ , B7-2, TGF- $\beta$ , LIF and LT
Chemosensitization genes
WAF1/Cip1 Cytosine deaminase/5-fluorocytosine
Radiosensitization genes
IR-responsive Egr1, p53, Cytokines (GM-CSF, IL-4, IL-12)
Other genes
Urokinase-type plasminogen activator, CEAFusogenic Membrane Glycoprotein Linamarase

**Table 2.** List of possible vectors and their modified techniques

Adenovirus
Fiber-mutant (F/K20) adenovirus
Pretreatment with protease
Single-chain antibodies targeting GFAP targeting
Hypoxic tumor targeting Herpes simplex virus-1
Vectors producing cells
Encapsulation
FasL microporous membranes)
Parvovirus
HVJ-liposomes
Semliki forest virus. Measles virus
Epstein-Barr virus, Newcastle disease virus
Mumps virus. Vesicular Stomatitis virus
Influenza virus, Reovirus and Poliovirus

**Table 3.** Various targeted viral and nonviral therapies in brain tumors

Vehicle	Immunogenicity	Advantages	Disadvantages
<ul style="list-style-type: none"> <li>•Nonviral</li> <li>•Polynucleotide</li> <li>•Plasmid</li> <li>•Cellular (pro/eukaryotic)</li> </ul>	Low	<ul style="list-style-type: none"> <li>•No replication or expression in target</li> <li>•Non immunogenic</li> </ul>	Unstable/transient/difficult delivery
<ul style="list-style-type: none"> <li>•Viral non integrating</li> <li>•Adenovirus</li> <li>•Herpes</li> </ul>	High	<ul style="list-style-type: none"> <li>•Large insert capacity.</li> <li>•Clinical experience, relative stability</li> <li>•Efficient transduction of dividing and nondividing cells.</li> </ul>	<ul style="list-style-type: none"> <li>•Toxicity esp. I.M. use</li> <li>•Production capacity limited</li> <li>•Recombination common and sequencing impractical</li> <li>•Persistence limited by immune response</li> </ul>
<ul style="list-style-type: none"> <li>Viral intergrating</li> <li>•Onco retroviral</li> <li>•Lentivirus</li> <li>•AAV</li> </ul>	Low	<ul style="list-style-type: none"> <li>•Moderate insert capacity</li> <li>•Persistence</li> <li>•Transduce dividing and non dividing cells.</li> <li>•Persistence</li> </ul>	<ul style="list-style-type: none"> <li>•Risk of insertional mutagenesis</li> <li>•Safety concerns</li> <li>•No clinical experience</li> <li>•Production capacity limited.</li> </ul>

**Table 4.** Phase trials involving targeted receptor therapies for brain tumors

	Targeted receptor	Phase	Company
EGFR tyrosine kinase inhibitors	PDGFR,	I/II	Novartis, Basel, Switz
	EGFR	I/II	Astra, Wilmington
	BGFR	I	OSI, CA
VbGF tyrosine kinase inhibitors	VBDF/PDGF	I/II	Pfizer
	VEGFR-2	I	Novartis
Farnesyl transferase inhibitors	K-ras	II	Johnson & Johnson
	K-ras,	II	Schering-Plough
Integrin antagonists	Av 3	II	Merck, Germany
	Av 3/5	I	Celegene, NZ
	Bfgf/vegf	II/III	Celegene, NZ
Bndothelin receptor antagonists	ET-A	I/II	Abbott lab
Metalloproteinase inhibitors	MMP-1,2,7,9	III	Schering
Phosphoinositide 3 kinase inhibitors	m-Tor	I/II	Wyeth, PA
Cyclooxygenase 2 inhibitors	Cox-2	I/II	Pfizer, NY
Proteosome inhibitors	26s proteosome		Millenium, Cambridge

Microbial genes (e.g. herpes simplex virus thymidine kinase) may be transferred into the tumors allowing prodrug activation (e.g. ganciclovir). Furthermore, cytokines or other immunomodulatory genes may be used for vaccination purposes, which frequently involves ex vivo transfection of autologous tumor cells with such genes.

Malignant gliomas were chosen for the first clinical study on new gene therapy approaches because these

tumors are non-metastatic and develop on the largely post-mitotic background of normal glial and neuronal tissue. Several molecular strategies have been tested, either in animal models or clinical trials: prodrug activating systems, introduction of tumor suppressor or cell-cycle-related genes, inhibition of growth factors and/or their receptors, inhibition of neovascularization, immunomodulatory maneuvers, oncolytic viruses, inhibition of matrix metalloproteinases, induction of toxic

agents and sensitization of tumors of local expression to chemotherapeutic agents and radiotherapy (Germano et al, 2003).

There are different physical methods for vector delivery to malignant primary brain tumors in experimental or clinical settings: stereotactic or direct intratumoral injection or convection-enhanced bulk-flow interstitial delivery; intrathecal and intraventricular injection; intravascular infusion with or without modification of the blood-tumor-barrier; and direct intratumoral delivery of anti-sense oligonucleotides (Rainov and Kramm, 2001). Critical evaluation of gene transfer and therapy studies has led to the conclusion that even using identical vectors, the anatomical route of the vector can dramatically affect both the efficiency of tumor transduction and its spatial distribution, as well as the extent of intratumoral and intracerebral transgene expression. The safety and efficiency of these therapeutic systems in humans has been confirmed by several controlled pre-clinical and clinical therapeutic trials (Ren et al, 2003).

## B. Genes

Prodrug Activating System HSV-TK Ganciclovir: Tumor cell transduction with the herpes simplex virus thymidine kinase (HSV-TK) gene and treatment with GCV is the most widely studied cancer gene therapy (Nafe et al, 2003). HSV-TK converts the prodrug GCV into a toxic nucleotide analogue, whose incorporation into cellular DNA blocks cell proliferation. Following repetitive ganciclovir (GCV) intraperitoneal or intravenous injection, effective killing of glioma cells in mouse brain is observed. There are several techniques described in the literature for the HSV-TK/GCV therapy with variations depending on the vector utilized, and the concentration of injection solution.

## C. Anti angiogenic factors

Inhibition of angiogenesis has been considered among the most promising approaches to treat highly vascularized solid tumors, such as high-grade gliomas. However, chronic systemic delivery of therapeutic proteins, such as inhibitors of angiogenesis, presents several difficult pharmacological challenges. The concept that targeted anti-angiogenesis, using virally mediated gene transfer, represents a promising strategy for delivering this anti-angiogenic factors (Tanaka et al, 1997).

## D. Angiostatin, Endostatin, and IFN- $\gamma$ (Davis et al, 1998)

Some researchers evaluated the effects of local production of three endogenous inhibitors of angiogenesis (angiostatin, endostatin, and interferon (IFN)- $\gamma$  (Davis et al, 1998)), using a stably transfected rat (9L) and human (GL15) glioblastoma cells, on tumor vascularization and growth in an *in vitro* assay system based on the implantation of tumor cells into organotypic brain slice cultures. Although all the three genes showed angiogenesis inhibitory effect, IFN- $\gamma$  demonstrated the most potent anti-angiogenic effect in organotypic brain

slice cultures. The mechanisms of this anti-tumor effect were most likely caused by the major anti-angiogenic action of the cytokine, because IFN- $\gamma$  (Davis et al, 1998) expression provoked a pronounced decrease in blood vessel density, which was accompanied by extensive necrosis in the tumors' body mass (De Bouard et al, 2003).

## E. p16 gene

Loss of p16 is a frequent event in the progression of malignant gliomas. High-grade gliomas are distinguished from low-grade gliomas by intense angiogenesis in addition to their frequent loss of p16. Infection with a recombinant replication-defective adenovirus vector containing the cDNA of wild-type p16, significantly reduced the expression of vascular endothelial growth factor, which is thought to be a pivotal mediator of tumor angiogenesis, in p16-deleted glioma cells. Restoring wild-type p16 expression into p16-deleted glioma cells markedly inhibited angiogenesis induced by tumor cells *in vivo*. Furthermore, wild-type p16 inhibited neovascularization more potently than did wild-type p53 transfer (Harada et al, 1999).

## II. Apoptosis related genes

p-53 gene: The p53 gene is thought to function abnormally in the majority of malignant gliomas, although it has been demonstrated to be mutated in only approximately 30%. This has led to studies in which adenoviral transduction with wild-type human p53 has been investigated in an attempt to slow tumor cell growth. Some authors demonstrated that multiple gene replacements with simultaneous exposure to adenovirus-containing p53 gene can produce additive effects in the treatment of glioma cell lines (Kim et al, 2002).

## A. Rb gene

Retinoblastoma tumor suppressor gene abnormalities are found in the majority of cancers, including, at least, 30% of malignant gliomas (Fueyo et al, 1998). These findings provide direct evidence that inactivation of the retinoblastoma protein is a critical event in gliomas and suggest that the restoration of wild-type retinoblastoma activity in these tumors through vector delivery gene therapy may have great therapeutic utility.

Other apoptosis-related genes: Some studies suggest that adenoviral vector-mediated delivery of other apoptosis-related genes may also be potentially useful in the gene therapy approach towards the treatment of human brain gliomas. The apoptosis-related genes already studied are: Fas/Fas ligand, caspase-8, p33ING1, p73, Bax, Apaf-1, caspase-9, I- BDN, NF- B, caspase-3, gas-1, Bcl-2, and Bcl-X (L) (Shinoura et al, 2003).

## III. Immunogenes

Cancer immunogene therapy is based on vaccination with radiated, autologous tumor cells transduced with immunostimulatory genes. It has been demonstrated that high-grade gliomas produce immunosuppressive factors, like TGF- $\beta$ , which reduce the anti-tumor response by peripheral blood effector cells. These immunosuppressive

factors could be neutralized to improve anti-tumor response (Ashley et al, 1998).

Vaccination treatment using genetically modified tumor cells to express certain cytokines consists in the following steps: First, glioma cells are cultured primarily from the patients' surgically resected tumor tissues. Afterwards, *in vitro* infection with a recombinant virus vector containing the gene of the cytokine is proceeded, and afterwards, the transduced cells are re-injected in the patient.

Vaccination therapy induces specific activation of cytotoxic T lymphocytes measured by cell-mediated cytotoxicity assay, suggesting the generation of a specific anti-tumor response and the potential for systemic immunity. This immunization results in the regression of the implanted cells, as well as the original brain tumor. Several cytokines have been studied: IL-12, IL-6, IL-4, IL-18, IFN- $\gamma$ , TGF- $\beta$ , TNF- $\alpha$ , GM-CSF, B7-2, TNF- $\beta$ , LIF and LT (Herrlinger et al, 2000).

## IV. Chemosensitizing genes

### A. Cell cycle regulator

WAF1/Cip1: Studies have shown that negative cell cycle regulator WAF1/Cip1 is often overexpressed in human gliomas and that WAF1/Cip1's overexpression makes glioma cells resistant to chemotherapy agents. These results show that the attenuation of WAF1/Cip1 expression initiated glioma cell death and sensitized glioma cells to apoptosis induced by 1,3-bis(2-chloroethyl)-1-nitrosourea and cisplatin. Thus, blocking WAF1/Cip1 production may serve as a useful chemosensitization regimen for treating glioma (Yamanaka et al, 2002a).

### B. Cytosine deaminase/5-fluorocytosine

Adenovirus (Ad) vector-mediated cytosine deaminase (CD)/5-fluorocytosine (5-FC) gene therapy has been proposed as a potential technique to overcome pharmacokinetic issues associated with systemic 5-FU and is particularly well suited to use with tumors in which local control is paramount, such as malignant gliomas. The bacterial enzyme CD catalyzes the conversion of 5-FC to the lethal 5-fluorouracil (5-FU). Cloning the CD gene from *Escherichia coli* and expression in human tumor cell lines enabled these cells to convert 3H-labeled 5-FC into 3H-5-FU.

Glioblastoma cell line T1115 became 200-fold more sensitive to 5-FC than the non-expressing parental cell lines. At least 90% of the cells are killed within 7 days. CD-expressing cells are able to kill non-expressing cells when grown in the same culture flask (bystander effect). The results of clinical studies in human patients with high-grade gliomas confirm the previous findings in rat models, demonstrating the potential clinical utility of Ad 5-FC gene therapy for gliomas. (Yamanaka et al, 2002a)

## V. Radiosensitizing genes

*Bax gene* The hypothesis that Ad-mediated transfection of proapoptotic Bax gene could enhance the cytotoxicity of radiotherapy (RT) in RT-refractory glioma

cells has been proposed. The results of *in vivo* experiments showed that apoptotic death may be enhanced by the combination of the treatment with Ad-containing Bax gene (Ad-Bax) and RT. Ad/Bax synergistically radiosensitizes glioma, with a seemingly favorable therapeutic index (Yamanaka et al, 2002b).

### A. Radiation-responsive gene promoters

Synthetic gene promoters, responsive to clinical doses of ionizing radiation (IR), have been developed for use in suicide gene therapy vectors. The crucial DNA sequences utilized are units with the consensus motif CC(A/T)(6)GG, known as CarG elements, derived from the IR-responsive *Egr1* gene. These elements had their sequences incorporated into a synthetic gene promoter and assayed for the ability to induce expression of a downstream reporter gene following irradiation. Exposure of cells to ionizing radiation resulted in the activation of these specific transcriptional control elements within the early growth response 1 (*Egr1*) gene promoter, leading to increased gene expression. Studies revealed that increasing the number of CarG elements up to a certain level, increases promoter radiation-response; specific alteration of the core A/T sequences caused an even greater positive response. (Inoue et al, 2004). These enhancers can be used to drive suicide gene expression from vectors delivered to a tumor within an irradiated field. These results demonstrate that the synthetic promoter is responsive to low doses of ionizing radiation, and therefore, isolated CarG elements function as radiation-mediated transcriptional enhancers outside their normal sequence context.

### B. p53 gene

Adenoviral vector-mediated expression of human wild-type p53 not only slows tumor cell growth but also enhances the radiosensitivity of malignant glioma cells that express native wild-type p53. RT2 tumor cells express native rat wild-type p53 before the transduction, and markedly overexpress human p53 following adenoviral p53 transduction. The combination of p53 transduction followed by radiation results in marked decreases of RT2 cell survival and increases in apoptosis at radiation doses from 2 to 6 Gy. The results support a new perspective in the p53 genetic therapy, showing the ability to enhance the radiosensitivity of malignant glioma cells that express wild-type p53 by using adenoviral transduction to induce overexpression of p53 and offer new hope for the p53 viral-mediated genetic therapy as a successful therapeutic strategy, not only in human gliomas that express mutant p53, but also in those that express wild-type p53 (Ruan et al, 1999).

### C. Cytokines

Some cytokine vaccination (GM-CSF, IL-4, IL-12) therapies have not only primary immunity generation against the tumor but also an important radiosensitization effect. In some studies with tumors treated with vaccination therapy and posteriorly, irradiation, about 80-100% of the glioma-bearing mice were cured.

## D. Other genes

Other genes which have been evaluated as candidates for genetic therapy in gliomas are: folypolyglutamyl synthetase gene, growth arrest-specific genes (gas1), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), cell cycle regulator WAF1/Cip1 gene, cytosine deaminase/5-fluorocytosine gene, Bax gene, carcinoembryonic antigen (CEA) gene, urokinase-type plasminogen activator gene and fusogenic membrane glycoprotein gene (Chiocca et al, 2003).

## VI. Vectors

Viruses (Table 2) have emerged on the genetic therapy scene and gained attention due to their ability to play essentially two roles: first, as vectors for therapeutic gene delivery and second, as engineered infectious agents capable of selectively lysing tumor cells. Oncolytic viruses have shown promising results in solid tumor treatment, including gliomas, but their potency must be improved if their full clinical potential is to be realized (Fu et al, 2003).

### A. Adenovirus

The local, intratumoral injection of adenovirus is an especially suitable strategy for gliomas because these tumors, although infiltrative, rarely metastasize. Two approaches have been used to generate tumor-selective replicative adenoviruses: use of tumor-specific promoters to regulate the expression of viral genes, and the deletion of the viral functions required for the cell cycle activation. Since normal cells surrounding gliomas are quiescent, the second strategy is particularly attractive to develop new treatments for brain tumors. Trials have showed that adenoviruses are more efficient than retroviruses in achieving *in vivo* gene transfer (Puumalainen et al, 1998).

### B. Targeted adenovirus

The application of adenoviral vectors in cancer gene therapy is hampered by low receptor expression on tumor cells and high receptor expression on normal epithelial cells. Targeted techniques with adenoviral vectors seem to be a promising tool for cancer gene therapy; they could provide an improved therapeutic index with efficient tumor transduction and effective protection of normal tissue.

### C. Single-chain antibodies targeting

Some authors proposed specific tumoral targeting by the use of doubly ablated adenoviral vectors, lacking coxsackie virus-adenovirus receptor and (v) integrin binding capacities, together with bispecific single-chain antibodies targeted toward human epidermal growth factor receptor (EGFR) or the epithelial cell adhesion molecule. These vectors efficiently and selectively targeted both alternative receptors on the surface of human cancer cells (Kuriyama et al, 2000).

### 1. GFAP targeting

In an attempt to limit the toxic effects on normal tissues, a recombinant adenoviral vector has been

constructed, in which the HSV-TK gene is driven by a 2.2 kb DNA promoter which controls expression for the encoding glial fibrillary acidic protein gene (GFAP), an intermediate filament protein expressed primarily in astrocytes (Martinet et al, 2003).

Hypoxic Tumor Targeting: New therapy targeting the hypoxic fraction of tumors is very useful as this population of cells is the most resistant to radio- and chemotherapies. Hypoxia-inducible factor (HIF) mediates transcriptional responses to hypoxia by binding to hypoxia-responsive elements (HRE) in target genes. Although this approach needs more experimental studies, the results suggest that it could be used to treat solid tumors that develop hypoxia, including the category of more malignant gliomas (Dupont et al, 2000).

### 2. Herpes Simplex Virus-1

The HSV-1 vectors are particularly useful because they can be genetically engineered to replicate and spread highly selectively in dividing tumor cells and can also express multiple foreign transgenes. If the viruses are directly injected into the brain, they might not be inactivated. The HSV-1 vectors have been recently utilized as oncolytic vectors instead of replication-defective vectors. The oncolytic HSV-1 have demonstrated cytopathic effect in rat's glioma models without damaging normal tissues, providing amplified gene delivery within the tumor, and inducing specific anti-tumor immunity. Different approaches are currently undertaken to improve the efficacy of oncolytic HSV-1 therapy which include: development of new generation vectors via further genetic engineering of existing safe vectors, combination with immune gene therapy, and combination with conventional therapies (Visted et al, 2000).

### 3. Other viruses

Other viral vectors, like parvovirus, hemagglutinating virus of Japan, Semliki forest virus, Measles virus, Epstein-Barr virus, Newcastle disease virus, Mumps virus, Vesicular Stomatitis virus, Influenza virus, Reovirus and Poliovirus have been tested in genetic therapy experiments *in vivo* and *in vitro* (Shah et al, 2003). However, they lack more investigation of their specific role in glioma therapy.

### 4. Clinical trials

In malignant glioma, standard gene therapy approaches employing non-replicating virus vectors failed to demonstrate significant benefit in clinical studies. Therapy with oncolytic viruses seems to hold more promise in early clinical trials than gene therapy with non-replicating virus vectors (Nestler et al, 2004).

The most studied candidates for gene therapy, which are in advanced stages of clinical trials include: the prodrug activating system HSVtk/GCV, utilizing either retrovirus vector producer cells or adenovirus vectors; the adenovirus-mediated p53 gene transfer; the adenovirus-mediated IFN- $\gamma$  gene transfer and studies with oncolytic therapy with herpes virus or adenovirus vectors. The other

vectors and genes previously discussed are still in cell or animal protocols investigation stage (Thomas et al, 2000).

There is an ongoing Phase I/II clinical study in adult patients with recurrent GBM which is aimed at evaluating biological safety, maximum tolerated dose, and anti-tumor efficacy of a cytokine vaccination model, using a genetically modified replication-disabled Semliki forest virus vector (SFV) carrying the human interleukin 12 (IL-12) gene and encapsulated in cationic liposomes (LSFV-IL12) (Sandmair et al, 2000). Several other Phase I and II clinical studies in patients with recurrent malignant glioma have shown a favorable safety profile and some efficacy of retroviruses (RV)-mediated gene therapy. More than 300 patients with glioma have already been treated in clinical trials with oncolytic viruses, and in most cases, the virus was administered directly into the tumor.

On the other hand, a prospective randomized Phase III clinical study of retroviral (RV) gene therapy in primary malignant glioma failed to demonstrate significant extension of the progression-free or overall survival times in RV-treated patients. (Puumalainen et al, 1998). The failure of this RV gene therapy study may be due to the low tumor cell transduction rate observed *in vivo*. Biological effects of the treatment may heavily depend on the choice of transgene/prodrug system and on the vector delivery methods.

RV clinical trials in malignant glioma have, nevertheless, produced a substantial amount of data and have contributed towards the identification of serious shortcomings of the non-replicating virus vector gene therapy strategy. New types of therapeutic virus vector systems are currently being designed, and new clinical protocols are being created based on the lessons learned from the RV gene therapy trials in patients with malignant brain tumors.

The long-term consequences of adenovirus-mediated conditional cytotoxic gene therapy for gliomas remain uncharacterized. Some studies reported detection of active brain inflammation 3 months after successful inhibition of syngeneic glioma growth. The inflammatory infiltrate consisted of activated macrophages/microglia and astrocytes, and T lymphocytes positive for leucosyalin, CD3 and CD8, and included secondary demyelination. (Mattei et al, 2005).

#### **IV. Targeted toxin therapies and drug delivery systems/convection enhanced delivery**

Most gliomas are incurable despite improvements in surgical techniques, radiotherapy, and chemotherapy. The therapeutic challenge is partially a result of diffuse tumor infiltration into surrounding brain tissue having an intact BBB. Along with the development of novel antineoplastic therapies with improved tumor specificity, innovative ways of delivering these agents to the brain tumor are also under investigation. Cotara (Peregrine Pharmaceuticals, Inc., Tustin, CA) is a <sup>131</sup>I-labeled chimeric monoclonal antibody (<sup>131</sup>I-chTNT-1/B Mab) specific for a universal intracellular antigen (i.e., histone H1 complexed to deoxyribonucleic acid) exposed in the necrotic core of

malignant solid tumors. This antigen provides an abundant, insoluble, nondiffusible anchor for the Mab. Once localized to necrotic regions of the tumor, Cotara delivers a cytotoxic dose of <sup>131</sup>I radiation to the adjacent living tumor cells. The intact BBB may not allow passage of Cotara, a high-molecular-mass protein (M<sub>r</sub> 150–170 kD), from the vascular compartment into the interstitium of tumor-infiltrated brain. Convection-enhanced delivery (CED) (U.S. Patent No. 5,720,720) provides one method of bypassing the BBB for regional delivery of large macromolecules, such as Cotara, into the interstitium of the brain tumor and infiltrated brain. CED was originally used as a tool for delivering other therapeutic modalities, specifically gene therapies. The technique was first used clinically to deliver a ransferring receptor-based diphtheria toxin, Tf-CRM-107, into recurrent primary and metastatic brain tumors in a Phase 1 study. Tf-CRM-107 was subsequently used via CED in a Phase 2 multicenter trial, the results of which have been presented in a preliminary report. Forty-four patients with recurrent or progressive anaplastic astrocytoma or glioblastoma multiforme were treated, and responses were seen in 21 patients. Eight of the 44 patients had symptomatic progressive cerebral edema, which was responsive to medical treatment. New-onset seizures occurred in 3 of the 44 patients treated. CED has similarly been used to deliver interleukin-4-Pseudomonas exotoxin chimeric fusion protein. A limited number of patients have been treated thus far. The dose-limiting toxicity is cerebral edema, which is treatable with medical or surgical methods, but the treatment protocol is still undergoing modification. Other CED-deliverable gene therapies under clinical trial include transforming growth factor-( ) and Pseudomonas exotoxin and interleukin-13 receptor-directed cytotoxin. CED uses a motor-driven pumping device to drive the flow of an infusate through a catheter tip that is stereotactically placed at the target site within the brain. The resulting pressure gradient drives the fluid through the interstitial space. Experimental studies show that CED can achieve a local drug concentration 10,000-fold greater than that achieved by intravenous drug administration without causing significant systemic exposure. The infused drug permeates the targeted region at a final concentration governed by such variables as infusion parameters, the flow resistance or hydraulic conductivity of the tissue, and the duration of treatment. CED can therefore effectively bypass the blocking effects of the BBB and deliver antitumoral compounds and agents to specific locations. Limited clinical studies using CED have been reported in humans. Pilot studies using this technique have been used to deliver tumor-targeting immunotoxin conjugates (e.g., TF-CRM107 and IL4-Pseudomonas exotoxin (NBI-3001)), chemotherapeutic agents (e.g., paclitaxel), and anti glioblastoma gene therapy (e.g., HSV-1-tk) to cancer patients (Patel Sunil et al, 2005).

Transforming growth factor (TGF 2) in tumor progression and immunosuppression of malignant glioma: Patients suffering from malignant glioma show a profound state of cellular immunodeficiency. The most important cause seems to be an increased release of the subtype TGF- by the glioma cells. Kjellman suggested that the

TGF-2 is specifically important in the later stages of malignancy, while TGF-1 and TGF-3 may be important during the earliest stages of tumor development (Kjellman et al, 2000). TGF-1 and TGF-2 were shown to have a negative growth regulating effect in low grade, near diploid gliomas. On the other hand, the majority of high grade tumors are either unresponsive or growth stimulated. Two additional pathomechanisms, in which tumor derived TGF-2 plays a role, may be associated with poor clinical prognosis (de Visser and Kast, 1999). (Figure 1) These include: increased production of extracellular matrices supporting invasive growth and infiltration of non affected tissue, and neovascularization, meaning denovo production of new blood vessels supplying tumor tissue. A knowledge of these 4 pathomechanisms of malignant glioma progression results in therapeutic strategies that counteract TGF-2 activities. A novel treatment approach has been developed based on specific inhibition of TGF-2 synthesis by antisense phosphorothioate oligonucleotides (S-ODN). Tumor cells are known to be an important source of TGF- production there is evidence that TGF- is an important promoter of malignant cell growth. Tumor growth in glioma seems to be due an increased release of TGF- 2 by the glioma tumor cells. TGF- 2 is the most potent immunosuppressant known.

TGF- 2 production may represent a significant tumor escape mechanism from host immunosurveillance. AP 12009 is a synthetic 18-mer antisense oligonucleotide targeted against TGF- (short strings of DNA/RNA down regulate gene expression by interfering with translation of encoded protein at mRNA level. A multinational multicentric open label, active controlled, randomized parallel group dose finding study –phase IIb study of which the senior author is one of the principal investigators) to evaluate the efficacy and safety of two

doses of antisense protein in adult patients with recurrent high grade glioma, administered intratumorally as continuous high flow microperfusion over a 7 day period every other week for 6 months, is currently carried out in 6 different countries. The preliminary results are quiet encouraging though final results are still awaited.

The poor prognosis of central nervous system malignancy is in part related to a lack of potent agents with adequate tumor specificity. Targeting to specific cell receptors provides the possibility of creating novel therapeutic agents with greater tumor specificity than conventional chemotherapy. Monoclonal antibodies against tumor associated antigens and other binding moieties, which provide tumor selectivity, have been conjugated with radionuclides and with various toxins. Investigators at National Institute Of Health (NIH) studied a targeted protein toxin, which uses the physiological binding of human transferrin (Tf) to transferrin receptors (TfR) expressed on metabolically active cells to achieve tumor specificity (Greenfield et al, 1987). This targeted protein toxin is transferrin-CRM-107, a conjugate of human Tf and diphtheria toxin with a point mutation which inactivates the nonspecific binding to mammalian cells. Another phase III multicentre study of intratumoral/interstitial therapy with Transmid (a conjugate of modified diphtheria toxin (CRM107) and human Tf joined by a stable, nonreducible thioether bond) compared to best standard of care in patients with progressive and/or recurrent, nonresectable glioblastoma multiforme patients is being carried out, the results are still awaited. TfRs transport iron into cells and are over expressed on rapidly dividing cells most notably on hematopoietic cells and various tumor cells, including glioblastoma cells. Studies have demonstrated higher expression of these receptors on gliolastoma and

## BLOCK OF PROTEIN EXPRESSION BY ANTISENSE

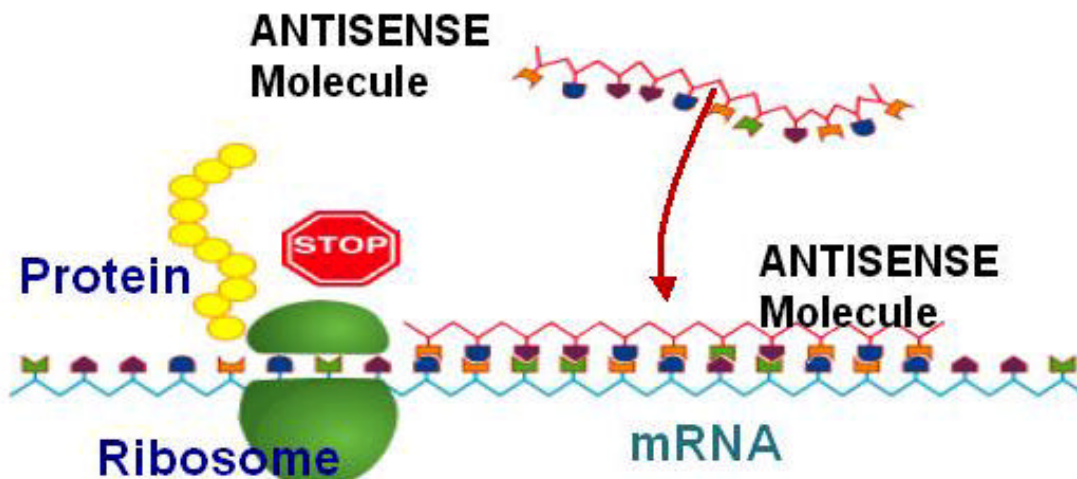


Figure 1. Schematic picture showing block of protein expression by antisense molecule.

medulloblastoma tumor cells lines in comparison to human erythroleukemia cell line K 562. In contrast to this, TFRs in normal brain tissue are sparse and are largely restricted to the luminal surface of brain capillaries. TranMID is being developed as a potential treatment for malignant brain tumors. A phase II clinical study of intratumoral infusions of TransMID in 44 patients with refractory and progressive GBM or Anaplastic Astrocytoma has been conducted in USA (drug delivered continuously over a period of 5-7 days via two catheters implanted in the tumors, given as two separate treatments between 4-10 weeks apart and good response was noted in 48% of patients (complete response in 11%, partial response in 16%, 21% had stable disease), it was concluded from these studies that the benefits of treatment of recurrent brain tumors with TransMID exceeds the risks and phase III trial is currently on its way for this drug (Laske and Rossi, 2002).

## V. Conclusions

Malignant gliomas remain a poorly understood form of cancer associated with high rates of morbidity and mortality. New treatment strategies are emerging that target steps in the molecular pathogenesis of these tumors. Antiangiogenesis agents, antisense oligonucleotides, and signal transduction inhibitors are all examples of such therapies now entering clinical trials. Future treatment strategies for malignant gliomas will likely involve synergistic combinations of agents aimed at different pathways in the molecular pathogenesis of this type of cancer. Major steps to improve gene transfer into the central nervous system and the efficacy of gene therapy for malignant brain tumors include: 1) the design of more effective vector systems; 2) the development of new or improved prodrug/suicide systems, gene replacement approaches, or strategies targeting the immune response or tumor angiogenesis; 3) the study of new techniques to enhance delivery of genetic vectors into brain tumors and for monitoring gene delivery into tumors. Further major advancements in virus designs, application modalities, and understanding of the interactions of the host's immune system with the virus, are clearly needed before oncolytic virus therapy of malignant brain tumors can be introduced to clinical practice. Finally, strategies to circumvent the BBB (polymers, bradykinin analogues, gene therapy) are important advances that have also shown efficacy in early clinical trials. With the present results, it is clear that gene therapy strategies for gliomas are quite promising but more critical research is required, mainly in the vector field. The ultimate molecular therapy will probably involve the application of multiple simultaneous (combinatorial) therapeutic modalities. The pace and breadth of discovery in molecular biology promise a steady supply of novel agents as well as refinements of existing ones. One of the important challenges for the future is the development and implementation of sound clinical research methods that will enable investigators to identify active treatment regimens.

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