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Gene- and Viral-Based Therapies for Brain Tumors

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Summary: Advances in understanding and controlling genes and their expression have set the stage to alter genetic material to fight or prevent disease with brain tumors being among one of the first human malignancies to be targeted by gene therapy. All proteins are coded for by DNA and most neoplastic diseases ultimately result from the expression or lack thereof with one or more proteins (e.g., coded by oncogenes or tumor suppressor genes, respectively). In theory, therefore, diseases could be treated by expression of the appropriate protein in the affected cells. Gene therapy is an experimental treatment that involves introducing genetic material (DNA or RNA) into cells, and it has made important advances in the past decade. Within this short time span, it has moved from the conceptual laboratory research stage to clinical translational trials for brain tumors. The most efficient approaches for gene delivery are based on viral vectors, which have been proven relatively safe in the CNS, despite occasional cases of morbidity and death in non-neurosurgical trials. However, the human response to various viral vectors can not be predicted in a reliable manner from animal experimentation, nor can size, consistency, and extent of experimental brain tumors in mouse models reflect the large, necrotic, infiltrative nature of malignant gliomas. Furthermore, the problem of delivering genetic vectors into solid brain tumors and the efficiency in situ gene transfer remains one of the most significant hurdles in gene therapy. Key Words: Glioma, gene therapy, oncolytic virus, clinical trial.

INTRODUCTION

Two crucial considerations in gene therapy relate to: 1) what gene should be delivered for expression, and 2) how to deliver it. In its simplest form, gene therapy is the process by which either defective or missing genes are replaced or new functions to the host’s cells are introduced. For this purpose, the genetic material is coupled to additional regulatory sequences (e.g., promoters and enhancers) and is packaged inside a gene delivery vehicle to enable transfer and expression of the intended gene product inside the cell (FIG. 1).

Although conceptually straightforward, the efficient expression of foreign genes is the most critical aspect for the success of in vivo gene therapy. The first step of gene therapy involves gene delivery to facilitate the expression of the therapeutic gene in the interior of a cell. The simplest method is the direct introduction of therapeutic DNA into target cells by physical (i.e., electroporation) or chemical techniques (i.e., lipofection).1 This approach still remains limited in its application, because it is relatively inefficient, it can be used only with certain tissues and requires large amounts of DNA. Furthermore, among the several barriers to successful gene delivery, foreign genes and/or the vectors used to deliver them can trigger a range of immune responses. However, sometimes, these immune responses have been harnessed behind the concept of using gene therapy as a vaccine (i.e., DNA vaccines).2

The next difficulty for the foreign genetic material is that once within the cell, it must escape intracellular degradation to enter the nucleus to be expressed (FIG. 1).3,4 Therefore, gene delivery systems (vectors) were designed to protect the genetic material. An ideal vector needs to meet three criteria: 1) it should protect the transgene against degradation by nucleases in the extracellular matrix, 2) it should bring the transgene across the plasma membrane and into the nucleus of target cells, and 3) it should have no detrimental effects. Currently, such vectors of gene transfer can be classified into two categories: 1) viral and 2) nonviral.5,6

GENE THERAPY APPROACHES FOR GLIOMAS: FIVE TYPES OF DELIVERY

Five glioma gene therapy approaches are currently being explored: 1) pro-drug activating gene therapy (also...
called suicide gene therapy, chemotherapy-sensitizing gene therapy, gene-directed enzyme pro-drug therapy; 2) transfer of tumor suppressor genes and cell cycle modulators; 3) genetic immune modulation; 4) anti-angiogenic gene therapy; and 5) oncolytic viruses (OVs).

1. Pro-drug activating

The pro-drug-activating approach is the most commonly used technique in clinical trials for brain tumors. It involves transducing tumor cells with a gene encoding an enzyme that can metabolize a nontoxic pro-drug to its toxic form.

**Herpes simplex virus type 1 (HSV-1) thymidine kinase.** The HSV-1 thymidine kinase (HSVtk) is an enzyme that metabolizes nontoxic nucleoside analogs, such as ganciclovir (GCV), acyclovir, or valacyclovir (VCV), into a cytotoxic molecule. The GCV metabolite is incorporated into DNA, causing DNA elongation to terminate and subsequently cause cell death. Because the effects of GCV are limited to DNA, it primarily targets the mitotic cells, much like the S phase-specific chemotherapeutic agents, but it also affects the ability of DNA to be repaired by DNA polymerases, and thus it theoretically could also affect quiescent cells with damaged DNA. *In vivo* efficacy was demonstrated in multiple animal studies.8–10 Furthermore, preclinical experiments demonstrated marked tumor elimination, despite gene transfer into only a small fraction of tumor cells.11 This cytotoxic effect of transduced cells on adjacent nontransduced cells is termed the bystander effect.12 The bystander effect is mediated mainly by the transfer of toxic phosphorylated forms of GCV to nontransduced cells, presumably via gap junctions.13 Another presumed mechanism contributing to the bystander effect is the targeting of mitotically active endothelial cells in tumor vessels.14 An immune-associated response against a nonhuman protein, such as HSVtk, leading to diffuse cell death that affects neighboring nontransduced cells has also been suggested.15 In addition to the bystander effect, tumor cells transduced to express HSVtk and treated with the antiviral agent acyclovir display enhanced sensitivity to radiation in culture and *in vivo*.16 Possible explanations for radiation enhancement could be that DNA that has incorporated acyclovir may be susceptible to radiation-induced strand breakage, and/or acyclovir might sensitize cells by inhibiting polymerase activity required for the repair of radiation-induced DNA damage.17 The HSVtk gene therapy approach was tested in human glioma trials (phase III) using RV vectors, and more recently adenovirus (AV) vectors. In fact, a phase III trial in Europe and Israel was recently completed under sponsorship by a British biotechnology venture (Ark Therapeutics) in which an AV-HSVtk vector and GCV were provided to patients with newly diagnosed glioblastomas (GBMs) before application of standard therapy (radiation and/or temozolomide). Interim results from this trial are available on the company’s website at www.arktherapeutics.com/main/products.php?content=products_cerepro. To take advantage of the possible synergy between radiation-induced damage of DNA and the inability of DNA with incorporated VCV metabolites to repair itself, we have recently completed a phase I clinical trial for patients with newly diagnosed malignant gliomas, in which AV-HSVtk and VCV is used in conjunction with radiation (Chiocca et al., unpublished).

**Cytosine deaminase/5-Fluorocytosine.** 5-Fluorocytosine (5-FC) is an agent used to treat fungal infections (such as *Candida albicans*). 5-FC is a pro-drug converted into the active agent 5-fluorouracil (5-FU) by cytosine deaminase (CD), which is uniquely expressed in certain fungi and bacteria. While 5-FU is nontoxic to human cells because of the lack of CD, 5-FU is used to treat cancers like colon, pancreatic, and breast cancer. The toxic effects of 5-FU are mediated by its intracellular metabolites, which cause DNA strand breakage leading to cell death.18 Two preclinical studies for glioblastomas using an adenoviral vector carrying the CD gene demonstrated promising results.19 However, no clinical trial for brain tumors has used this gene transfer strategy so far.

**Cyclophosphamide.** Cyclophosphamide (CPA) is a pro-drug that is activated by liver-specific enzymes of the cyclophosphamide P450 family. The active form of CPA (i.e., phosphoramid mustard) is an alkylating agent that generates DNA cross links and consecutive DNA strand breaks. The efficacy of CPA in treating brain tumors has been limited by the fact that (although CPA crosses the blood-brain barrier) its active metabolites are poorly transported across the blood-brain barrier.20 The rat cyclophosphamide P450 2B1 (CYP2B1) activates CPA with high efficiency,21 and gene therapy
using CYP2B1 to activate CPA was designed primarily for use in brain tumors, because other malignancies have ready access to the active metabolites of CPAs. The implantation of CYP2B1 expressing RV vectors was shown to induce regression gliomas in animal studies.22,23

Replacement of parts of the HSV-1 genome with the CYP2B1 gene has led to the design of an HSV-1 vector (rRp450) that can kill tumor cells through three modes: 1) using viral oncolysis, 2) rendering the infected cell sensitive to CPA, and 3) rendering the infected cell sensitive to GCV. Animal studies using subcutaneous tumors established from glioma cell lines in immunodeficient mice showed tumor regression only when the animals were treated with rRp450, CPA, and GCV.18 Currently, no P450/CPA clinical trials for brain tumors have been conducted.

2. Transfer of tumor suppressor genes and cell-cycle modulators

Advances in understanding the underlying molecular and genetic mechanisms of gliomas provide a rational framework for the development of new treatments.24 The p53 tumor suppressor protein regulates cell-cycle progression and apoptosis in response to many external insults (e.g., DNA damage and oncogenic mutations).25 Mutation in the p53 gene (TP53) resulting in loss of its function are common in astrocytomas, and are also associated with tumor progression from low-grade astrocytoma to glioblastoma.26 Accordingly, the TP53 gene became an attractive candidate for gene transfer in an attempt to restore cell cycle regulation in TP53-mutated cells and induce apoptosis, even in tumors with intact functional genes, by causing enhanced expression of the gene product.27,28 Another commonly affected cell-cycle pathway in gliomas is the retinoblastoma protein/cyclin-dependent kinases/cyclin-dependent kinase inhibitors (CDKN) circuit.29,30 Preliminary studies restoring the genomic region of CDKN2A/CDKN2B in glioblastoma significantly glioma inhibition by using a RV vector to deliver the gene for vascular endothelial growth factor receptor, which forms inactive dimers with wild-type vascular endothelial growth factor receptor.45 In addition to being one of the gene replacement strategies described earlier, p53 might also have an anti-angiogenic effect due to the discovery that inducing wild-type p53 expression in astrocytomas causes release of an angiogenesis inhibitory factor.46 The intense neovascularization in malignant gliomas may enable a new intravascular modality of gene therapy for this disease. Although genetic vectors administered intravascularly are unlikely to penetrate the blood-brain barrier, intravascular delivery of vectors may target endothelial cells in the brain. So far, no trials have been conducted using this approach in gliomas.

3. Genetic immune modulation

Gene therapy approaches using genetic immune modulation enhance the immune response against tumors by expressing cytokines and lymphokines. The frequently used cytokines to achieve genetic immune modulation of tumors are interleukin (IL)-2,34,35 IL-4,36 interleukin-12,37 interferon (IF)-β,38 IF-γ,39 and granulocyte-macrophage colony stimulating factor.40 Several studies have been performed by infecting tumor cells ex vivo with cytokine genes, arresting cell growth by irradiation, and then reimplanting the cells to sustain paracrine secretion of cytokines within the tumor.34,41 Another model introduces RV producer cells carrying immune modulating genes into the tumor so infection occurs in situ.30 Several phase I trials are currently underway using this strategy. However, for IL-2 and IFN-γ severe CNS toxicity has been reported when these cytokines were secreted by tumor cells intracranially.42 One clinical trial in humans with recurrent gliomas has been conducted where an AV vector was employed to deliver the gene for human IF-β.43 Gene therapy can also be used to generate tumor vaccines by inducing tumor antigen presentation by antigen-presenting cells. Antigen-presenting cells (i.e., dendritic cells) can be harvested from peripheral blood or brain tumor biopsy specimens, which are transduced with the DNA, mRNA, and/or proteins coding for the tumor antigen(s), and then expanded in vitro before administration to the patient. Clinical trials using gene-based approaches for GBM are currently in process.

4. Anti-angiogenic gene therapy

Neovascularization is a feature of malignant gliomas and is dependent on several potent angiogenic factors secreted by tumor cells. Vascular endothelial growth factor is an important overexpressed angiogenic factor in gliomas. It has been shown that in vivo transfer of a recombinant AV vector carrying the gene for vascular endothelial growth factor in an antisense orientation into gliomas inhibits tumor growth.44 Antisense oligonucleotides are single strands of DNA or RNA that can bind to a complementary RNA sequence. If binding takes place, this DNA/RNA hybrid can be degraded, subsequently preventing protein synthesis. Another study showed significant glioma inhibition by using a RV vector to deliver a signaling-defective vascular endothelial growth factor receptor, which forms inactive dimers with wild-type vascular endothelial growth factor receptor.45 In addition to being one of the gene replacement strategies described earlier, p53 might also have an anti-angiogenic effect due to the discovery that inducing wild-type p53 expression in astrocytomas causes release of an angiogenesis inhibitory factor.46 The intense neovascularization in malignant gliomas may enable a new intravascular modality of gene therapy for this disease. Although genetic vectors administered intravascularly are unlikely to penetrate the blood-brain barrier, intravascular delivery of vectors may target endothelial cells in the brain. So far, no trials have been conducted using this approach in gliomas.

5. Oncolytic viruses and gene delivery vectors

There are several methods for nonviral gene transfer. However, these methods have been relatively inefficient in their capacity to transfer genes to a sufficiently elevated number of cells, especially for in vivo gene delivery into brain tumors. So far, the most effective way to transfer DNA into somatic cells remains the use of a viral-based delivery system. Vectors for brain tumor therapy can be divided
into two categories: 1) replication-defective vectors, which we will name as a vector from here on, and 2) replication-competent (ie, replication-conditional and oncolytic viruses), which we will name as oncolytic viruses (OVs) from here on. In the first instance, the vector is derived from a virus from which all or most of the viral genes have been removed to minimize virus-mediated toxicity. In the second instance, selected viral genes are deleted or mutated so that viral targeting and/or replication can occur selectively in tumor versus endogenous neural cells. So far, the replication-defective vectors used in gene therapy trials of brain tumors have been based on retrovirus and AV. In terms of replication-competent (oncolytic) viruses, the ones used in clinical trials of brain tumors have been HSV, AV, reovirus and Newcastle disease virus (Table 1). However, experimentally, almost any type of virus has been used either in a replication defective or replication-competent fashion.

**Retroviruses.** Retroviruses (RVs) are a class of enveloped viruses containing a single-stranded RNA mole-

| Vector or OV                     | Advantages                                                                                   | Disadvantages                                                   |
|----------------------------------|-----------------------------------------------------------------------------------------------|-----------------------------------------------------------------|
| Retrovirus (Vector)              | • Low immunogenicity                                                                         | • Insertional mutagenesis                                       |
|                                  | • Persistent gene expression                                                                  | • Infects dividing cells only                                    |
|                                  | • Specific for mitotic cells                                                                  |                                                                |
| Adenovirus (Vector)              | • Infects dividing and nondividing cells                                                     | • Short-term gene expression                                    |
|                                  | • Nonintegrating                                                                             | • Very immunogenic                                              |
|                                  | • Widely available experience in its use, production, and widely developed by biotechnology  |                                                                |
|                                  | industry                                                                                    |                                                                |
| Adenovirus (OV)                  | • Safe in human brain                                                                        | • Low transgene capacity                                        |
|                                  | • Mutants that target p16/RB, p53 and other tumor suppressor pathways have been engineered    | • Cell lysis is slow                                             |
|                                  | • Large experience in generation of clinical product                                          | • High number of viral particles to cell are needed for efficient |
|                                  | • Most widely developed by biotechnology industry                                            | replication                                                   |
|                                  | • One mutant has been approved for use in human head and neck cancer by the FDA-equivalent   | • Relatively low number of viral progeny produced per cell     |
|                                  | of China                                                                                    | • Immunity to the virus is widely present in the human population|
|                                  | • Targeted mutants have been developed to receptors present in human tumor cells             |                                                                |
|                                  | • The function of all of its viral genes is known                                             |                                                                |
| Herpes simplex virus (OV)        | • Safe in human brain                                                                        | • Very immunogenic                                              |
|                                  | • Mutants that target p16/RB or MEK have been used                                            | • Immunity to the virus is widely present in human population   |
|                                  | • High transgene capacity to allow for combination of OV-based and gene-therapy based therapy| • Little experience in production for clinical trials           |
|                                  | • Rapid cell lysis                                                                           | • Function of many viral genes is still unknown                 |
|                                  | • Low number of viral particles per cell are required for efficient infection/replication     | • Relatively low number of viral progeny produced per cell     |
|                                  | • Effective drugs are available to limit undesirable viral infection                          | • Immunity is widely present in the human population           |
| Reovirus (OV)                    | • Safe in human brain                                                                        | • Experience and use for clinical trials confined to one        |
|                                  | • Mutant strain targets cells that overexpress the oncogene ras                               | biotechnology company                                          |
|                                  | • High number of viral progeny produced per cell                                             | • RNA virus with high rate of mutation of viral genome          |
|                                  | • Function of all viral genes is known                                                       | • Avian virus                                                  |
| Newcastle disease virus (OV)     | • Safe when administered intravenously for human brain tumors                                | • RNA virus with high rate of mutation of viral genome          |
|                                  | • Immunity not present in humans                                                             | • Little experience in production for clinical trials          |
|                                  | • Strain of virus replicates in tumor cells due to their defective interferon response compared to normal cells |                                                                |
|                                  | • High yield of viral progeny for infected cells                                             |                                                                |

Table 1. Comparison of Viral Vectors and OVs Used for Clinical Trials
cule as the genome. After infection, the viral RNA genome is reverse transcribed into a double-stranded DNA, which can be integrated into the chromosomes of host cells and expressed as proteins. RVs have been primarily used as vectors, although there are preclinical studies and a likely future attempt for a clinical trial using a retroviral-based OV.

Recently, a replication-competent retroviral (RCR) vector based on the Moloney murine leukemia virus was found to replicate with kinetics similar to those of wild-type Moloney murine leukemia virus, and was also found to be stable through multiple serial passages in cultured cells. Injection of the RCR vector into established subcutaneous and intracranial tumors in mice resulted in highly efficient transmission of the transgene, and in some cases this resulted in transduction of the entire tumor. The following vector systems have been tested in preclinical studies RCR-HSVtk/ganciclovir and RCR-CD/5-FC. One such RCR-CD vector is being developed for possible use in a clinical trial (Tocagen Inc.).

The advantages of RV vectors are that they are relatively easy to manipulate for gene therapy purposes and have been used widely. The available long-term experience with low toxicity to normal brain tissue makes this vector a safe candidate for CNS gene therapy. One of the problems of RV vectors is that the viral genome can be inserted randomly in the genome of the host. If the insertion happens to be in the middle of one of the host genes, this gene will be disrupted (insertional mutagenesis). If the disrupted host gene is involved in regulating cell division, uncontrolled cell division (i.e., cancer) can occur.

Other disadvantages of RV vectors are low titers, instability of the viral particles, and small transgene capacity (the maximum amount of DNA that can be packaged into a retrovirus allows only for 7.5 kb of foreign DNA). Another drawback of RV vectors is the requirement that the target cell should be dividing for integration and expression of viral genes. This restricts gene therapy to proliferating cells only. Although RV vectors were used in the early gene therapy trials, their use has been greatly reduced in more recent gene therapy trials of cancer.

Adenoviral vectors. Adenoviruses (AV) are non-enveloped viruses containing a linear double-stranded DNA genome responsible for respiratory and eye infections in humans. For gene therapy purposes, the commonly used AV vectors are derived from a subgroup that can be manipulated to produce replication-deficient vectors. AVs are the most commonly used gene therapy vectors; in fact, they have become a common tool in the kit of the molecular biology lab whenever a gene needs to be expressed in a mammalian cell. The vector can be extensively modified to target it away from its usual receptor (present only in some cells) to other receptors present to a desired target cell. Once bound to its receptor, the virus enters the cell in endosomal vesicles that fuse to lysosomes. Then the virus is able to liberate itself and escape to the cell nucleus for gene expression. Viral DNA does not generally integrate into the host genome and survives as an extrachromosomal element.

In terms of gene delivery to brain tumors, the vector has been used to deliver almost any type of anticancer gene. However, in clinical glioma trials, AV-mediated delivery of the genes for HSVtk, p53, and human IF-β have been published so far.

In summary, AV has been used widely as vectors in gene therapy trials, and the advantages to their use are relative ease of manipulation and ability to be produced at high titers. Furthermore, AV vectors are very efficient at transducing a wide variety of cells, both dividing and nondividing in vitro and in vivo. AVs do not usually integrate genetic material into the host genome, rather they replicate as episomal elements in the nucleus of the host cell with a transient gene expression, and consequently there is no risk of insertional mutagenesis.

The limitations of AV vectors are their short-term gene expression and their production of toxic acute-phase responses. As clinical trials have begun to progress, it has become apparent that AV vectors may cause a significant innate immune response.

Oncolytic adenovirus. Oncolytic AVs are engineered or naturally occurring strains of virus that replicate better in tumor cells versus normal cells. This selectivity can occur at three general levels. First, every time a virus infects a cell, an antiviral response occurs inside the cell consisting of numerous “stress” signals (FIG. 2). The overall effect of these signals is to limit the ability of the infecting virus to replicate and thus limit the number of viral progeny that could go on to infect neighboring cells. These “stress” signals consist of genes involved in the IF pathways, NF-kB, toll-like receptor responses, PKR, and others. In some cases, tumor cells have disabled some of these responses, because these responses tend to be pro-apoptotic and anti-proliferative.

Therefore, tumor cells that have such disabled responses provide better targets for viral replication than normal cells with intact antiviral responses. Second, in tumor cells, several genes involved in cell-cycle regulation and apoptosis signaling are disrupted. These disruptions in tumor cell factors can be exploited to rationally engineer viral mutants that can not replicate well in normal cells with intact cell-cycle/apoptosis controls, but that will replicate in tumor cells that harbor these disruptions. Finally, viral mutants can be re-engineered so that they will target cell surface receptors present on tumor versus normal cells. For instance, an engineered oncolytic adenovirus (named ONYX-015) lacks a viral gene (E1B) that encodes for a protein that inactivates the cellular tumor suppressor protein p53. This mutant AV was believed to replicate in and lyse p53-deficient human tumor
viral genes (UL39 or UL40) that encode for the viral monomers needed mutants for glioma therapy. The two most commonly used types have revolved around deletions of the p14ARF to make it one the most commonly mutated sides in gliomas.

50% of human GBMs carrying a p53 mutation. It may possess anticancer effects against the remaining tumors, because p53 function is modulated by p14ARF protein with a gene (CDKN2A) that is frequently deleted in gliomas.

However, subsequent experiments with this virus have revealed that the mechanism of tumor selectivity is not based on the lack of p53 tumor suppressor function, rather on a more complicated mechanism related to the nuclear export of viral mRNAs in tumor cells. Other oncolytic AVs include those that target a gene (CDKN2A) that is frequently deleted in gliomas.

HSV-1. HSV-1 is an enveloped neurotropic DNA virus. The wild type HSV-1 is able to proceed into a lytic life cycle after infection or persist as an intranuclear episome in a latent state. Interest in the use of HSV-1 as a cancer attacking agent became high in the 1990s after reports showed that a genetically engineered form was oncolytic in brain tumor models. Since then, numerous reports have detailed various types of genetically engineered mutants for glioma therapy. The two most commonly used types have revolved around deletions of the viral genes (UL39 or UL40) that encode for the viral protein, ICP6. This protein possesses the function of a ribonucleotide reductase, and recent reports have linked this defect to an ability to replicate more selectively in cells with a defect in the p16 tumor suppressor gene. The other type has focused on a mutation of the viral gene that encodes for the viral protein ICP34.5. This viral gene counteracts a host cell response (based on the PKR enzyme) that promotes cellular apoptosis during infection. Mechanisms of tumor selectivity of this mutant virus have elicited several reports. In one case, authors have linked this viral defect to enhanced replication in tumor cells with elevated levels of Ras activity. However, others have disputed this claim and have linked mutant OV replication to activity of the MEK pathway present in the tumor more than normal cells.

In addition, it is also possible that tumor cells may have lost some PKR function, thus enabling this mutant virus to replicate. Another genetically engineered HSV-1 (termed G207) was designed such that it is a replication competent in GBM, it has attenuated neurovirulence, it is temperature sensitive, and it can be eliminated with ganciclovir. It possesses deletion of the viral genes that encode both ICP6 and ICP34.5, and thus it may selectively target both cells with defects in p16 and with overexpression of MEK. Intraneoplastic inoculation with G207 decreased tumor growth and prolonged survival in animal models with no significant toxicity to the normal brain. Based on these results, two phase I studies using G207 in patients with anaplastic astrocytoma and GBM was completed with promising results.

HSV-1 as an OV offers several advantages: 1) potential for incorporating a large load of foreign DNA; 2) infects human cells with high efficiency and low viral particle-to-cell ratio; 3) sensitivity to antiherpetic agents, such as ganciclovir or VCV providing a safety mechanism by which viral replication could be eliminated, if needed; and 4) the fact that HSV-1 never integrates into the host genome guarantees that the risk of insertional mutagenesis posed by RV vectors is not an issue with HSV-1. However, there are major challenges working with HSV-1. The genetic manipulation of HSV-1 is more difficult than that of AV due to the large size of the viral genome, and gene delivery could be affected due to pre-existing HSV immunity of the host (with as many as 90% of adults who have been exposed to HSV-1), and experience and interest in preclinical development by the biotechnology industry is limited. Moreover, the potential neurotoxicity of HSV-1 vectors could cause life-threatening encephalitis from primary infection or from reactivation of latent virus.

Measles virus. The measles virus (MV) belongs to the Paramyxoviridae family, which are enveloped, single stranded RNA viruses. Numerous viruses are now being considered as potential cancer therapeutics, including Edmonston’s vaccine strain of MV. The Edmonston vac-
vaccine strain enters cells predominantly through the CD46 receptor, which interacts with cellular surface glycoproteins.\textsuperscript{85,86} The CD46 receptor is frequently overexpressed in tumors including gliomas, but it is expressed in low levels in the normal brain.\textsuperscript{87} In contrast to wild type virus, which can result in serious disease, vaccine strains of MV have an excellent safety record with millions of vaccine doses having been administered. Measles has been tested against a variety of cancer models, including gliomas, showing significant biologic effects. A phase I trial is currently being conducted using the MV in recurrent GBMs (E. Galanis, Mayo Clinic, personal communication).

\textbf{Reovirus.} Reovirus is a small RNA virus that produces self-limiting infections of the respiratory or intestinal tract, and it is endemic to the human population because most newborns get exposed to it. A strain of the virus was discovered to replicate in cells with an overactive Ras pathway, and this property has been exploited by attempting to define the safety and efficacy profile of this virus in clinical trials of humans with GBMs, as well as other cancers.\textsuperscript{88}

As with most RNA viruses, reoviruses can replicate to very high levels in an infected cell, and thus in theory it could provide an increased therapeutic "punch," because the large number of viral progeny could infect a large number of neighboring tumor cells. Some of the issues that limit use of this virus relate to its small size and virological properties, which render genetic manipulation difficult. RNA viruses are also notoriously, highly mutable, which can also be problematic for production and use. The clinical experience is currently being supported by one biotechnology venture (Oncolytics Biotech Inc., Calgary, Alberta, Canada).

\textbf{Newcastle disease virus (NDV).} This virus (NDV) is an avian RNA virus. Several strains have been adapted for growth in tumor cells, and a phase I trial of intravenous infusion of an oncolytic NDV mutant in glioma patients has been reported.\textsuperscript{89} The advantages and disadvantages of this virus are similar to those of reovirus. However, differences with this virus exist; immunity to this avian virus does not exist in humans, and thus initial administration of the virus would not be limited by the adaptive arm of the host’s immune reaction. This could also be a disadvantage, because the zoonotic nature of the virus and its administration into the human species could generate mutant species that become adapted to human infection and cause rapid pandemics, such as those that have occurred in the past with coronaviruses and HIV. The mechanism of selectivity of the virus for tumor cells is also not well understood. It may be due to the impaired IF and/or other host cell antiviral responses in tumor versus normal cells.

\section*{Glioma Gene Therapy Trials}

Six viruses (two replication-deficient and four replicating) have been studied in clinical brain tumor trials in which the results have been published (e.g., replication-deficient retrovirus, replication-deficient adenovirus, replicating adenovirus, replicating HSV-1, replicating reovirus, and replicating NDV), and one replicating virus (measles) has been studied in glioma patients in a trial that is still ongoing and whose results have yet to be published.

RV and AV have been genetically modified to express HSV\textsubscript{tk} with concurrent GCV administration. HSV and NDV have been used as an oncolytic agent. A selection of clinical trials is summarized in Table 2. The first study using therapy in glioma patients used stereotactic intratumoral inoculation of an RV vector carrying HSV\textsubscript{tk} in 15 patients with recurrent malignant brain tumors.\textsuperscript{90} Although this study showed some promising results in terms of antitumor efficacy, it was not a controlled or randomized protocol. Two subsequent phase I and II studies in patients with recurrent GBM were performed using an RV/HSV\textsubscript{tk} approach followed by GCV administration. The virus was delivered via vector-producing cells that were injected during tumor resection. The first study involved 12 patients and no treatment-related adverse effects were noted. There was an overall median survival of 6.8 months, with three patients surviving longer than 12 months and one patient who was recurrence-free 2.8 years after treatment.\textsuperscript{91} A comparable international, multicenter, uncontrolled study included 48 patients with recurrent gliomas. The median survival time was 8.6 months, with a 12-month survival rate of 27%. Tumor recurrence was absent on MRI in seven patients for at least 6 months and in two patients for 12 months, and one patient remained recurrence-free at 24 months.\textsuperscript{92} A similar phase I study was performed in 12 children (aged 2 to 15 years) with recurrent malignant supratentorial tumors. This study also used an RV/HSV\textsubscript{tk}/GCV approach. Disease progression occurred at a median time of 3 months after treatment, and the longest time until progression was 24 months with no adverse effects noted.\textsuperscript{93} A large controlled phase III trial seemed necessary for an ultimate confirmation of the efficacy of the RV/HSV\textsubscript{tk}/GCV approach. This study used an adjuvant gene therapy protocol to the standard therapy of maximum surgical resection and irradiation for newly diagnosed GBM. After 4 years of follow-up of 248 patients who were divided into a gene therapy and a control arm, survival analysis showed no advantage of gene therapy in terms of tumor progression and overall survival.\textsuperscript{94}

There have been several clinical trials using AV vectors for malignant gliomas. Representative AV trials are briefly discussed herein. One clinical trial compared hu-
mans with recurrent gliomas who were treated with direct stereotactic injection of either an RV vector or an AV vector that expressed the *E. coli* LacZ gene. They showed that the latter showed more widespread distribution of gene expression, and thus they concluded that AV vectors may be superior in their ability to distribute the transferred gene into brain tumors. A phase I/II trial evaluated an AV vector expressing the HSV tk gene in patients with primary or recurrent high-grade gliomas. This study was performed in a controlled randomized fashion on 36 patients (i.e., 17 in the treatment arm and 19 in the control group). All patients underwent surgical resection and AV injection, followed by intravenous GCV on postoperative day 5 for 14 days. The control group underwent resection without gene therapy and GCV injections. The median survival in the gene therapy group was significantly longer than the control group (62 vs. 37 weeks). As discussed previously, a large phase III trial has been conducted in Europe and Israel with this agent (Cerepro) and a recent statement from the company (Ark Therapeutics, Inc., London, United Kingdom) suggested evidence of efficacy, although published data would allow for independent evaluation of this claim. A recent phase I trial of AV-HSV tk delivered at the time of surgical resection of newly diagnosed malignant gliomas followed by oral VCV, combined with standard therapy (external field radiation to 6000 cGy and temozolomide) has also recently concluded, showing relative safety, whereas the data is maturing regarding any evidence of efficacy (Chiocca et al., unpublished data). An AV vector delivering a wild-type copy of the p53 transgene was also evaluated in humans with recurrent gliomas. The vector was initially introduced by an implanted catheter placed in the middle of the tumor bed, followed by resection of the tumor to allow for studies related to p53 gene delivery and distribution. Again, while the treatment was well tolerated, the distribution of p53 gene into tumor was relatively low. One recent trial evaluated an AV vector in 11 patients with recurrent high-grade glioma introduced by stereotactic injection, followed by surgical resection and an additional injection of the vector into the tumor bed. The vector was well-tolerated in all but one patient who experienced confusion after the postoperative injection, which was believed to be caused by local brain toxicity. Tumor resection after the viral treatment demonstrated a dose-related inflammation and necrosis suggestive of a biologic effect from the transferred gene.

The next virus to undergo clinical trials in brain tumors was HSV-1. A phase I trial evaluated the safety of an oncolytic HSV-1 in nine patients with recurrent GBMs. Direct intratumoral injection was performed with no induction of encephalitis or adverse clinical symptoms, nor reactivation of latent HSV occurring, thus appearing as though tumor progression was controlled with some efficacy. Further investigations of the oncolytic HSV-1 mutant demonstrated that this vector is nontoxic when it is delivered into the tumor or into the adjacent brain. It was also shown that explanted glioma cells could still support replication of the oncolytic HSV-1, but that 20% of cells did not lyse immediately, but rather shedded low levels of virus for up to 31 days after which they lysed. A large phase III trial in Europe using HSV-1 is being conducted, which is sponsored by Crusade Laboratories (www.crusadelabs.com).

| Phase | Strategy | No. of Patients | Median Survival (months) | Ref. |
|-------|----------|----------------|--------------------------|------|
| I     | Stereotactic injection | 15 | 8 | 90 |
| I/II  | Freehand injection | 48 | 9 | 91 |
| III   | Freehand injection | 248 (124 RV, 124 control) | 12.2 (RV) | 94 |
|       |           |               | 11.8 (control) |      |
| I     | Freehand injection | 11 | 4 | 43 |
| II/III| Freehand injection | 36 (17 AV, 19 control) | 16 (AV) | 96 |
|       | HSVtk/GCV |               | 9 (control) |      |
| I     | Stereotactic injection | 9 | 9 | 97 |
| I     | Stereotactic injection | 21 | 6 | 83 |
| I     | HSV-1 | 6 | 6.6 | 84 |
| I     | HSV-1 | 15 | 10 | 33 |
| I     | AV-p53 | 24 | 6 | 103 |

AV = adenovirus; GCV = ganciclovir; HSVtk = herpes simplex virus thymidine kinase; RV = retrovirus; VPC = vector producer cells.
ical trial using a different HSV-1 mutant included 21 patients with recurrent glioma with similar results as they relate to safety.83

CONCLUSIONS

The rapid evolution of recombinant DNA technology enables us to develop new therapeutic modalities, including gene therapy. However, screening of new approaches is based on animal models that are far from representative of the analogous clinical scenarios, as shown in the discrepancy between the experimental animal studies and the clinical trials. As for brain tumors, the size, consistency, and extent of tumor models do not reflect the large, necrotic, infiltrative nature of GBMs. Approaches that aim at immune enhancement but use animal models that are poorly syngeneic to the implanted tumor are unlikely to reliably predict the human response. Accordingly, many ingenious gene therapy strategies effective in pre-clinical studies may not fulfill their expectations in the clinic. Furthermore, the problem of the delivery of genetic vectors into solid brain tumors and efficient in situ gene transfer remains one of the most significant hurdles in gene therapy. The efficiency of transduction could be improved by modifying vector-producing cells, such as migratory vector-producing cells, which have the ability to track even single tumor cells invading the surrounding brain tissue.100,101 The currently used manual injection of vectors, which is limited in volume and can be injected at any given time, and the efflux that occurs along needle tracks, might be improved by the use of three-dimensional neuroradiation techniques and convection-enhanced delivery methods; these delivery methods involve the infusion of therapeutic agents via surgically implanted catheters, and they use a pressure gradient to achieve a greater volume of distribution compared with that evident with diffusion alone.102

The completed brain tumor gene therapy trials have offered some promising results. However, the results of most clinical studies have not lived up to the expectations created by experimental data. Although gene delivery to human patients seems to be safe, these studies have not yet translated into benefits in the clinic. At present, gene therapy is being studied in trials for brain tumors, and so far it is not available outside of a clinical trial.

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