Treatment of progressive or recurrent pediatric malignant supratentorial brain tumors with herpes simplex virus thymidine kinase gene vector--producer cells followed by intravenous ganciclovir administration

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Object. The outcome for children with recurrent malignant brain tumors is poor. The majority of patients die of progressive disease within months of relapse, and other therapeutic options are needed. The goal of this Phase I study was to evaluate the safety of in vivo suicide gene therapy in 12 children with recurrent, malignant, supratentorial brain tumors.

Methods. After optimal repeated tumor resection, multiple injections of murine vector--producing cells shedding murine replication--defective retroviral vectors coding the **herpes simplex virus thymidine kinase type 1 (HSV-Tk1)** gene were made into the rim of the resection cavity. Fourteen days after the vector-producing cells were injected, ganciclovir was administered for 14 days. The retroviral vector that was used only integrated and expressed **HSV-Tk1** in proliferating cells, which are killed after a series of metabolic events lead to cell death. The median age of the patients was 11 years (range 2--15 years). Treated brain tumors included seven malignant gliomas, two ependymomas, and three primitive neuroectodermal tumors. The patients were treated with one of three escalating dose concentrations of vector-producer cells. Four transient central nervous system adverse effects were considered possibly related to the vector-producing cells. In no child did permanent neurological worsening or ventricular irritation develop, and tests for replication-competent retroviruses yielded negative findings.

Conclusions. This Phase I study demonstrates that in vivo gene therapy in which a replication-defective retroviral vector in murine vector--producing cells is delivered by brain injections can be performed with satisfactory safety in a select group of children with localized supratentorial brain tumors.

Key Words * pediatric brain tumor * gene therapy * astrocytoma * glioblastoma multiforme *

retroviral treatment * children

Abbreviations used in this paper: HSV-Tk1 = herpes simplex virus thymidine kinase type 1; KPS = Karnofsky Performance Scale; MR = magnetic resonance; PCR = polymerase chain reaction; PNET = primitive neuroectodermal tumor; RCR = replication-competent retrovirus.

Nervous system tumors are the leading cause of cancer-related death in children.[9] Although progress has been made in the management of some forms of childhood brain tumors, the majority of patients who have recurrent malignant brain tumors die within months of tumor recurrence. Treatment options are limited and consist primarily of attempts at repeated tumor resection, chemotherapy, and, in some cases, additional localized radiation therapy.[7] Alternative means of treatment, such as immunotherapy, have resulted in minimal success. The primary tumor site remains the most common location for tumor recurrence, and new means to enhance local tumor control are needed for many forms of malignant childhood brain tumor, including high-grade gliomas, ependymomas, and a significant proportion of PNETs.

Recent advances have made it possible to consider using gene transfer techniques for the treatment of cancer.[4] A variety of different vectors and delivery techniques are being studied.[4] One approach is to use retrovirus-mediated in vivo gene transfer, which, theoretically, has multiple advantages when used in the central nervous system.[3,8] Retroviral vectors result in complementary DNA, which can integrate into chromosomal DNA only in proliferating cells. These transduced cells express the transgene coded by the retroviral vector. In the brain, the tumor contains the most mitotically active cells, with only macrophage-derived cells, blood cells, some glial cells, and endothelial cells placed at minimal risk of transduction. In humans the majority of mitotic activity leading to the generation of neurons has ceased by birth. The brain is a partially immunologically privileged site that may allow a somewhat longer survival time for xenogeneic murine cells, which can be used as vector-producer cells in the brain and may lead to a greater transduction frequency of the tumor.[3,8]

The retroviral vector used in this study was derived from the Moloney murine leukemia virus. The vector transfers **HSV-Tk1**, a suicide gene promoting negative cell selection. This vector, which results in integration of DNA predominantly in tumor cells, is replication incompetent. The **Tk1** gene is a negative selective marker or suicide gene, and, when exposed to ganciclovir, the ganciclovir acts as a substrate for the phosphorylation of HSV-Tk1, resulting in a monophosphate form of the drug. Cellular kinases convert the ganciclovir monophosphate to ganciclovir triphosphate and inhibit DNA polymerase, resulting in the inability of the cell to proliferate. The end result is cell death for those cells that take up the viral vector and are exposed to ganciclovir. Surrounding cells that are not transduced by the vector may also be killed via the "bystander" effect.[2,3,8]

In the present study, we treated children harboring recurrent supratentorial brain tumors by using this genetic approach followed by ganciclovir infusion. The study was designed to determine the safety of using such an approach in children with surgically accessible recurrent malignant brain tumors that were separate from the ventricle. In this Phase I study, three different concentrations of vector-producing cells were used. The patients were also monitored to determine if they suffered any toxicity, evidence of viral infection, or systemic illness secondary to treatment, and for biosafety testing.

CLINICAL MATERIAL AND METHODS

Children between the ages of 2 and 18 years with progressive or recurrent malignant supratentorial brain tumors were eligible for study. Patients had to harbor a surgically accessible lesion, as estimated by pretreatment radiological evaluation, and must have received primary surgical treatment and completed radiation therapy at least 45 days prior to study entry. The patients had to have completed chemotherapy at least 28 days before study entry. There had to be evidence of progressive disease on contrast-enhanced MR images obtained in the patients within 30 days before initiation of treatment, and measurable disease had to be documented before the second surgical debulking. Before treatment commenced, those patients deemed eligible had to have a KPS score[5] of 60 or greater and adequate renal, liver, and hematological function. Patients with tumors that involved the brainstem or both hemispheres or those with multifocal disease were excluded from study. Tumor had to be located at least 1 cm away from the ventricular surface.

Viral Vector

The G1Tk1SvNa.7 is a retroviral vector derived from the Moloney murine leukemia virus.[3,8] The vector results in **HSV-Tk1** gene complementary DNA under transcriptional control of the viral long terminal repeat promoter and a bacterial neomycin-resistant gene under control of an internal SV 40 promoter. This G1-base vector was modified for increased safety by altering the gag protein start codon to a stop codon and by eliminating viral sequences needed in trans for the formation of the virus particle. These modifications have been shown to minimize the potential for the development of an RCR via recombination events in producer cells that contain the vector. The murine vector--producer cells (PA317 cells) have been tested in vitro and in human gene transfer therapy experiments.[3,6,8]

Injection of Vector-Producing Cells and Infusion of Ganciclovir

Initially, a craniotomy was performed and a biopsy specimen was obtained. After analysis of a frozen section of tissue confirmed the presence of tumor cells, optimal tumor removal was undertaken. The surgical margin of the tumor cavity was infiltrated at multiple sites with the **HSV-Tk1** vector--producer cells; a maximum volume of 10 ml was injected, although the amount of producer cells injected ($\frac{3}{4}$ 10 ml) was at the discretion of the operating neurosurgeon. Fourteen days later, ganciclovir was administered via a 1-hour intravenous infusion at 5 mg/kg twice daily for 14 days.

In this Phase I study, the patients were treated with different concentrations of murine virus--producer cells, although the maximum volume allowed remained constant. At dose Level 1, the patients were treated with 10^7 cells/ml; at dose Level 2 with 5×10^7 cells/ml; and at dose Level 3 with 10^8 cells/ml.

At completion of the ganciclovir infusion, MR imaging, hematological studies, serum chemical analysis, and complete neurological and physical examinations were repeated. After completion of the treatment cycle, the patients underwent follow-up clinical MR imaging and examinations at 1, 2, 3, 5, 7, 9, and 12 months posttreatment, for every 3 months for the 2nd year posttreatment, and at least annually thereafter for life.

Retroviral Gene Therapy Biosafety Monitoring

Before the initiation of treatment, after completion of the ganciclovir treatment, and at follow-up Months 1, 2, 3, 7, 9, and 12, blood was obtained to evaluate: 1) the presence of an antibody to retroviral protein; 2) the presence of RCR via DNA PCR analysis of peripheral blood leukocytes; and 3) RCR coculture by using **Mus dunni** (performed only if positive antibody to retroviral protein was found). In addition,

antibodies to producer cells were assayed on specimens collected at screening and within 24 hours before the start of ganciclovir therapy. Vector DNA PCR analysis of peripheral blood leukocytes was performed only on specimens collected at screening, within 24 hours before the start of ganciclovir therapy, and on Day 28 of treatment. If repeated surgery was required, DNA PCR analysis of tissue was performed to determine the presence of RCR.

Statistical Methods and Methods of Evaluation

The first three patients treated received vector-producer cells at dose Level 1. If one of three patients at dose Level 1 experienced a Grade 3 or 4 adverse event that was determined to be related to the vector-producer cells, two additional patients were enrolled at that same dose level. If at least two of the first three patients or three of the first five patients treated at dose Level 1 developed a Grade 3 or 4 adverse event that appeared to be related to treatment, accrual in the study was stopped. If none of the first three patients or no more than two of the first five patients treated at dose Level 1 experienced a Grade 3 or 4 adverse event that appeared to be related to the treatments, the dose of vector-producer cells was to be escalated to the next level, and so forth, until Level 3 was reached.

The MR imaging study obtained on Day 28 of treatment (after completion of ganciclovir infusion) was used as the baseline study. However, this study was also compared with the MR imaging study performed 24 to 48 hours after the surgery and injection of the viral vector. Determination of local progression during follow up was based on the volume of the contrast-enhancing lesion compared with the volume of the contrast-enhancing lesion observed on imaging obtained at the end of ganciclovir infusion. For those patients in whom disease could be evaluated on the 28th day of the study, responses were characterized as complete response (disappearance of all active disease); partial response ($\geq 50\%$ decrease in tumor volume); minor response (25--49% decrease in tumor volume); or stable disease ($< 25\%$ decrease or $\frac{3}{4}$ 25% increase in tumor volume). Patients were considered to have progressive disease when there was a greater than 25% increase in the volume of the treated lesion compared with that observed on the baseline image (Day 28) or if a new lesion developed that was not contiguous with the original lesion.

RESULTS

Twelve children 2 to 15 years of age (median 11 years) harboring brain tumors were treated between November 1995 and December 1997. The brain tumors included malignant gliomas in seven patients (five anaplastic gliomas, one anaplastic ganglioglioma, and one glioblastoma multiforme), anaplastic ependymomas in two patients, and solitary cortical PNETs in three patients. Tumor locations included the frontal lobe in four patients (left frontal lobe in three and right frontal lobe in one), temporal lobe in three patients (left temporal lobe in all cases), temporoparietooccipital region in four patients (left side in all cases), and the insular region in one patient. Before surgery for tumor debulking and subsequent injection of the murine virus--producer cells, the median volume of the tumors was 4.5 cm³ (range 0.85--90 cm³). In the majority of patients following surgery, there was demonstrable tumor (as seen on the enhanced MR image) only in the rim of the surgical cavity, and volume assessment for residual tumor cells was impossible.

All patients had been treated previously, and the initial form of treatment had failed. Initial therapies included attempts at surgical debulking in all patients, with four patients having undergone at least two previous resections. Subsequent postsurgical treatments included radiotherapy alone in two patients (including one who had undergone two courses of radiotherapy before being treated with this protocol);

two regimens of multiagent chemotherapy without radiotherapy in the youngest child treated in this study; and treatment with both radiotherapy and chemotherapy in nine patients. No child was within 6 months of completion of a course of radiation therapy when gene therapy was initiated.

All patients treated at dose Level 1 received the full 10 ml of vector-producer cells. At the discretion of the treating surgeon, the first three patients treated at dose Level 2 received a reduced volume of vector-producer cells (0.8, 2.4, and 2 ml), and two additional patients were treated at this level with the full 10-ml dose. At dose Level 3, all patients received the full 10-ml dose; however, because a herpes zoster infection requiring ganciclovir treatment developed in one patient approximately 1 week earlier than planned, a fourth patient was enrolled to receive this concentration level.

Treatment Toxicity

In four patients significant toxicity (that is, a serious adverse event) developed, which was thought to be related to the experimental treatment (injection of vector-producer cells or ganciclovir exposure). Not unexpectedly, these serious adverse events involved the central nervous system. At dose Level 1, one child experienced a seizure, altered consciousness, and left-sided weakness 2 days after receiving vector-producer cells; all of these events resolved spontaneously within 24 hours. A second patient at dose Level 1 experienced headache, lethargy, nausea, vomiting, and increased left-sided weakness, which were thought to be caused by increased intracranial pressure; the results of MR imaging studies in this case were suggestive of increased peritumoral edema. The event occurred 15 days after injection of vector-producer cells and 1 day after the course of ganciclovir commenced. This adverse event required a temporary increase in the dose of glucocorticoid therapy (dexamethasone), which was ultimately tapered within 2 weeks after the event without residual sequelae. Because both of these events were transient and were thought to be due to the surgery or tapering of corticosteroid medications, the decision was made to escalate to dose Level 2.

In one patient a focal seizure and cerebral edema developed 23 days after injection of vector-producer cells and during the course of ganciclovir (9 days after initiation of ganciclovir therapy). This toxicity resolved in response to glucocorticoid therapy. One patient experienced headache, fever, a focal seizure, and cerebral edema 23 days after injection of vector-producing cells and 1 day after the conclusion of a 14-day course of ganciclovir.

There were other serious adverse events, which were not believed to be associated with treatment with vector-producer cells or ganciclovir: sepsis thought to be secondary to a central-line infection in one child who required brief hospitalization 27 days after treatment; shunt malformation occurred in one patient 3 days after surgery and again 24 days following vector-producer cell injection; and dermatological manifestations of a herpes zoster infection appeared in a third child 6 days after treatment with vector-producer cells, prompting early initiation of ganciclovir therapy.

Neuroimaging Findings

The neuroimaging features of the tumors were difficult to evaluate. In three cases, the MR image obtained on Day 28 demonstrated more enhancement around the tumor rim than the image obtained immediately after surgery (Fig. 1).

Fig. 1. Enhanced sagittal T₁-weighted MR images obtained in a 15-year-old girl 2 days (A), 1 month (B), 2 months (C), and 4 months (D) after she underwent resection of a recurrent anaplastic temporal glioma. The image obtained 1 month after surgery (B) reveals new, intense enhancement along the posterior edge of the surgical bed, which is consistent with an acute demyelinating or inflammatory process. This enhancement is resolved 2 months after surgery (C). Four months after surgery, new enhancement has developed along the superoposterior aspect of the surgical bed (D). Pathological investigation revealed recurrent anaplastic astrocytoma.

In addition, enhancement and/or signal change could be noted around the needle tracks in one patient (Fig. 2). Because minimal enhancement was observed on MR images in many patients, possibly reflecting residual disease after surgery and injection of vector-producer cells, tumor response could not really be assessed.

Fig. 2. Gadolinium-enhanced axial T₁-weighted MR images obtained in a 10-year-old girl after she underwent resection of a recurrent superficial left insular malignant glioma. The images were obtained immediately (A and D), 2 months after surgery (B and E), and 8 months after surgery (C and F). Postoperative enhancement is present in the surgical bed (long arrow in A). Two months after surgery, the operative site shows a decrease in the postoperative enhancement; areas of enhancement are now seen along the tracks of needle injection of vector-producer cells in the brain tissue deep into the surgical bed (arrows in B) and in the temporal lobe (arrows in E). These areas demonstrate complete resolution 8 months after surgery (C and F). This vector enhancement is not seen in the image obtained immediately postoperatively (A and D).

Patient Outcome and Short-Term Quality of Life

Ten of 11 patients experienced progressive disease at a median time of 5 months after treatment. One patient, who was clinically and radiologically stable, underwent focal radiation therapy 28 days following viral vector injection (at the discretion of the treating institution) and was deselected for evaluation. Disease progression, determined by clinical or radiologic changes, occurred at a median time of 3 months after initiation of treatment. One child with an anaplastic glioma is still alive and free of progressive disease 24 months posttreatment. This child previously had undergone two surgical resections and both radiotherapy and chemotherapy. Four other children had somewhat longer disease control lasting between 5 and 10 months. One child in this study, with a cortical PNET, exhibited evidence of disease progression outside the primary tumor site concurrent with local disease progression.

At the time treatment commenced, the patients' overall level of function was quite good; 10 patients had

KPS scores of 100, and the remaining two patients had KPS scores of 90. One month following treatment, in one child the KPS score improved from 90 to 100, and in two children, one of whom exhibited clear-cut progressive disease, the KPS score had fallen from 100 to 90.

All patients were receiving dexamethasone at the time of initial treatment. In one child, the dexamethasone could be completely tapered within 28 days of treatment, and in nine children the dexamethasone dose could be tapered (although not eliminated) by Day 28. One patient received a stable dose of dexamethasone through the first 28 days of therapy; in the remaining child, data concerning dexamethasone therapy were too incomplete to be evaluated.

Biosafety Testing

Throughout the study, in no patient was there evidence suggesting systemic illness due to vector-producer cell injection or evidence of RCR on blood tests in 50 posttreatment samples. Of 52 posttreatment samples tested for vector DNA sequences in peripheral blood leukocytes, 21 were positive in eight patients. Samples were intermittently positive (three patients), transiently positive (two patients), or always positive (three patients) in all posttreatment samples. In none of the 12 patients was there evidence of antibody (tested by Western blot analysis) to the retroviral p30 protein. In five patients brain tumor biopsy specimens were collected at repeated surgery performed at varying times (14 days--8 months) posttreatment after vector-producer cells were injected. All four patient samples tested for RCR by using PCR after vector-producer cells were injected proved negative. Only one of five patient samples indicated the presence of vector DNA according to PCR analysis. This positive result was seen 6 months after treatment.

DISCUSSION

The results of this study demonstrate that gene therapy in which a replication-defective retroviral vector in murine vector--producing cells is delivered by brain injection can be performed with satisfactory safety in children with recurrent brain tumors. This study was conducted at five centers, and the eligibility criteria were relatively restrictive. Only one half of all childhood brain tumors occur supratentorially, and, even in those patients, only a subgroup would be eligible because of the frequent midline origin of pediatric brain tumors.[7] By choosing only those patients with tumors farther than 1 cm from a ventricular margin, the possibility of their developing ventriculitis or ventricular irritation was limited. However, this restriction also further reduced the patient population eligible for treatment. Deep-seated lesions, such as those seen in patients with thalamic and chiasmatic tumors and, by study design, brainstem tumors were also excluded from study because it was not likely that they could be debulked adequately to leave only a rim of tissue before vector-producer cell injection. Direct infusion techniques might allow some patients with such tumors to be eligible for future studies in which viral vectors are used. In addition, by selecting only patients whose tumors were amenable to extensive resections, so that only a rim of tumor remained, tumor "response" to treatment could not be assessed.

Previously, this vector approach had been used in 15 adult patients with recurrent malignant tumors.[3,8] A decrease in tumor volume and loss of enhancement at the treated site was noted. Following this, a Phase II study in 30 patients with recurrent glioblastoma multiforme was completed.[1] In the Phase II study, an Ommaya reservoir was placed at the time of surgical resection, and 28 days following initial vector-producer cell injection those patients not demonstrating disease progression after initial injection were given additional treatment via the Ommaya reservoir. Patients could receive up to four cycles of treatment. In three patients ventricular irritation developed, presumably caused by inadvertent

administration of vector-producing cells into the cerebrospinal fluid. This method of vector-producer cell administration was also associated with Ommaya reservoir blockage and infection involving this delivery device. Transient neurological symptoms were noted in 11 patients within days to weeks after vector-producer cell injections and in three patients during ganciclovir treatment. The median survival of 29 eligible patients was 7 months, and five were alive 17 months or longer after treatment. A Phase III study of patients with newly diagnosed disease has recently been completed and the data are being analyzed. Because of difficulties in assessing responses and, possibly, increased toxicity due to slippage of the shunt tip into the ventricular region with resultant aseptic ventriculitis, the approach using multiple treatments through an Ommaya reservoir was not chosen in our Phase I study.

In the pediatric population, such a local approach to treatment will probably be of benefit only for patients harboring high-grade cortical gliomas and, possibly, anaplastic ependymomas. In this study, patients with cortical PNETs were also eligible for enrollment. However, in retrospect, because PNETs may have a higher proclivity to disseminate in the neuraxis (as was seen in two of our patients), such patients would not seem to be good candidates for our experimental approach. This local approach also severely limits patient accrual. Only two to three patients were treated per site during the time of study.

A probable major limiting factor is the vector transduction rate. In previous experimental studies, in which the same viral vector was chosen, widely ranging transduction rates of tumor cells were observed with this type of direct injection approach.[3,8] It was hoped that the injection of murine producer cells, being injected into a partially immunologically privileged site such as the brain, would allow more vectors to get to the tumor cells, making them sensitive to the ganciclovir. In both in vitro and in vivo studies, despite incomplete transduction rates of tumor cells after direct **HSV-Tk1** injection, complete elimination of the tumor could be documented. This was thought to be due to a bystander effect.[3,6,8] The explanation of this phenomena is still unclear but is thought to be related to the passage of the ganciclovir triphosphate through intercellular communication networks, perhaps via gap junctions, immune system--mediated effects, and/or damage to transduced vascular endothelial cells. It has been postulated that direct cell--cell contact is required in vitro for bystander killing to take place. Approaches with other viral vectors such as herpes virus and adenovirus are under study and may result in a higher transduction rate and potentially greater efficacy.[4] However, these other vectors may result in greater toxicity from transduction infection of normal cells intermixed with or surrounding the tumor. Another potential risk of the approach used in this study was that the retroviral vector, although replication incompetent, would result in DNA integration into nontumor cells and, potentially, be mutagenic.

Overall, the experimental therapy procedure and any swelling caused by it, including reactions to the vector-producer cells, the retroviral vectors, or the transgenes, or subsequent treatment with ganciclovir, were well tolerated. Four serious adverse events thought to be related to the experimental treatment were observed. These adverse events occurred predominantly within 1 month following the administration of vector-producer cells and most frequently occurred near the time of ganciclovir administration. Whereas one serious adverse event developed soon (2 days) after administration of vector-producer cells, three serious adverse events developed during (two patients) or immediately after (one patient) the conclusion of the course of ganciclovir. The adverse events occurring during or near the time of ganciclovir therapy were presumably related to ganciclovir-induced inflammation caused by the ganciclovir being phosphorylated, incorporated into newly synthesized DNA, killing cells, and eliciting an inflammatory response. Not unexpectedly, these adverse events were often associated with cerebral edema. In all cases, the neurological complications resolved spontaneously and/or in response to glucocorticoid management, and it is unclear whether the events were related to the tumor, its resection, or, specifically, to the

injection of the viral vector and subsequent treatment.

Our experience with biosafety testing indicated that no patient exhibited evidence of RCR. This was similar to a large series of adults receiving intracerebral vector-producer cells for treatment of brain tumors.[6] In several patients there was evidence of the presence of the vector DNA sequence in peripheral blood leukocytes (approximately 40% of all posttreatment samples). Conceivably, some patients with intermittently positive samples exhibited decrements of transduced cells followed by clonal expansion of cells. Alternatively, positive levels may have been observed intermittently because the level was near the limit of assay detection. In one patient, a positive vector DNA sequence in peripheral blood leukocytes disappeared after the course of ganciclovir was completed, as would be expected if positive cells were replicated at the time of ganciclovir therapy and, thus, sensitive to ganciclovir killing. Persistence of the positive specimens indicated an insensitivity to ganciclovir killing. Perhaps these cells were not mitotic during ganciclovir therapy, or they may have been transduced by a nonfunctional version of the **HSV-Tk** gene. Finally, only one of five tumor biopsies was positive for vector DNA. This observation further supports the concern that transduction efficiency is low when using this approach.

Outcome for patients in this study was somewhat disappointing, but not unexpected. In 10 of the 11 patients who could be evaluated, progressive disease ultimately developed, although stable disease was confirmed in at least five patients for at least 5 months after injection of the viral vector. One patient continues to be alive and free of progressive disease 18 months after treatment, despite experiencing disease progression prior to treatment after having undergone two surgeries and both radiotherapy and chemotherapy.

Given the need for total or near-total resection before injection of the viral vector--producer cells, the associated difficulty in evaluating postoperative and posttreatment imaging to determine a response, and the relatively limited number of patients available for evaluation, a Phase II pediatric study may be difficult to complete and evaluate. In our study, most patients did not have disease that could be measured and evaluated following surgical resection, and the image obtained on Day 28 often demonstrated more edema and enhancement than the baseline image obtained 2 days after surgery, with a subsequent decrease in the amount of enhancement. Thus, evaluation for an objective response to treatment would be difficult, and the most reliable measure would be time to progression or overall survival time. An alternative approach using this type of treatment would be to treat patients with newly diagnosed disease and poor prognoses at the time of initial resection with gene transfer therapy. Initially, this would need to be done as a toxicity trial focusing on safety, to determine the feasibility of using gene therapy in combination with radiotherapy and/or chemotherapy in such patients. Later, survival times in patients treated with gene therapy would need to be compared with patient outcome after standard treatment. However, given the poor prognosis of children with high-grade supratentorial tumors, especially malignant gliomas including glioblastoma multiforme, the need for new means to improve local control, and the satisfactory safety observed in this gene therapy study, future pediatric studies of gene therapy are warranted.

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