

Phase I Study of Adenoviral Delivery of the HSV-tk Gene and Ganciclovir Administration in Patients with Recurrent Malignant Brain Tumors

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Between December 1996 and September 1998, 13 patients with advanced recurrent malignant brain tumors (9 with glioblastoma multiforme, 1 with gliosarcoma, and 3 with anaplastic astrocytoma) were treated with a single intratumoral injection of 2×10^9 , 2×10^{10} , 2×10^{11} , or 2×10^{12} vector particles (VP) of a replication-defective adenoviral vector bearing the herpes simplex virus thymidine kinase gene driven by the Rous sarcoma virus promoter (Adv.RSVtk), followed by ganciclovir (GCV) treatment. The VP to infectious unit ratio was 20:1. Our primary objective was to determine the safety of this treatment. Injection of Adv.RSVtk in doses $\leq 2 \times 10^{11}$ VP, followed by GCV, was safely tolerated. Patients treated with the highest dose, 2×10^{12} VP, exhibited central nervous system toxicity with confusion, hyponatremia, and seizures. One patient is living and stable 29.2 months after treatment. Two patients survived >25 months before succumbing to tumor progression. Ten patients died within 10 months of treatment, 9 from tumor progression and 1 with sepsis and endocarditis. Neuropathologic examination of postmortem tissue demonstrated cavitation at the injection site, intratumoral foci of coagulative necrosis, and variable infiltration of the residual tumor with macrophages and lymphocytes.

Key Words: gene therapy; HSV-tk; ganciclovir; glioblastoma; astrocytoma; adenovirus; stereotaxic technique.

INTRODUCTION

The American Cancer Society estimates that 16,800 individuals will develop and 13,100 individuals will die from primary tumors of the nervous system in 1999, with the majority of these cases being malignant gliomas (1). Although there have been significant advances in neurosurgery, neuroradiology, radiation therapy, and medical oncology, malignant brain tumors remain resistant to all current therapies, and survival is often less than 1 year (2–4). Even when conventional treatments extend survival, malignant brain tumors almost invariably recur

because of surviving tumor cells that have invaded the surrounding brain. All treatments must balance effective tumor killing against toxicity to surrounding neural tissue. The prognosis after recurrence of a malignant brain tumor is particularly poor (3, 5).

Transduction of tumor cells with the herpes simplex virus thymidine kinase (HSV-tk) gene, which activates the nucleoside analog prodrug ganciclovir (GCV), has been one of the most effective approaches in treating experimental brain tumors (6–12). This therapy selectively kills dividing cells, an advantageous feature in the brain where most normal cells are not actively dividing. Treatment efficacy may be enhanced by the “bystander effect,” whereby surrounding nontransduced tumor cells are also killed through a variety of mechanisms which are believed to include transfer of toxic metabolites via gap

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junctions (13–15), induction of apoptosis (16), and activation of a host immune response (17–19).

Toxicity studies of intracerebral injection of an adenoviral vector, followed by GCV treatment, in cotton rats (20), which are permissive to adenoviral infection, and in other rodent models (7, 10, 21) have shown no clinical or systemic pathological sequelae. Our and other centers have also conducted preclinical toxicity studies in primates (21–23). Although clinical symptoms have not been observed following intracerebral injection of the vector at lower doses, dose-dependent toxicity has been observed in animals receiving higher doses of the vector and GCV (21, 22). The goal of the present study was to determine the safety of Adv.RSVtk + GCV treatment for recurrent advanced malignant brain tumors in humans and to determine the maximal safely tolerated vector dose for intratumoral injection at a single site.

MATERIALS AND METHODS

Permission for this Phase I clinical trial was granted by the Baylor College of Medicine Institutional Review Board (IRB), the Food and Drug Administration (FDA), and the National Institutes of Health Recombinant DNA Advisory Committee (RAC) on the basis of the efficacy and toxicity studies we performed in animal models (7–10, 20, 22) and after production and testing of clinical grade adenoviral vector. Adverse events and patient deaths were reported to the IRB, FDA, and RAC according to the recommended guidelines.

Patient Selection

Adults (≥ 18 years of age) with recurrent and clinically progressive malignant glial or metastatic tumors were eligible for enrollment. Histological confirmation of the diagnosis was required, and tumors had to be identifiable on neuroimaging studies. Subjects eligible for the study had received and failed conventional treatments, including surgery and radiation therapy. A Karnofsky performance score ≥ 60 was required. In addition, adequate systemic organ function was required as determined by the following criteria: serum creatinine of ≤ 1.5 mg/dl or creatinine clearance ≥ 45 ml/min/m², platelet count $\geq 100,000$ /mm³, absolute neutrophil count ≥ 8.5 /mm³, normal PT and aPTT, and bilirubin ≤ 2.5 mg/dl.

Specific exclusion criteria included acute infection (including HIV infection), progressive systemic malignancy, or unacceptable anesthesia risk. Patients with tumors with cavities likely to communicate with either a subarachnoid space or the ventricular system were excluded. Patients with tumors causing significant mass effect or increases in intracranial pressure and requiring surgical debulking were not eligible. Pregnant patients were excluded, and female subjects of child-bearing age were required to practice birth control for the duration of the study. Patients were informed that the treatment was experimental, and detailed informed consent was obtained at enrollment.

Prior to the gene therapy treatment, each patient underwent a complete history and physical examination, laboratory testing, magnetic resonance imaging (MRI) of the brain, and neuropsychological testing. The neuropsychological test battery was tailored to each patient's level of functioning, area of brain involvement, and endurance, with the goal being detection of significant changes from the baseline examination. Tumor histology was reviewed by the study neuropathologist (J.C.G.) to confirm tumor diagnosis. Pretreatment presence of serum-neutralizing antibodies to wild-type adenovirus and of adenovirus in serum, urine, and nasal mucosa was determined for each patient.

Creation of the Adv.RSVtk Vector

Construction of the replication-deficient viral vector has been described in detail previously (10, 24). The adenovirus E1A region was replaced with the HSV-tk gene under the control of the Rous sarcoma virus (RSV) long-

terminal repeat and grown in 293 cells. The vector was produced at the Baylor College of Medicine Gene Vector Laboratory using Good Laboratory Practices. In addition to standard quality control procedures used in vector production, we verified that the vector preparation did not contain measurable wild-type contamination or EIA recombination from 293 cells by polymerase chain reaction (PCR) for the wild-type EIA sequence. The vector preparation was certified to be free of replication-competent adenovirus at < 1 per 10^{10} VP. Additionally, the failure of cotton rats to sustain productive adenoviral infections after intracerebral delivery of the vector and the failure of adenoviral proliferation on HeLa cells in culture established that there was no significant replication-competent viral contamination. The sensitivity of the HeLa culture assay was one wild-type genome per 75,000 vector genome equivalents.

Adv.RSVtk Vector Dose

Based on our previous toxicity trials (22), we designed a dose-escalation protocol to test the safety of four Adv.RSVtk vector doses: 2×10^9 , 2×10^{10} , 2×10^{11} , and 2×10^{12} VP. The VP to infectious unit (IU) ratio, measured using the cytopathic effect (CPE) assay (25), was determined to be 20:1. A minimum of two patients were injected with each dose, beginning with the lowest dose, 2×10^9 VP (1×10^8 IU); if no toxicity was observed over the next month, subsequent patients received the next higher dose.

Injection of the Adv.RSVtk Vector

A Leksell stereotactic frame was applied to the patient's skull using local anesthesia and monitored anesthesia care. Tumor coordinates were generated after computerized tomography (CT) or MRI with the Leksell frame in place. Patients were returned to the operating room, and an 18-gauge stainless steel injection cannula was directed to the target through a twist-drill craniotomy or burr hole. One milliliter of vector solution was injected through the cannula over 5 min. The cannula was left in place for an additional 5 min and then withdrawn. A central venous catheter was placed in most patients to facilitate subsequent intravenous access.

Patients were monitored in the neurosurgical intensive care unit and then transferred to a private room on the neurosurgical unit. A postoperative MRI was obtained within the first 24 h to confirm injection placement and to observe if swelling or hemorrhage had occurred. Standard perioperative medications were administered, including antibiotics, dexamethasone, anticonvulsants, and analgesics.

Ganciclovir Treatment

Twenty-four hours after vector injection, intravenous GCV administration was begun, using a dose of 5 mg/kg over 1 hour, every 12 h, for a total of 28 doses over 14 days. During GCV treatment, patients underwent daily physical and neurological examinations and frequent laboratory studies including complete blood counts, electrolyte levels, and liver function tests. Serum, urine, and nasal swab samples were monitored for vector shedding, and serum was tested for neutralizing antibodies.

Toxicity following vector injection and during GCV treatment was graded using the Common Toxicity Criteria published by the Cancer Therapy Evaluation Program of the National Cancer Institute (26). Permanent Grade 3 or recurrent Grade 4 toxicity was the criterion for cessation of treatment.

The GCV (Cytovene) used in this trial was manufactured by Syntex Corp. (Palo Alto, CA). The drug is approved for the treatment of cytomegalovirus (CMV) retinitis in immunocompromised patients and for the prevention of CMV disease in transplant patients at risk for CMV disease.

Posttreatment Assessment

Patients underwent a neuropsychological evaluation approximately 2 weeks after vector injection. After discharge from the hospital, patients were seen as outpatients every 2 weeks for the first 2 months and then monthly for the first year. Physical examination and laboratory studies were obtained at each visit. A second neuropsychological evaluation was performed at 2 months postinjection. MRI scans were obtained in the first week after completion of HSV-tk/GCV treatment and every 12 weeks

TABLE 1
Patient Pretreatment Characteristics

PT	Age at DX (years)	Age at GT (years)	DX-GT period (months)	Sex	Tumor path	Side	Loc	KFS %	No. Pretreatment resect	XRT	Chem
1	33.2	34.8	19.3	M	GBM	L	Fr	60	1	Y	Y
2	46.2	52.4	74.6	M	AA	R	P	60	3 + 1P	Y	Y
3	57.2	57.8	7.3	M	GBM	L	O	70	2	Y*	Y
4	42.4	53.8	136.7	M	AA	R	FrT	80	1	Y*	Y
5	67.3	68.6	15.7	M	GBM	R	Fr	60	1	Y	YY
6†	41.0	41.8	9.8	F	GBM	L	Fr	60	2	Y*	YY
7	38.3	39.3	12.2	M	GBM	L	P	90	2 + 1P	Y + 1P	Y
8	64.1	65.3	14.9	M	GS	B	Fr	80	2	Y	N
9	64.3	64.9	7.5	M	GBM	R	T	60	1	Y	Y
10	47.6	48.5	10.7	M	GBM	R	PO	80	3	Y	N
11	46.2	50.0	44.6	M	GBM	L	T	80	2	Y	YY
12	37.3	38.3	12.3	F	GBM	R	P	80	1	Y	Y
13	43.9	45.0	13.6	M	AA	L	Fr	60	2	Y	Y
	Mean	Mean	Mean	11 M	9 GBM	7 L	5 Fr	6 60%	2 3r	13 Y	11 Y
	48.4 ± 11.2	50.8 ± 11.0	29.2 ± 34.4	2 F	1 GS	6 R	3 P	1 70%	6 2r		2 N
					3 AA		2 T	5 80%	5 1r		
							1 O	1 90%			
							1 FrT				
							1 PO				

Note. Abbreviations used: AA, anaplastic astrocytoma; B, bilateral; Chem, chemotherapy; DX, diagnosis; F, female; Fr, frontal; FrT, frontotemporal; GBM, glioblastoma multiforme; GS, gliosarcoma; GT, gene therapy; KFS, Karnofsky scale; L, left; Loc, tumor location; M, male; N, no; O, occipital; P, parietal; PO, parietooccipital; PT, patient; DX-GT period, time between tumor diagnosis and GT; r, resect, resections; R, right; T, temporal; XRT, X-radiation treatment; Y, yes; YY, two courses of chemotherapy; ±, standard deviation; *, subsequent XRT boost to tumor; +1P, also underwent a post-GT resection or XRT; †, patient diagnosed and treated (resection, radiation, and chemotherapy) for oligodendroglioma 4 years before GBM, for which she also underwent resection, radiation (focused), and chemotherapy.

thereafter. The MRIs were read independently by a neuroradiologist and by a neurosurgeon. Tumor response was monitored by clinical criteria and by MRI. Palliative and therapeutic options were discussed and made available to patients who deteriorated due to tumor progression.

Neutralizing serum antibodies to adenovirus type 5 (AV5) were determined before and at 14, 28, 42, 56, and 84 days after vector injection in most patients and thereafter at outpatient follow-up visits in patients with extended survival. The assay used to measure AV5-specific neutralizing serum antibodies has been described in detail previously (20). Neutralizing antibody titers were expressed as the \log_2 of the reciprocal of the last dilution of antiserum that inhibited virus-induced cytopathic effects by 100%.

Serum, urine, and nasal swabs were obtained at 1, 2, and 4 weeks after vector injection and examined by plaque assay for evidence of adenovirus shedding. A detailed description of the plaque assay has been published previously (20). Minimum detection in these assays was 20 plaque-forming units (pfu).

The presence of viral vector DNA in urine was determined by PCR and Southern blot hybridization of the PCR product. The sensitivity was 10 VP/ml of urine. Urine was analyzed daily for vector DNA during hospitalization and at 2 and 6 weeks after vector injection. The procedure used has been previously described (22).

RESULTS

Thirteen patients were enrolled into the study between December 1996 and September 1998 (Table 1). The mean age was 50.8 years (range 34.8 to 68.6 years). There were 11 males and 2 females. Pretreatment Karnofsky scores ranged from 60 to 90. All patients had primary central nervous system neoplasms. Tumor histologies were nine

glioblastoma multiformes (GBM), one gliosarcoma, and three anaplastic astrocytomas (AA). The mean intervals between initial diagnosis and adenoviral treatment for patients with a GBM or GS were 15.5 (\pm SD 11.6) and 14.9 months, respectively; for patients with an AA the mean interval between initial diagnosis and Adv.RSVtk treatment was 74.9 (\pm SD 61.5) months.

All patients had undergone previous tumor resection and had received conventional external beam radiation therapy. Eleven of the 13 patients had received chemotherapy, and 2 patients had received additional chemotherapy after tumor recurrence. Three patients had received conformal radiation boosts, with 2 of the 3 receiving the boost after tumor recurrence. Eight patients had undergone reoperation for tumor debulking.

Tumor characteristics on imaging were typical of advanced recurrent gliomas, with complex configurations, cystic areas, and brain invasion. Quantitative descriptions are difficult in this setting, but the cross-sectional diameter was greater than 3 cm in the majority of patients.

Clinical Course during Treatment

Table 2 summarizes the clinical courses of all patients enrolled. The sequence of dose escalation was as follows: 2 patients each received 2×10^9 and 2×10^{10} VP; 3 patients received 2×10^{11} VP; and 2 patients received $2 \times$

TABLE 2
Gene Therapy and Outcome

PT	VP	Cog (2 weeks)	GT Tox	Postoperative adverse events (days 1–14)	Post-DX survival (months)	Post-GT survival (months)
1	2×10^9	—	N	Perioperative focal seizures, small intratumoral hemorrhage	47.6	28.4
2	2×10^9	↓	N	Slight rash	78.2	3.5
3	2×10^{10}	—	N		11.3	4.0
4	2×10^{10}	—	N		162.6	25.9
5†	2×10^{11}	↓	N	Increased left hemiparesis, thrombocytopenia	16.8	1.1
6	2×10^{11}	—	N	Seizure day 6	40.0**	29.2**
7	2×10^{11}	↑	N	Thrombocytopenia	21.5	9.3
8	2×10^{12}	↓↓*	Y	Lethargy, confusion, mild hyponatremia, fever, leukocytosis, intratumoral hemorrhage day 6	16.9	2.0
9^	2×10^{12}	↓↓*	Y	Air in ventricle, lethargy, confusion, fever, hyponatremia, leukocytosis, increased CSF protein, hydrocephalus	10.1	2.6
10	2×10^{11}	↓	N		16.2	5.5
11	2×10^{11}	↓	N	Increased right hemiparesis, lethargy	45.7	1.1
12	2×10^{11}	—	N	Thombocytopenia, increased liver enzymes	14.3	2.0
13	2×10^{11}	—	N	Increased right hemiparesis, hyponatremia	20.9	7.4
					M 38.4 ± 42.1 (median 20.9)	M 9.2 ± 10.5 (median 4.0)

Note. Abbreviations used: Cog, change in cognition from baseline at 2 weeks after Adv.RSVtk injection; DX, diagnosis; GT, gene therapy; M, mean; N, no; Tox, toxicity; VP, vector particles (20:1 VP:IU ratio); Y, yes; ↑, slightly better; —, same; ↓, slightly worse; ↓↓, worse; ^, completed 4 of 28 GCV treatments; †, received 25 of 28 GCV treatments; *, unable to undergo formal testing due to neurological decline; **, patient is alive at time of publication.

10^{12} VP. After evidence of toxicity at 2×10^{12} VP, the 4 subsequent patients were treated with 2×10^{11} VP. Eleven of the patients completed the full 2-week course of treatment with GCV. Patient 9 received 4 of the 28 GCV doses. Patient 5 received 25 of the 28 GCV doses.

No patient who received 2×10^9 VP ($N = 2$) or 2×10^{10} VP ($N = 2$) experienced any significant treatment-related toxicity other than brief perioperative focal seizures in patient 1 who had a known seizure disorder at the time of enrollment. This patient also developed a small intratumoral hemorrhage that resolved without treatment. Patient 2 developed a slight rash that may have been caused by the GCV. The rash resolved without treatment and without interruption of the GCV treatment.

In the 2×10^{11} VP group ($N = 7$), three patients (patients 5, 11, and 13) experienced a mild increase in pre-existing hemiparesis during GCV treatment. Patient 13 also developed transient hyponatremia (131 meq/liter on day 7 after vector injection). This patient had required Na tablets for hyponatremia prior to gene therapy. Patient 12 developed thrombocytopenia (platelet count = 132,000/ mm^3 on day 13), and the platelet counts of two patients (patients 5 and 7) with low baseline platelet counts (132,000 and 144,000/ mm^3 , respectively) declined during GCV treatment (101,000/ mm^3 on day 11 and 78,000/ mm^3 on day 12, respectively). Platelet counts returned to baseline following GCV treatment; platelet transfusion was not required.

Three (patients 5, 11, and 12) of the seven patients who received 2×10^{11} VP had rapidly deteriorating clinical courses (patient 5 withdrew from the study near the com-

pletion of GCV treatment) and died within 2 months of gene therapy. There was no evidence of treatment-related toxicity in these three patients. Patients 5 and 11 had evidence of rapid tumor progression that continued during treatment. Patient 12 developed pneumonia 5 weeks after injection, followed by disseminated intravascular coagulation (DIC), adult respiratory distress syndrome (ARDS), sepsis, and multiorgan failure. She died 2 months after vector injection; postmortem examination revealed marantic endocarditis.

Both patients treated with 2×10^{12} VP of the Adv.RSVtk vector demonstrated clinically significant toxicity during the treatment period. The first patient (patient 8) had been treated for a left frontal gliosarcoma that had recurred in the right frontal lobe after two previous resections. Twenty-four hours after injection of the vector into the right frontal tumor mass, the patient exhibited confusion and lethargy. He improved and GCV was started on day 2. Six days after vector injection, the patient had a generalized seizure, and a CT scan demonstrated a right frontal hematoma. The patient improved after the postictal period, but did not return to his neurological baseline. He had hyponatremia ($\text{Na} = 129$ meq/liter on day 12), leukocytosis, and low-grade fever (38.7°C on day 11). He improved, completed GCV treatment, and was discharged from the hospital. He remained stable after discharge until 6 weeks after vector injection when he presented with status epilepticus. He did not regain consciousness. A CT scan demonstrated severe bifrontal edema suggestive of tumor progression. The patient's family asked that care be withdrawn. He died 2 months after vector injection. Post-

mortem examination of the brain showed cavitation in the right frontal lobe along the injection tract, surrounding edema, and mass effect. Gross residual tumor was seen in both frontal lobes. The residual right frontal tumor was heavily infiltrated with lymphocytes, and foci of coagulative necrosis were present. Scattered perivascular lymphocytes were seen in the white matter of the injected hemisphere and in the pons, and there was also patchy denudation of the ependyma on the injected side. No demyelination or macrophage infiltration was seen in association with the perivascular lymphocytes. There was no evidence of brain herniation.

The second patient (patient 9) who was treated with 2×10^{12} VP had a recurrent right temporal GBM. He complained of a severe headache in the immediate postinjection period, and a CT scan demonstrated air within the ventricular system, raising the possibility of vector entry into the cerebrospinal fluid. Twelve hours after the injection the patient became obtunded, was unable to follow commands, and developed a fever of 38.9°C. He subsequently recovered rapidly to his neurological baseline, and GCV treatment was initiated. Three days postinjection the patient developed hyponatremia ($\text{Na} = 126$ meq/liter) and obtundation. Ganciclovir treatment was stopped. He initially improved after correction of the hyponatremia, but later deteriorated. A CT scan demonstrated hydrocephalus, and a ventriculoperitoneal shunt was placed on day 14. CSF protein was found to be markedly elevated (624 mg/dl). The patient subsequently gradually improved until tumor progression supervened. The patient died 2.6 months after vector injection. An autopsy was not performed.

One patient (patient 6) with a GBM is alive 29.2 months after vector injection. She is clinically stable and her left frontal tumor has changed little over the 2-year period (Fig. 1). Two other patients, one with a GBM (patient 1) and one with an AA (patient 4), survived for 28.4 and 25.9 months, respectively. A transient reduction in tumor dimensions was observed in patient 4, and both patients had a posttreatment interval of reduced steroid requirements. Survival times to date range from 1 to 29.2 months (median 4.0, mean $9.4 \pm \text{SD } 10.8$ months) (Fig. 2).

Neuropathologic examination of six brains from patients who received 2×10^{10} VP (patients 3 and 4), 2×10^{11} VP (patients 7, 10, and 12), or 2×10^{12} VP (patient 8) disclosed recurrent viable malignant glioma in all specimens. Minimal to moderate intratumoral inflammation and foci of coagulative necrosis were observed in all specimens. As noted earlier, minimal extratumoral inflammation was seen in the brain of patient 8, who received the highest vector dose. Patient 7, who received 2×10^{11} VP, underwent tumor debulking at 2 months and died 9 months after vector injection. Neuropathologic examination of the resected tumor at 2 months showed heavy macrophage and lymphocyte infiltration and areas of coagulative necrosis within the GBM. No inflammation was observed in more viable areas of the tumor. At autopsy, considerable coagulative necrosis was found in the recurrent tumor but minimal intratumoral inflammation. No

inflammation was seen in the brain outside of the tumor bed. Detailed neuropathologic studies will be reported separately.

No adenovirus was detected by plaque assay in any of the serum, urine, or nasal swab samples obtained after vector injection or in any of the urine samples examined by PCR.

Posttreatment elevations in adenoviral antibody titers, relative to pretreatment levels, were detected in 10 of the 12 patients studied (Fig. 3A). In 3 patients with extended survival whose antibody titers were followed for more than 100 days after vector injection (Fig. 3B), 2 patients (patients 4 and 6) maintained significantly elevated titer levels. The third patient (patient 1) had titer levels around baseline throughout his treatment.

Neuropsychological testing was performed shortly before vector injection in all patients, at 2 weeks (at the completion of GCV treatment) in 11 of the 13 patients (testing could not be performed for patients 8 and 9), and at 2 months after vector injection in 6 of the 9 patients alive at 2 months. Because of significant differences in the patients' cognitive abilities and because of the deteriorating clinical course of most of the patients, meaningful neuropsychological data for the group as a whole could not be obtained. Comparison of each patient's performance before and at the end of GCV treatment (Table 2) identified no obvious treatment-associated effect on neuropsychological performance.

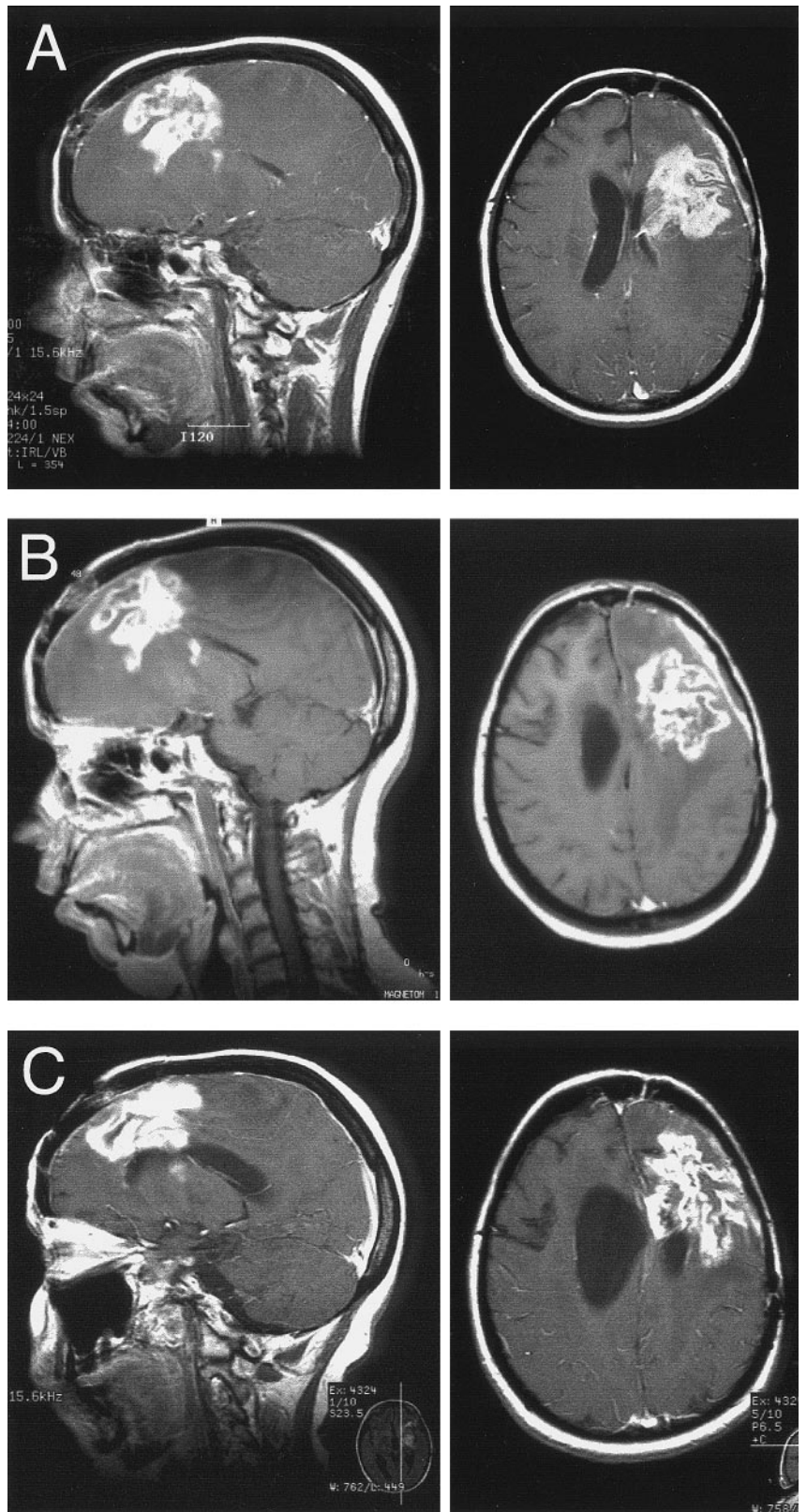
DISCUSSION

Genetic approaches to treating neoplasia have included the transduction of tumor cells with genes or gene products that activate cytotoxic prodrugs (27), upregulation of tumor suppressor genes such as p53 (28, 29), stimulation of immune response (30–32), and enhancement of cellular response to other therapies such as radiation (33, 34).

A number of viral and nonviral vectors are available for gene delivery. Experimental brain tumors have been successfully treated with retroviral (35–39) and adenoviral (6, 10–12, 28) vectors, and clinical trials using these approaches to treat malignant brain tumors have been and are being conducted (40–45). Adenoviral vectors are attractive for use in gene therapy because of their efficiency in transducing many cell types, ease of high titer production, episomal genome location, and ability to transduce nondividing cells.

Our Phase I trial examined the safety of the injection of Adv.RSVtk into a single intratumoral site, followed by intravenous GCV, in patients with advanced malignant brain tumors. Pre- or posttreatment tumor resection was not included as a part of this protocol although two patients underwent postinjection tumor debulking because of tumor growth. Multiple or repeat vector injections were not carried out. The trial was designed in this manner for the following reasons: (i) the simple design offered the best opportunity to establish the safe/toxic dose levels, (ii) any treatment effect would not be confounded with other treatments, and (iii) the design com-

FIG. 1. Forty-two-year-old female (patient 6) with a recurrent left frontal glioblastoma multiforme who received 2×10^{11} VP (1×10^{10} IU) of the Adv.RSVtk vector, followed by intravenous GCV. (A) Pretreatment (1 day before injection of Adv.RSVtk). (B) Two months after vector injection. (C) One year after vector injection. (D) Two years and 2 months after vector injection.



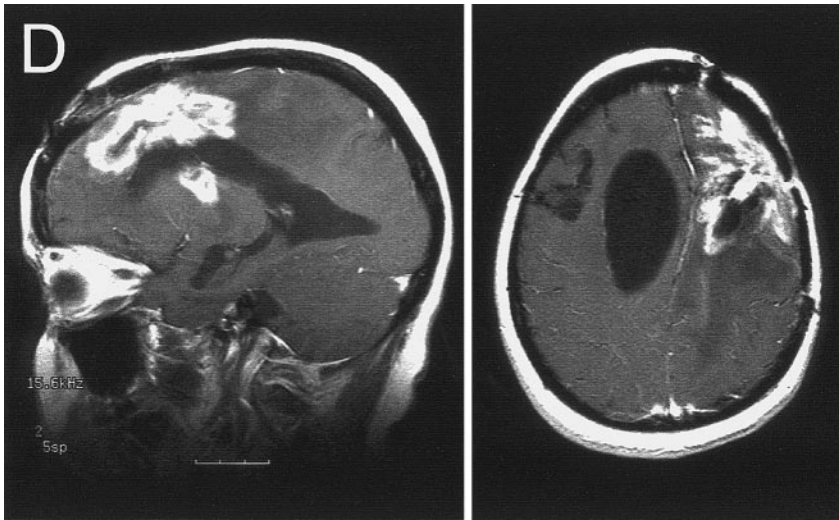


FIG. 1—Continued

plemented another Phase I trial of patients with advanced brain tumors in which resection and multiple adenoviral vector injections were being used (44).

We conclude that the form of gene therapy used in our Phase I trial is safe when the vector dose does not exceed 2×10^{11} VP (1×10^{10} IU). There was significant toxicity in patients 8 and 9 who were treated with 2×10^{12} VP. Factors that may have potentiated toxicity in these two patients include older age (age 65), bilateral tumor and hemorrhage in patient 8, and possible intraventricular injection in patient 9.

Toxicity of the nature we observed was not seen in another Phase I brain tumor protocol using an adenoviral vector which is being carried out at the University of Pennsylvania (personal communication, Stephen L. Eck, University of Pennsylvania Medical Center, October 1999). In that trial, patients received two intratumoral injections, 1 week apart, using an adenoviral vector dose of 1×10^{11} pfu. Whether the differences in toxicity observed reflect dissimilarities between the vectors, the doses administered, the patients, or the protocols is currently not known. Although the highest dose we used [2×10^{12} VP (1×10^{11} IU as determined by CPE)] appears similar to that used in the University of Pennsylvania study, comparison of vector doses used in different studies is difficult. Nyberg-Hoffman and associates have demonstrated that the various assays and methodologies used to titer adenoviral vectors can produce markedly different estimates of the number of infectious units in a vector sample (25). In addition, toxicity following injection of an adenoviral vector may relate not only to the infectious dose employed, but also to the physical number of vector particles injected. Standardization of the methods used to determine adenoviral titers is needed in order to compare vector doses and to better assess toxicity.

The toxicity associated with adenoviral vectors appears to be the result of a multifactorial cellular and humoral immune response, which may also play an important role in the destruction of malignant neoplasms. In our Phase I

study, neuropathological examination of tumor after vector injection demonstrated marked intratumoral infiltration with inflammatory cells, suggesting that recruitment of an antitumor response of the immune system may play a role in killing tumor cells. Dewey and associates have recently reported long-term inflammation in normal brain in a rat tumor model in which the rats were injected intratumorally with an adenoviral vector (46). We did not observe such severe or widespread inflammatory changes in normal brain tissue in any of the specimens examined.

Three patients in our study, 2 with a GBM (patients 1 and 6) and 1 with an AA (patient 4), survived >25 months, considerably beyond the expected survival for patients with recurrent malignant gliomas. A recent survival study followed a group of 130 patients with glioblastoma multiforme, some of whom underwent chemotherapy (74%) or stereotactic radiation (7%) but not resection, following tumor recurrence (3). These patients had, upon initial diagnosis, undergone resection of the tumor and had received external beam radiation therapy, usually with concomitant chemotherapy. The median survival

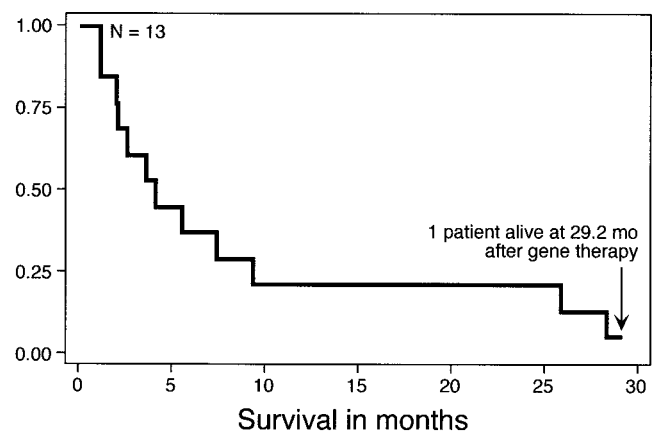


FIG. 2. Patient survival.

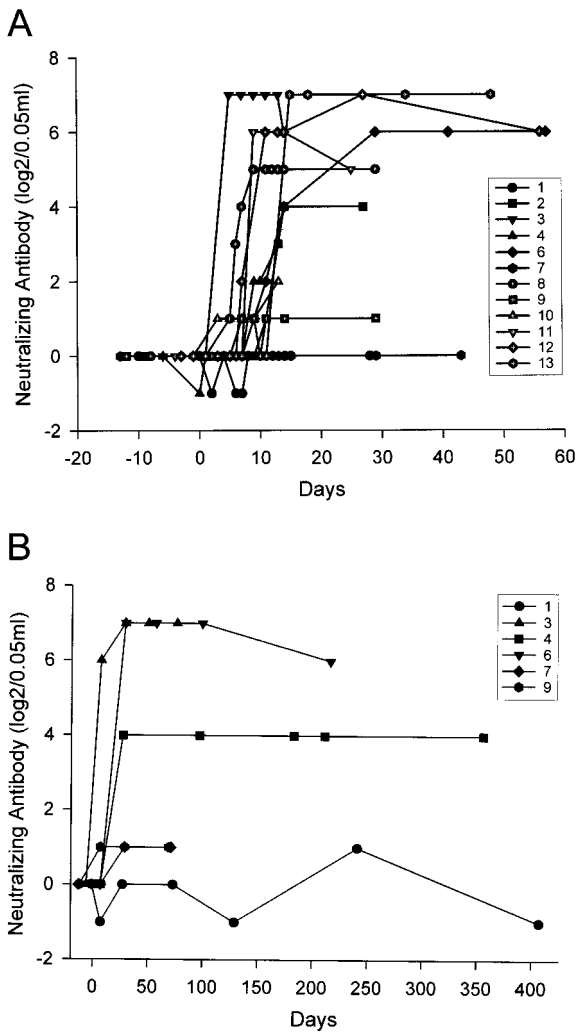


FIG. 3. Adenovirus antibody titers relative to levels immediately before gene therapy (day 0). (A) Titers for 12 patients over the first 2 months after Adv.RSVtk injection. (B) Titers for 6 patients with extended survival after Adv.RSVtk injection.

time after tumor recurrence for these 130 patients was 23 weeks (5.75 months). None of our 3 patients received any other treatment following Adv.RSVtk injection and GCV for their tumor. Each had one or more favorable prognostic factors for longer survival: younger age, AA in 1, and longer pretreatment survival in 2. One patient (patient 6) with a GBM is currently living with minimal tumor progression at 29.2 months after treatment (Fig. 1). Her age at treatment, 41.8 years, is comparatively young. Four years earlier she had been diagnosed with an oligodendroglioma in the same location and had undergone resection and radiotherapy. Subsequent to the diagnosis of GBM, she underwent another resection, chemotherapy, and focused radiation therapy but tumor progression continued. She underwent vector injection 9.8 months after diagnosis of GBM. The relative contribution of favorable prognostic factors and of Adv.RSVtk + GCV treatment cannot be determined in this case.

Current development of gene therapy for malignant brain tumors is being addressed in a number of areas: improving vector efficiency and minimizing immunologic response to the vector (47, 48), exploration of vector constructs utilizing different deletions as well as different promoters, and improvements in the construct or delivery of GCV or similar prodrugs that may extend the bystander effect and reduce GCV-mediated toxicity (14, 15, 49). In addition, the optimal delivery technique must be determined in order to more uniformly distribute the vector throughout the tumor. Possibilities include multiple stereotactic injections, injection of postresection tumor margins, and vector delivery in conjunction with blood-brain barrier disruption. Several centers have recently developed models that improve vector delivery to brain tumors by selectively modifying the blood-brain barrier (50, 51).

Finally, the best results may be obtained when this treatment is combined with other modalities such as radiation therapy, chemotherapy, and other gene therapy approaches. Improved tumor killing has been observed in several cancer models by combining HSV-tk/GCV treatment with radiotherapy (52, 53).

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