Journal of Neuro-Oncology **65:** 279–289, 2003.

Clinical Study

Adenovirus/herpes simplex-thymidine kinase/ganciclovir complex: preliminary results of a phase I trial in patients with recurrent malignant gliomas

Isabelle M. Germano[1], Jennifer Fable[1], S. Humayun Gultekin[2], and Adam Silvers[3]
[1]*Department of Neurosurgery,* [2]*Department of Pathology (Neuropathology),* [3]*Department of Radiology (Neuroradiology), Mount Sinai School of Medicine, New York, NY, USA*

Key words: adenovirus vector, anaplastic astrocytoma, ganciclovir, gene therapy, glioblastoma, thymidine kinase

Summary

The management of patients with glioblastoma remains challenging with an average survival of 32–56 weeks. We report on a clinical trial of patients with recurrent glioblastoma treated with adenovirus/herpex simplex-thymidine kinase/ganciclovir (ADV/HSV-tk/GC).

Entry criteria for this study included: recurrent malignant glioma after surgical resection and conventional radiation therapy. At the time of recurrence, computerized volumetric resection of the tumor was performed and the ADV/HSV-tk complex was injected in the tumor bed. GC was administered 24 h after surgery (10 mg/kg/day) for 7 days. Patients were divided into 3 ADV/HSV-tk dose-escalating cohorts.

Adenoviral vector shedding, and local or systemic toxicity did not occur in this study. Magnetic resonance imaging showed lack of increased brain edema in the treated patients. Histological examination of the 5 patients that had repeated surgery after gene therapy treatment showed lack of tissue toxicity. Additionally, PCR for HSV-tk was negative in the brain 3 months after injection. The patients' Karnofsky score was maintained $\geq$70 in 8/10 patients (80%) and 5/9 patients (55%) 3 and 6 months respectively, after gene therapy. Ten of 11 patients survived $\geq$52 weeks from diagnosis with an average survival of 112.3 weeks. One patient is still alive 248 weeks from diagnosis.

These data show that the ADV/HSV-tk/GC complex at the dose used in this study is safe. Additional dose escalation is currently in progress.

Introduction

Glioblastoma multiforme (GBM) accounts for 20% of primary brain tumors [1] Despite the tremendous emphasis that the neuroscientist community has made in the application of new therapies during the last decade, patients survival remains practically unchanged since the early 1970s [2]. Available surgical, radiotherapeutic and chemotherapeutic options achieve at best transient responses in a minority of patients treated, with a median survival ranging from 32 to 56 weeks [3,4]. Tumor recurrences are located in the margin of the previous resection cavity in more than 90% of the patients [5,6]. Innovative therapeutic approaches to this tumor are clearly needed.

Gene therapy characterized by direct introduction of therapeutic genes into malignant cells *in vivo* may provide an effective treatment for solid tumors of the central nervous system (CNS). One of the first examples, introduced by Moolten in 1986, was the transfer into malignant cells of the herpes simplex-thymidine kinase (HSV-tk) gene which converts the non-cytotoxic nucleotide analog ganciclovir (GC) into phosphorylated compounds that halt the transcription of DNA in dividing cells [7]. This technique referred to as 'suicide gene therapy' has shown promising results in numerous animal studies [8–15]. Animal experiments indicate that this strategy causes selective tumor cytotoxicity and spares the quiescent non-neoplastic neuronal and glial cells [15]. Glioblastomas are particularly appropriate targets for gene therapy approach. This is due to the non-dividing characteristics of the tissue from which the tumor arises. Furthermore, GBMs have a very low frequency of multicentric dissemination.

Therefore, to lengthen the interval to tumor progression local control is the therapeutic goal for most patients. Additionally, the 'bioavailability' of the vector in the brain compared with other tissues, could be extended [15,16].

The use of adenoviral vectors provides several theoretical advantages over their retroviral counterparts. The transfection efficiency for adenovirus vectors greatly exceeds that of retroviruses. In addition, retroviral-mediated gene introduction is limited to those cells within the target tumor which are actively dividing [17]. Since the proliferation index of malignant gliomas, including GBM, is small and not all the cell lines might be in active replication [18], retrovirus vectors may fail to introduce the suicide gene into a sufficient fraction of tumor cells to produce a clinical response. Adenoviruses infect both dividing and non-dividing cells [19], so that the large percentage of cells in G0 will still be susceptible to infection with subsequent sensitivity to GC when they divide. Additionally, adenoviral gene therapy in the brain leads to transfection of ependymal, choroid plexus, neurons and isolated glia and neurons without clinical toxicity [20–24]. This vector is also capable of producing gene expression without integration into the host genome, eliminating the oncogenecity secondary to insertional mutagenesis [25].

We report on an ongoing phase I clinical trial of gene therapy for recurrent GBM using the ADV/HSV-tk/GC complex. The primary study end point is to find the maximum tolerated dose of virus that can be safely administered after surgical resection of recurrent tumor, using an escalating dose protocol. The secondary end points include assessment of quality of life and survival after treatment with ADV/HSV-tk/GC.

Patients and methods

Regulatory compliance

This study was conducted in accordance with the Institutional Review Board of Mount Sinai School of Medicine, Food and Drug Administration (FDA) Regulations under appropriate IND, and NIH/OBA/RAC regulation. All patients signed informed consent prior to enrollment in the study. The study was monitored by an external regulatory monitoring agency, by a Data Safety Monitoring Board, and was audited by the FDA.

Clinical end points

The primary study end point is to find the maximum tolerated dose of virus that can be safely administered after surgical resection of recurrent tumor, using an escalating dose protocol. The secondary end points include assessment of quality of life, tumor progression and survival after treatment with ADV/HSV-tk/GC.

Study design

Inclusion criteria were as following: (1) Patients $\geq$18 years of age with histologically confirmed malignant glioma, defined as GBM, anaplastic astrocytoma (AA), anaplastic oligodendroglioma (AO), anaplastic mixed oligo-astrocytoma (AOA) who failed conventional external beam radiation and possibly chemotherapy (GBM, AA, AOA) and external beam radiation and chemotherapy (AO), (2) radiographic evidence of recurrent tumor was considered any increase in size of the gadolinium-enhancing area on serial brain magnetic resonance imaging (MRI) scans, (3) completion of brain radiation therapy 8 weeks before enrollment, completion of chemotherapy, if applicable, 6 weeks before enrollment, (4) solitary surgically resectable tumor, defined as: a lesion that can be resected without unacceptable neurological deficits; a lesion that the surgeon anticipates could be resected leaving $<$2 cm in maximum diameter of tissue enhancing with gadolinium on MRI scan, (5) Karnofsky performance score $\geq$70 at the time of recurrence, (6) adequate baseline organ function, (7) histological confirmation at the time of surgery of recurrent primary malignant CNS GBM confirmed by neuropathological review.

Exclusion criteria included: (1) Active infection defined as: any acute viral, bacterial or fungal infection which requires specific therapy; HIV positive patients, (2) pregnancy, (3) progressive systemic malignancy, (4) severe systemic diseases considered to be indicative of an unacceptable anesthetic/operative risk, (5) tumor characteristics including multiple tumors and tumors infiltrating the corpus callosum, (6) entry of the ventricular system at the time of surgery.

Adenovirus concentration and dose escalation

Dose escalation cohorts are outlined in Table 1. The initial dose was 2.5×10^{11} virus particles (vp). Dose increments were planned at 0.5 log after 3 patients

Table 1. ADV/HSV-tk dose escalation cohorts

Cohorts	Number of patients entered	ADV concentration (VP)
I	5	2.5×10^{11}
II	3	3.0×10^{11}
III	3	9.0×10^{11}
IV	Pending	2.7×10^{12}
V	Pending	5.6×10^{12}

had received the same dose. If grade 3 or 4 neurologic or non-neurologic toxicity, NCI Common Toxicity Criteria, occurred related or non-related to the study additional 2 patients would be treated at that dose.

All preclinical experiments using ADV/HSV-tk were based on viral concentrations expressed in plaque forming units (pfu) per ml. Due to the great variability between biological assay of infectious adenovirus titer, viral titers in this and other clinical study using the same vector were expressed in vp per ml. The value for vp is based on optical density of viral preparation and is much more consistent between different labs than the biological assay to determine pfu or iu (infectious units). The approximate ratio of vp to pfu is generally 50 to 1. The FDA allowed us to start at the initial dose indicated in Table 1 as there was evidence in animal studies that this would be unlikely to cause tissue toxicity (see Discussion). The maximum dose of ADV/HSV-tk to be administered to each patient in the final cohort is limited by: (1) The clinical protocol, with FDA approval to inject a volume of fluid not to exceed 1 ml, (2) the concentration of vector in the clinical lot (5.6×10^{12} vp/ml).

Vector construction

The viral construct was described by Graham and Prevec [26] and Chen et al. [10]. The adenovirus-thymidine kinase (ADV-tk) vector used in this study was prepared by inserting HSV-tk into the plasmid pADL.1/RSV, which contained the Rous sarcoma virus long-terminal-repeat promoter (RSV-LTR) to generate pADL.1/RSV-tk [10]. Recombinant adenovirus was produced by co-transfecting 293 human kidney cells which contain the E1 region of the adenovirus genome with pADL.1/RSV-tk and a plasmid, pJM17, containing the adenovirus genome. Replication-defective adenovirus was produced by homologous recombination [26].

Surgical resection, intraoperative administration of ADV/HSV-tk and GC

All surgeries for gene therapy were performed by the same surgeon (IMG) using image-guided computer assisted techniques, as previously published [27–29]. Briefly, all patients underwent a pre-operative contrast-enhanced MRI the day of the surgery with skin adhesive fiducial markers. The pre-operative MRI was acquired using a conventional frameless protocol (axial T1-weighted images, 2 mm thick). Patients were then brought to the operating room and anesthesia induced. The StealthStation (Medtronic SNT, Louisville, CO) was used in all cases for frameless guidance. In cases involving tumor near eloquent cortex, intraoperative electrophysiological mapping was also used. In all patients, intraoperative frozen sections were sent to confirm the presence of recurrent tumor. All patients were treated perioperatively with dexamethasone, antibiotics and anti-seizure medications.

After gross total surgical resection was accomplished, a Surgicel grid was tailored to the tumor cavity and marked at 5 mm intervals. Each patient was injected with 1 ml of viral solution in the tumor bed. Injection of the virus was performed using a microsyringe in 5 mm as guided by the grid, at a depth of 10 mm. GC treatment began 24 h post-virus injection at 5 mg/kg IV over 1 h every 12 h for 7 days (14 doses).

Primary end point: safety monitoring

Adenovirus toxicity monitoring

Adenoviral toxicity was followed to monitor viral shedding and brain toxicity.

Viral shedding. After patients received the intratumoral ADV/HSV-tk treatment, they were admitted to the neurosurgical intensive care unit in a single patient module with respiratory and body fluid precautions. Beginning 24 h after the vector administration and prior to the first GC infusion, nasal swab, urine and blood specimens were obtained. When the screening immunofluorescent assay identified adenovirus in the patients' body fluids, the isolates were plated in a quantitative fashion in plaque forming assays. The viral isolates were analyzed by PCR amplification, Northern hybridization with probes specific for the recombinant HSV-tk sequences and direct sequencing

to determine if the viral isolates represent recombinant ADV/HSV-tk or a concurrent unrelated adenoviral infection.

Brain toxicity. Potential brain toxicity of the ADV/HSV-tk/GC complex includes brain edema and inflammatory response [30]. Brain edema was measured by evaluation of increased water content on T2-weighted brain MR images obtained 24 h (early effects) and 7 days after surgery (delayed effects). Assessment of brain edema was performed by the same Neuro-Radiologist in all cases (AS) comparing the pre-operative and post-operative images (early effects) and the immediate post-operative with the delayed images (delayed effects). A three point semi-quantitative scale was used to score the differences (same, better, worse).

In addition, when tissue became available after gene therapy, brain toxicity was measured by histological and immunohistochemical examination. This was focused on detecting abnormalities consistent with inflammatory changes. Immunohistochemistry for glial fibrillary protein (GFAP), T and B cell markers, and macrophages was performed in all cases. In addition PCR for HSV-tk was performed for the brain and all other organs in all autopsy cases.

GC toxicity monitoring

Potential toxicity caused by intravenous administration of GC includes: neutrophilia, thrombocytopenia, anemia, hepatic/renal insufficiency [30,31]. These were monitored according to the patient follow-up schedule summarized in Table 2. Patients were followed up as outpatients at 2-week intervals for the first 2 months and on a monthly basis for one year or as clinically indicated. Evaluations included laboratory, radiographic and neurologic examination.

Secondary end points: quality of life, tumor progression and survival

Quality of life was monitored by assessing the Karnofsky performance score at each patient follow-up visit as summarized in Table 2. Tumor progression, assessed on follow-up MR images as summarized in Table 2, was defined as increase in signal intensity on images after gadolinium. Survival is reported in weeks after first diagnosis at the time of the first surgery and after the administration of the ADV/HSV-tk.

Table 2. Post-operative patients' follow-up after ADV/HSV-tk/GC complex treatment

Interval	Tests
Day 1	CBC, LFTs, ADV, MRI
Day 3	CBC, LFT's, ADV
Day 5	CBC, LFT's, ADV
Day 7	CBC, LFT's, Chem 7, ADV, MRI
2 weeks	PE, CBC, LFT's, ADV
4 weeks	PE, CBC, LFT's, ADV
6 weeks	PE, CBC, LFT's, ADV
8 weeks	PE, CBC, LFT's, ADV
12 weeks	PE, MRI, ADV
16 weeks	PE, ADV
20 weeks	PE, ADV
24 weeks	PE, MRI, ADV
28 weeks	PE
32 weeks	PE
36 weeks	PE, MRI
40 weeks	PE
44 weeks	PE
48 weeks	PE
52 weeks	PE, MRI

CBC – Cell blood count with platelets and differential; LFT's – Liver function test; ADV – Adenovirus cultures (blood, urine, nasal sawb) and adenovirus serum antibody titer; MRI – Brain magnetic resonance image; Chem 7 - Blood chemistry (glucose, sodium, potassium, chloride, carbon dioxide, BUN, creatinine).

Statistical analysis

Linear regression analysis was used to analyze blood work results. A 95% confidence interval was used to ensure that the average change lies between the lower and upper limit of the confidence interval.

Results

Patients characteristics

Patients' demographics and baseline characteristics are outlined in Table 3. A total of 11 patients with diagnosis of recurrent malignant gliomas were enrolled. There were 5 women in this study. Nine patients had initial diagnosis of glioblastoma. The mean age of all patients was 55 years, ranging from 33 to 75 years. The average Karnofsky score at the time of enrollment was 88, ranging from 100 to 70. The average time interval between the initial surgery and the surgery for gene therapy was 41 weeks, ranging from 18 to 72 weeks. All patients underwent resection of the enhancing area as outlined in the entry criteria and documented by post-operative

Table 3. Patients' demographics

Patient no.	Age	Sex	Symptom	Tumor location	Weeks between first surgery and gene therapy	KPS at enrollment
1	43	M	Seizures	R temporal	32	90
2	63	F	Hemiparesis	R frontal	42.5	80
3	73	M	Decreased MS	R parietal	36	90
4	33	M	Seizures	L frontal	20	90
5	69	F	Seizures	R temporal	29	80
6	48	M	HA	L parietal	18.5	90
7	62	M	HA, Decreased MS	L temporal	60	90
8	47	M	HA, Decreased MS	L frontal	72.5	90
9	57	F	Seizures	L parietal	48	100
10	49	F	HA	L parietal	64	70
11	64	F	Hemiparesis	R frontal	31.5	100

MRI with contrast obtained 24–48 h after the surgery.

GC toxicity monitoring

There were no significant changes in hematological parameters for the evaluation of myelotoxicity. In particular, administration of GC did not result in neutrophilia, thrombocytopenia or anemia. Similarly, liver function and renal function tests were not significantly altered in this study.

Adenoviral toxicity monitoring

Adenoviral shedding
Adenoviral antibody blood titers and body fluid cultures for the vector used (ADV type V) were negative in all patients.

Local toxicity: radiographic results
Early and delayed assessment of brain edema evaluated as described in Methods, revealed no change in 6/11 patients and improvement in 5/11 patients in the early and delayed phases. Worsening of cerebral edema was not noticed in any of the patients (Figure 1).

Local toxicity: histological findings
All patients had histology consistent with GBM at the time of tumor recurrence and ADV/HSV-tk treatment. In addition, one patient had some features suggestive of gliosarcoma component. In all examined specimens, there was no evidence of intraparenchymal toxicity, including encephalitis and ventriculitis. PCR analysis of brain sections at the injection site and distant to the administration of gene therapy and of peripheral organs failed to reveal persistent transgenic expression. Figure 2 shows illustrative histological findings.

Serious adverse events

Serious adverse events are listed in Table 4. Six patients experienced 11 serious adverse events. Four patients required hospitalization for tumor progression. Three of these patients required re-operation for resection of recurrent tumor 12 weeks after gene therapy. Only one of 3 patients was symptomatic. One patient leaked CSF from the surgical incision 2 weeks after surgery. This was successfully managed conservatively. One patient had perioperative status epilepticus followed by prolonged need for intubation, sepsis, and death 4 weeks after gene therapy. Ten of 11 patients have died at the time of this report (see below).

Quality of life, tumor progression monitoring, survival

The Karnofsky performance score remained unchanged at 12 and 24 weeks after gene therapy in 8/10 patients (80%) and 5/9 patients (55%), respectively (Table 5).

Two patients had radiographic evidence of tumor control for over 12 months after gene therapy, 72 and 118 weeks respectively (Figure 3). Radiographic evidence of tumor progression was found in 6 of 10 patients 12 weeks after gene therapy. Three of these patients underwent re-operation. Histological

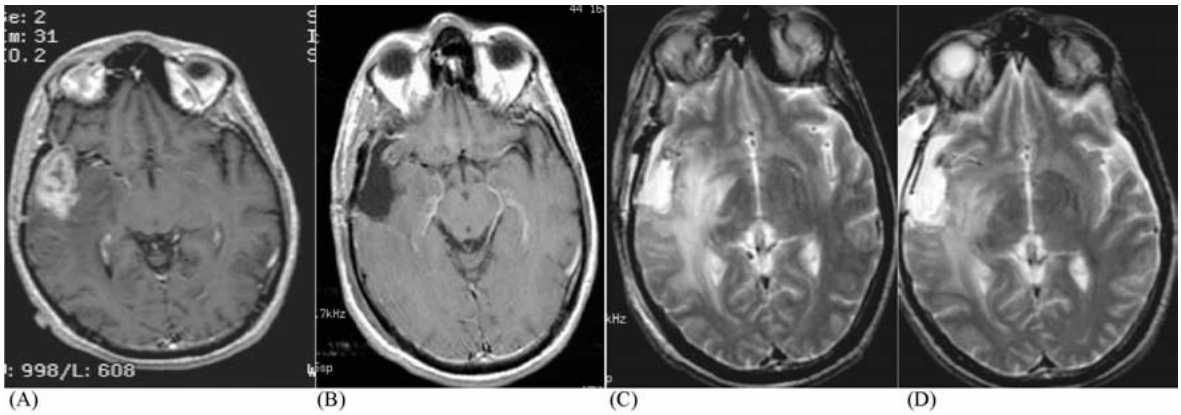

Figure 1. Magnetic resonance (MR) axial images of a patient with recurrent glioblastoma at the time of recurrence prior to gene therapy (A), 24 h after surgery and administration of gene therapy (B,C) and 7 days after gene therapy. The amount of edema was unchanged from pre-operative MRI (data not shown) and improved 7 days after surgery compared to immediately post-operative images (C,D). A,B = gadolinium-enhanced T1-weighted images; C,D = T2-weighted images.

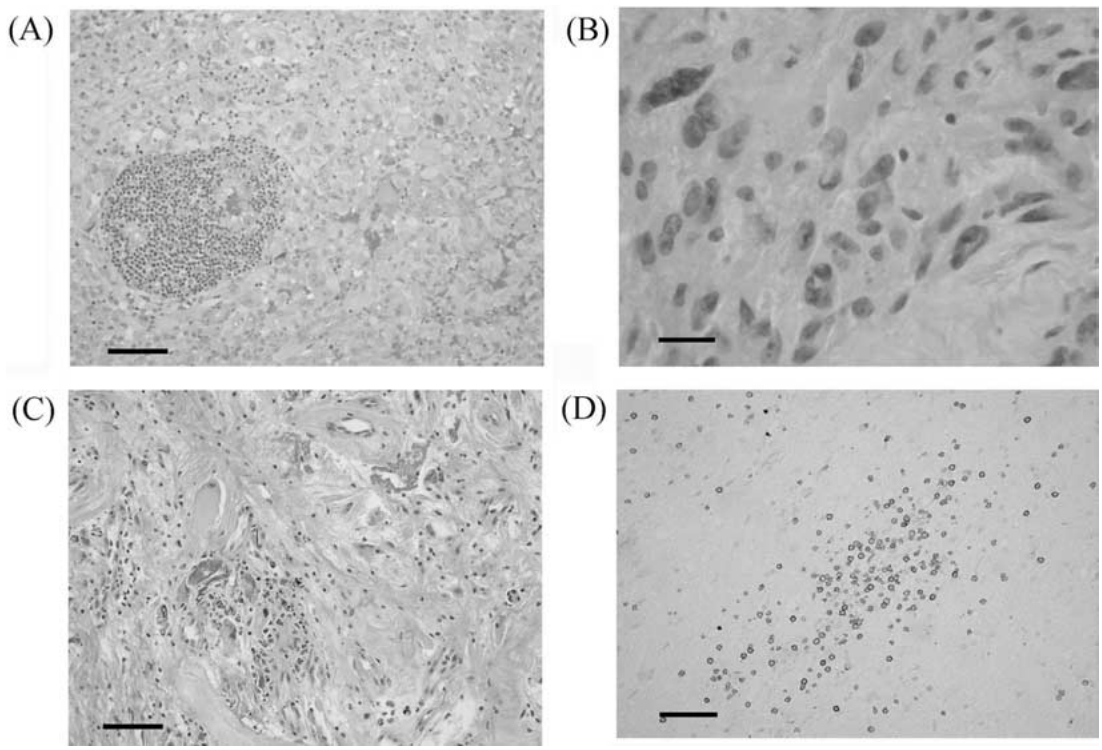

Figure 2. Microphotographs of glioblastoma at initial presentation (A), after radiation-therapy before the administration of gene therapy (B) and 12 months after gene therapy (C,D). Note the perivascular lymphocytic cuffing seen in most glioblastomas at first presentation (A). Note the radiation-induced changes characterized by nuclear atypia, hyalinized vessel wall, and decreased inflammatory component after radiation therapy (B). Twelve months after gene therapy, histological and immunohistochemical stains did not reveal increased inflammatory response (C,D) A,B,C: Hematoxylin and Eosin; D: leukocyte common antigen immunohistochemistry. Magnification: (A,C,D) 200×; (B) 400×; Magnification bar: (B) 100 μm; (A,C,D) 200 μm.

Table 4. Summary of serious adverse events

Patient ID no.	Serious adverse event	Relationship to protocol	Action	Time interval after gene therapy(weeks)
1	CSF leak	Not related	Hospitalization	2
1	Left hemiparesis	Not related	Hospitalization	12
2	Status epilepticus	Possibly related	Hospitalization	24 h
2	Sepsis	Not related	Hospitalization	3
1	Lethargy	Not related	Hospitalization	8
3	Head trauma	Not related	Hospitalization	32
3	Deep vein thrombosis	Not related	Hospitalization	50
5	Radiographic recurrent GBM	Not related	Hospitalization	72
9	Radiographic recurrent GBM	Not related	Hospitalization	12
9	Radiographic recurrent GBM	Not related	Hospitalization	32
11	Radiographic recurrent GBM	Not related	Hospitalization	12

Table 5. Karnofsky performance score

Patient no.	Age	Sex	KPS at enrollment	KPS at 12 weeks	KPS at 24 weeks	KPS at 36 weeks	KPS at 48 weeks	Survival after gene therapy surgery(weeks)
1	43	M	90	50	50	50	No visit	51.4
2	63	F	80	n/a	n/a	n/a	n/a	5
3	73	M	90	90	80	60	50	48
4	33	M	90	100	100	100	100	227
5	69	F	80	90	90	90	90	118.2
6	48	M	90	90	90	No visit	n/a	45.1
7	62	M	90	60	No visit	n/a	n/a	28
8	47	M	90	No visit	n/a	n/a	n/a	16.2
9	57	F	100	100	90	No visit	n/a	51
10	49	F	70	80	No visit	n/a	n/a	25
11	64	F	100	80	No visit	n/a	n/a	33.1

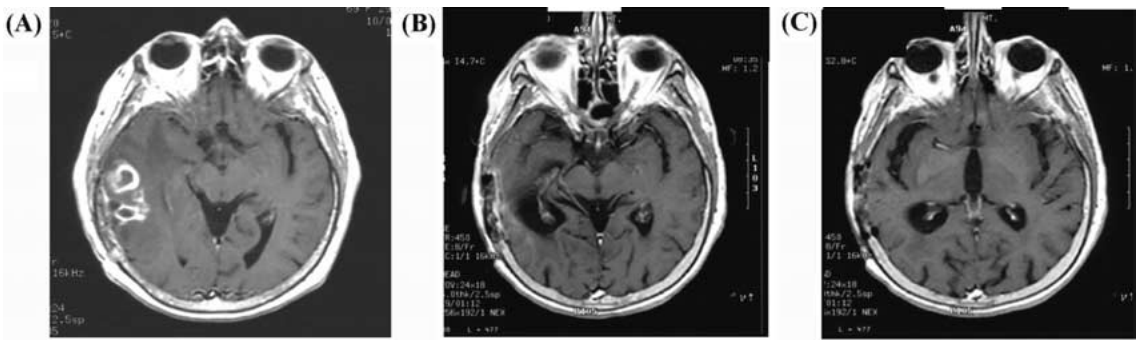

Figure 3. Magnetic resonance (MR) axial gadolinium-enhanced T1-weighted images of a patient with recurrent glioblastoma at the time of the tumor recurrence (A) and 12 months after administration of the ADV/HSV-tk/GC complex (B,C). Note that radiographic tumor control was persistent 12 months after gene therapy.

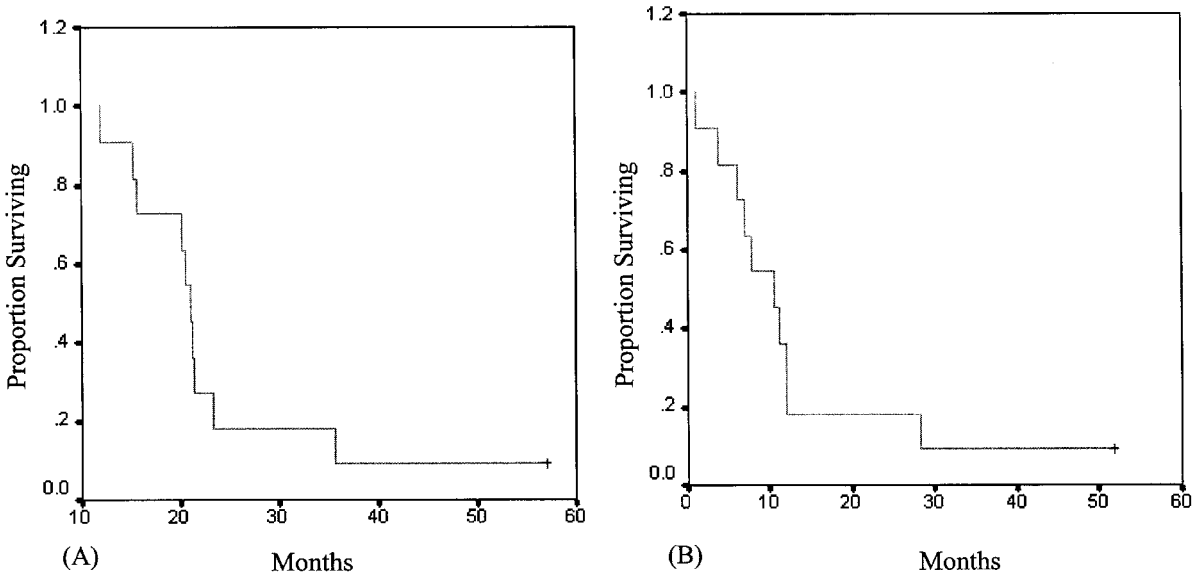

Figure 4. Kaplan–Meier survival curves of patients with malignant astrocytomas since first diagnosis (A) and administration of ADV/HSV-tk/GC complex (B). Data is reported in months (see text).

exam showed recurrent tumor in two of these patients. Survival data is reported in Figure 4. The average survival from initial surgery and diagnosis was 102.4 weeks, ranging from 51 to 248 weeks. The average survival from gene therapy was 58.9 weeks, ranging from 5 to 118 weeks. The 50% survival from initial diagnosis was 22 months and from gene therapy 12 months.

Discussion

Tissue toxicity

Although gene therapy is potentially useful in treatment of many inherited [32] and acquired disorders, it currently suffers from a number of limitations. Two of the main limitations attributed to the ADV/HSV-tk/GC complex are the safety of the vector *in vivo* and the efficiency of gene transfer [33]. One of the primary concerns for the use of adenoviral vectors in cancer therapy is the inflammatory response that the virus might trigger in the brain and the specific immune response to adenoviral proteins that can be triggered. Inflammatory response to viral vectors has been previously documented. Animal and clinical studies by MRI and histological exam [15,17,34,35] reported brain toxicity in the rat characterized by necrosis, gliosis and focal astrocytosis with an adenoviral viral dose of 1.5×10^{11} pfu. This is a dose lower than that used in our study. In addition, these authors reported evidence of increased edema on MRI of rhesus monkeys' brain [34]. Similarly, in a clinical trial using retroviral vector, increased edema on MR images in 2 of 10 patients was reported [35]. Rainov et al. [17] reported that in their clinical trial the number of tumor-infiltrating lymphocytes found after gene therapy was not significantly increased compared with the primary tumors. Ram et al. [15] reported lack of increased edema in rhesus monkeys' brain MRI after administration of HSV-tk/GC using a retroviral-mediated vector.

On the other hand, Klatzman et al. [36] and Shand et al. [37] did not report increased brain edema in 12 and 48 patients, respectively, who received retroviral-mediated gene transfer with HSV-tk followed by GC administration. Similarly, Goodman et al. [38] reported small MRI abnormalities in the primate brain treated with ADV/HSV-tk/GC complex. These authors, however, reported microscopic foci of brain necrosis found at autopsy. In our study we examined seven human brain specimens after gene therapy. There was no evidence of significant increase in inflammatory response in any of the cases examined. In addition, in the patient who experienced perioperative status epilepticus and subsequent death 4 weeks after gene therapy surgery, there was no evidence of increased mononuclear

infiltrate or other inflammatory response. These histological data is corroborated by clinical observation. None of the patient in this study showed any clinical symptoms or signs of inflammatory reaction. Thus, we suggest that at the dose used in this clinical study, the ADV/HSV-tk/GC complex does not cause tissue toxicity. It must be noted, however, that the dose escalation of this study is still in progress.

Viral shedding

The escape of the adenovirus from the brain tissue into the systemic circulation is of great concern when administering gene therapy. Although this is a theoretical risk, to the best of our knowledge, at the present time there has been no documentation of viral shedding after use of the adenovirus vector in the brain. Similarly, in our study we did not document any viral shedding in the body fluids examined, including blood. These findings suggest that viral shedding should not be a concern when using the adenovirus vector for recurrent brain tumors at the dose reported in our study.

Systemic toxicity

Ganciclovir is an FDA-approved medications for treatment of cytomegalovirus retinitis in immunocompromized individuals [31]. The most common side effects include granulopenia and thrombocytopenia. Other side effects occurring in approximately 2% of the population include anemia, fever, rash, abnormal liver and kidney functions, myelotoxicity and liver or kidney insufficiency [30]. In this study we did not detect any side effects attributable to the administration of GC. A previous clinical report had suggested that one of 124 patients receiving GC experienced neutropenia, probably attributable to GC [17]. A previous clinical study had reported the evidence of six intracerebral hemorrhages after administration of HSV-tk/GC complex in 48 patients [37]. This was attributed to GC toxicity. In our study, we did not detect any intracerebral hemorrhages.

Similar to other studies [17,36], the present clinical trial did not disclose any evidence of neurological side effects at the time of the GC administration when the local gene therapy induced cell destruction should occur. These were previously observed in a series of 12 children treated with retrovirus/HSV-tk/GC complex [39].

Adverse events, radiographic recurrence, quality of life, and survival

The serious adverse events reported in this study were all attributable to tumor progression. One patient experienced CSF leak 2 weeks after gene therapy. CSF leak in re-operated patients is a well-known complication in patients undergoing re-operation for malignant astrocytoma [40]. Compared with chemotherapy for malignant brain tumors, the ADV/HSV-tk/GC complex appears to offer a better clinical safety profile as regards to the incidence of adverse events with chemotherapy [41,42]. Furthermore, the overall quality of performance of the patients enrolled in this study was maintained for at least 3 months in 70% of the patients. This compares favorably with other clinical reports on patients with glioblastoma who did not receive gene therapy [3,17].

Radiographic control was not a primary end point of this dose escalation study. Nonetheless, it is encouraging to see that radiographic control was achieved in 2 patients of the first cohort 12 months after gene therapy. Similarly, other clinical trials had reported radiographic control on a few patients after gene therapy treatment. Shand et al. [37] showed radiographic evidence of tumor control in 7 of 48 patients using retroviral-mediated HSV-tk transfer followed by GC 3 months after gene therapy. Klatzman et al. [36] reported 4 of 12 patients with radiographic control 4 months after gene therapy. Deliganis et al. [43] reported decreased tumor volume in 3 of 7 patients treated with retrovirus-mediated HSV-tk/GC complex. Our results taken together with those of other trials suggest that there is a subpopulation of patients with glioblastoma that responds to gene therapy. Although at the present time there are no factors identified to predict responders to treatment, additional studies focused on the cellular biology of these tumors might bring additional insights. In addition, strategies to improve vector delivery and enhance viral-mediated transfection efficiency are currently investigated and provide promising preliminary results [44,45].

Clearly, quality of life is a major concern in patients with malignant brain tumors. Adjuvant treatments with intracavitary brachytherapy or intravenous chemotherapy have been associated with detrimental impact on quality of life [42]. On the other hand, newer approaches using oral chemotherapy seem to be better tolerated [46]. In our study, we showed that a high Karnofsky performance score ($\geq$70) was maintained

by 80% of patients 3 months after gene therapy and by 55% of patients 6 months after gene therapy. These results compare favorably with those for other glioblastoma patients reported in the literature.

The prognosis quod vitam of patients with glioblastomas remains practically unchanged since the early 1970s [2]. Available surgical, radiotherapeutic and chemotherapeutic options achieve at best transient responses in a minority of patients treated, with a median survival ranging from 32 to 56 weeks [3,4]. The survival found in our paper compares favorably to that reported in the literature. It should be noted, however, that the sample size is relatively small and therefore, this improved survival can be attributed to selection biases.

Conclusions

The interim data of our clinical trial shows that the ADV/HSV-tk/GC complex is safe to use in the clinical setting at the dose reported in our paper. The survival rate and quality of life of the patients in our study compares favorably with that of other glioblastoma patients reported in the literature. Altogether, we believe that our results warrant further clinical investigation of this therapeutic strategy for the treatment of glioblastoma.

In particular, the efficiency of gene transfer needs to be assessed in a phase II clinical trial.

Acknowledgements

Supported in part by NIH-1R03-CA-82804-01. The Authors wish to thank the members of the Data Safety Monitoring Board for their time and comments: Dr. Kalmon D. Post, MD; Jeffrey Bruce, MD; Meryl Mendelson, MD; Alberto Paciucci, MD. In addition, the Authors whish to thank Dr. Hunaldo Villalobos, Dr. Jamie Ullman, Dr. Marcia Mesenek, Dr. Ruth Abramson, Dr. Savio Woo, Dr. Seiji Kondo, Dr. Susan Margello, and Dr. Yasuko Kondo for their participation in matters pertinent to this study.

References

1. Salcman M: Glioblastoma multiforme. Am J Med Sci 279(2): 84–94, 1980
2. Legler JM, Ries LA, Smith MA, Warren JL, Heineman EF, Kaplan RS, Linet MS: Cancer surveillance series: brain and other central nervous sytems cancers: recent trends in incidence and mortality. J Natl Cancer Inst 91: 1382–1390, 1999
3. Westphal M, Hilt DC, Bortey E, Delavault P, Olivares R, Warnke PC, Whittle IR, Jaaskelainen J, Ram Z: A phase III trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (Gliadel wafers) in patients with primary malignant gliomas. Neuro-Oncology 5: 1–10, 2003
4. Wrensch M, Minn Y, Chew T, Bondy M, Berger M: Epidemiology of primary brain tumors: current concepts and review of the literature. Neuro-Oncology 4: 278–299, 2002
5. Albert FK, Forsting M et al.: Early postoperative magnetic resonance imaging after resection of malignant glioma: objective evaluation of residual tumor and its influence on regrowth and prognosis [see comments]. Neurosurgery 34(1): 45–61, 1994
6. Choucair AK, Levin VA et al.: Development of multiple lesions during radiation therapy and chemotherapy in patients with gliomas. J Neurosurg 65: 654–658, 1986
7. Moolten FL: Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: paradigm for a perspective cancer control strategy. Cancer Res 46: 5276–5281, 1986
8. Barba D, Hardin J et al.: Thymidine kinase-mediated killing of rat brain tumors. J Neurosurg 79(5): 729–735, 1994
9. Boviatsis EJ, Chase M et al.: Gene transfer into experimental brain tumors mediated by adenovirus, herpes simplex virus, and retrovirus vectors. Hum Gene Ther 5(2): 183–191, 1994
10. Chen SH, Shine HD et al.: Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer *in vivo*. Proc Natl Acad Sci USA 91(8): 3054–3057, 1994
11. Colak A, Goodman JC et al.: Adenovirus-mediated gene therapy for experimental spinal cord tumors: tumoricidal efficacy and functional outcome. Brain Res 691(1–2): 76–82, 1995
12. Izquierdo M, Cortes M et al.: Long-term rat survival after malignant brain tumor regression by retroviral gene therapy. Gene Ther 2(1): 66–69, 1995
13. Moolten FL, Wells JM: Curability of tumors bearing herpes thymidine kinase genes transferred by retroviral vectors. J Natl Cancer Inst 82(4): 297–300, 1990
14. Perez-Cruet MJ, Trask TW et al.: Adenovirus-mediated gene therapy of experimental gliomas. J Neurosci Res 39(4): 506–511, 1994
15. Ram Z, Culver KW et al.: Toxicity studies of retroviral-mediated gene transfer for the treatment of brain tumors. J Neurosurg 79(3): 400–407, 1993
16. Russell DW, Berger MS et al.: The effects of human serum and cerebrospinal fluid on retroviral vectors and packaging cell lines. Hum Gene Ther 6(5): 635–641, 1995
17. Rainov NG, the GL1328 International Study Group: A phase III clinical evaluation of herpes simplex virus type I thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. Hum Gene Ther 11: 2389–2401, 2000
18. Hoshino T, Wilson CB: Cell kinetic analyses of human malignant brain tumors (gliomas). Cancer 44(3): 956–962, 1979
19. Shine HD, Wyde PR et al.: Neurotoxicity of intracerebral injection of a replication-defective adenoviral vector in a semipermissive species (cotton rat). Gene Ther 4(4): 275–279, 1997

20. Akli S, Caillaud C et al.: Transfer of a foreign gene into the brain using adenovirus vectors [see comments]. Nat Genet 3(3): 224–228, 1993
21. Bain D, Wilkinson GW et al.: Adenovirus vectors to transfer genes into neurones: implications for gene therapy of neurological disorders Gene Ther 1: S68, 1994
22. Le Gal La Salle G, Robert JJ et al.: An adenovirus vector for gene transfer into neurons and glia in the brain. Science 259(5097): 988–990, 1993
23. Neve RL: Adenovirus vectors enter the brain. Trends Neurosci 16(7): 251–253, 1993
24. Shenk T, Flint J: Transcriptional and transforming activities of the adenovirus E1A proteins. Adv Cancer Res 57: 47–85, 1991
25. Viola JJ, Ram Z et al.: Adenovirally mediated gene transfer into experimental solid brain tumors and leptomeningeal cancer cells. J Neurosurg 82(1): 70–76, 1995
26. Graham FL, Prevec L: Methods for construction of adenovirus vectors. Mol Biotechnol 3(3): 207–220, 1995
27. Benveniste R, Germano IM: Evaluation of factors predicting accurate resection of high-grade gliomas using frameless image-guided stereotactic guidance. Neurosurgical Focus 14: 1–4, 2003
28. Germano IM, Villalobos H, Silvers A, Post K: Clinical use of the neurostation for intracranial computer-assisted neuronavigation. Neurosurgery 45: 261–270, 1999
29. Germano IM, Kondo S: Image-guided tumor resection. In: Germano IM (ed) Advanced Techniques in Image-Guided Neurosurgery. Thieme Publisher, NY, 2001, pp 1–10
30. Burhies WC, Mastre BJ, Tinker AJ, Strand V, Koretz SH, the Syntex Collaborative gancyclovir treatment Study Group: Ganciclovir treatment of sight-threatening cytomegalovirus infection: experience in 314 immunocompromised patients. Rev Infect Dis 10: 495–506, 1988
31. Oldfield EH, Ram Z et al.: Gene therapy for the treatment of brain tumors using intra-tumoral transduction with the thymidine kinase gene and intravenous ganciclovir. Hum Gene Ther 4(1): 39–69, 1993
32. Horellou P, Vigne E et al.: Direct intracerebral gene transfer of an adenoviral vector expressing tyrosine hydroxylase in a rat model of Parkinson's disease. Neuroreport 6(1): 49–53, 1994
33. Chiocca AE: Oncolytic viruses. Nat Rev Cancer 2: 938–950, 2002
34. Smith: Hum Gene Ther 8: 943–954, 1997
35. Weber FW, Floeth FW, Reifenberger G, Felsberg J, Aulich A, Kiwit JCW, Bock WJ: MRI characteristics and histological changes in glioblastoma after gene therapy. Front Radiat Ther Oncol 33: 244–252, 1999
36. Klatzmann D, Valery CA et al.: A phase I/II study of herpes simplex virus type 1 thymidine kinase 'suicide' gene therapy for recurrent glioblastoma. Study Group on Gene Therapy for Glioblastoma. Hum Gene Ther 9(17): 2595–2604, 1998
37. Shand N, Weber F, Mariani L, Bernstein M, Gianella-Borradori A, Long Z, Soresen AG, Barbier N: A phase 1–2 clinical trial of gene therapy for recurrent glioblastoma multiforme for tumor transduction with herpes simplex thymidine kinase gene followed by ganciclovir. Hum gene Ther 10: 2325–2335, 1999
38. Goodman JC, Trask TW et al.: Adenoviral-mediated thymidine kinase gene transfer into the primate brain followed by systemic ganciclovir: pathologic, radiologic, and molecular studies. Hum Gene Ther 7(10): 1241–1250, 1996
39. Packer RJ, Raffel C, Villablanca JG, Tonn JC, Burdach SE, Burger K, Landfond d, McComb JG, Cogen PH, Vezina G, Kapkala LP: Treatment of progressive or recurrent pediatric malignant supratentorial brain tumors with herpex simplex virus thymidine kinase gene vestor-producer cells followed by intravenous ganciclovir administration J Neurosurg 92, 249–254, 2000
40. Brem H, Ewend MG, Piantadosi S, Greenhoot J, Burger PC, Sisti M: The safety of interstitial chemotherapy with BCNU-loaded polymer followed by radiation therapy in the treatment of newly diagnosed malignant gliomas: phase I trial. J Neuro-Oncol 26: 111–123, 1995
41. Kramer ED, Packer RJ, Ginsberg J, Goldman S, Thompson S, Bayer LA, Shen V, Harris R, Khan S, Finlay JL: Acute neurologic dysfunction associated with high dose chemotherapy and autologous bone marrow rescue for primary malignant brain tumors. Pediatric Neurosurg 27: 230–237, 1997
42. Prados MD, Russo C: Chemotherapy of brain tumors. Semin Surg Oncol 14: 88–95, 1998
43. Deliganis AV, Baxter AB et al.: Serial MR in gene therapy for recurrent glioblastoma: initial experience and work in progress. AJNR 18(8): 1401–1406, 1997
44. Colombo F, Zanusso M et al.: Gene stereotactic neurosurgery for recurrent malignant gliomas. Stereotact Funct Neurosurg 68(1–4): 245–251, 1997
45. Palu G, Cavaggioni A, Calvi P, Franchin E, Pizzato M, Boschetto R, Parolin C, Chilosi M, Ferrini S, Zanusso A, Colombo F 6: 330–337, 1999
46. Gilbert MR, Friedman HS, Kuttesch JF, Prados MD, Olson JJ, Reaman GH, Zaknoen SL: A phase II study of temozolomide in patients with newly diagnosed supratentorial malignant glioma before radiation therapy, Neuroncology 4: 261–267, 2002
47. Osamura T, Mizuta R et al.: Isolation of adenovirus type 11 from the brain of a neonate with pneumonia and encephalitis. Eur J Pediatr 152(6): 496–499, 1993
48. Rainov NG, Kramm CM, Aboody Guterman K, Chase M, Ueki K, Louis DN, Harsh GR 4th, Chiocca A: Retrovirus-mediated gene therapy of experimental brain neoplasms using the herpes simplex virus-thymidine kinase/ganciclovir paradigm. Cancer Gene Ther 3: 99–106, 1996
49. Ram Z, Culver KW et al.: Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells [see comments]. Nat Med 3(12): 1354–1361, 1997
50. Rosenberg SA, Blaese RM et al.: Human gene Marker/therapy clinical protocols. Hum Gene Ther 7(13): 1621–1647, 1996
51. Takamiya Y, Short MP et al.: An experimental model of retrovirus gene therapy for malignant brain tumors. J Neurosurg 79(1): 104–110, 1993

Address for offprints: Isabelle M. Germano, Department of Neurosurgery, Box 1136, One Gustave L. Levy Place, New York NY 10510, USA; Tel.: 212-241-9638; Fax: 212-831-3324; E-mail: isabelle.germano@msnyuhealth.org