

# A Phase I Open-Label, Dose-Escalation, Multi-Institutional Trial of Injection with an *E1B*-Attenuated Adenovirus, ONYX-015, into the Peritumoral Region of Recurrent Malignant Gliomas, in the Adjuvant Setting

E. Antonio Chiocca,<sup>1,\*</sup> Khalid M. Abbed,<sup>1</sup> Stephen Tatter,<sup>1</sup> David N. Louis,<sup>1</sup> Fred H. Hochberg,<sup>1</sup> Fred Barker,<sup>1</sup> Jean Kracher,<sup>1</sup> Stuart A. Grossman,<sup>1</sup> Joy D. Fisher,<sup>1</sup> Kathryn Carson,<sup>1</sup> Mark Rosenblum,<sup>1</sup> Tom Mikkelsen,<sup>1</sup> Jeff Olson,<sup>1</sup> James Markert,<sup>1</sup> Steven Rosenfeld,<sup>1</sup> L. Burt Nabors,<sup>1</sup> Steven Brem,<sup>1</sup> Surasak Phuphanich,<sup>1</sup> Scott Freeman,<sup>2</sup> Rick Kaplan,<sup>3</sup> and James Zwiebel<sup>3</sup>

<sup>1</sup>The NABTT CNS Consortium, Baltimore, MD 21231, USA

<sup>2</sup>Onyx Pharmaceuticals, Redmond, CA, USA

<sup>3</sup>Cancer Therapy Evaluation Program, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

\*To whom correspondence and reprint requests should be addressed c/o The NABTT CNS Consortium, 1650 Orleans Street, Room G93, Baltimore, MD 21231. Fax: +1 410 614 9335. E-mail: jfisher@jhmi.edu.

Available online 24 August 2004

ONYX-015 is an oncolytic virus untested as a treatment for malignant glioma. The NABTT CNS Consortium conducted a dose-escalation trial of intracerebral injections of ONYX-015. Cohorts of six patients at each dose level received doses of vector from  $10^7$  plaque-forming units (pfu) to  $10^{10}$  pfu into a total of 10 sites within the resected glioma cavity. Adverse events were identified on physical exams and testing of hematologic, renal, and liver functions. Efficacy data were obtained from serial MRI scans. None of the 24 patients experienced serious adverse events related to ONYX-015. The maximum tolerated dose was not reached at  $10^{10}$  pfu. The median time to progression after treatment with ONYX-015 was 46 days (range 13 to 452 + days). The median survival time was 6.2 months (range 1.3 to 28.0 + months). One patient has not progressed and 1 patient showed regression of interval-increased enhancement. With more than 19 months of follow-up, 1/6 recipients at a dose of  $10^9$  and 2/6 at a dose of  $10^{10}$  pfu remain alive. In 2 patients who underwent a second resection 3 months after ONYX-015 injection, a lymphocytic and plasmacytoid cell infiltrate was observed. Injection of ONYX-015 into glioma cavities is well tolerated at doses up to  $10^{10}$  pfu.

**Key Words:** glioma, brain tumor, clinical trial, ONYX-015, gene therapy, oncolytic virus, experimental therapy, virotherapy, adenovirus, conditionally replicating adenoviruses

## INTRODUCTION

Current therapies such as surgery, radiotherapy, and chemotherapy have had only limited success in treating patients with malignant gliomas [1], and thus their prognosis remains grim [2]. Novel treatment strategies are needed [3–5]. Oncolytic viral therapy is one such strategy [6,7]. This consists of the use of replication-conditional viruses, genetically altered to render their replication selective for tumor cells. An example of a replication-conditional virus is ONYX-015, an adenovirus mutant that is thought to replicate more efficiently in cells with disruptions in the p53 tumor suppressor pathway (such as tumor cells) [8], although this mechanism remains controversial since other mechanisms of

replicative selectivity may be operative [9]. ONYX-015 has been tested in clinical trials for a variety of cancers, including head and neck, ovarian, prostate, and lung [10–23].

The standard therapy for patients with a suspected malignant brain mass is surgical excision, if possible. Resected tissue is analyzed and a diagnosis of glioma is made dependent on the presence of histologic features. The histologic features of the more malignant forms of glioma include nuclear atypia, endothelial proliferation, necrosis, and mitoses. Based on how many of these features are present, further classification of a malignant glioma into an anaplastic astrocytoma (World Health Organization grade III astrocytoma), an anaplastic oligo-

dendroglioma (World Health Organization grade III oligodendroglioma), or a glioblastoma multiforme (World Health Organization grade IV astrocytoma) is made. Because these tumors are characterized by extensive infiltration of single and multiple cells throughout the brain, which obviously cannot be resected, further treatment of patients with radiation is started 2 weeks after surgical excision. A total of 5400–6000 cGy is delivered to the tumor cavity and to the margins over a period of 6 weeks. At the end of this treatment, addition of chemotherapy with alkylating agents, such as BCNU, may provide additional therapeutic benefit. Throughout this time, patients are usually maintained on corticosteroids to reduce brain edema and anticonvulsants to reduce the incidence of seizures. Almost all tumors will recur during or after the above treatments, usually locally at the margin or site of the previous resection or, less often, at a distance from the main tumor mass. Generally, phase I/II experimental therapies are reserved for this group of patients.

The objectives of our study were to determine the safety and evaluate the efficacy of multiple injections of escalating doses of an E1B-attenuated adenovirus, ONYX-015, into the margins of a recurrent malignant glioma that has been resected in adult patients. We found that injection was very well tolerated without evidence of toxicities attributable to ONYX-015, up to a dose of  $10^{10}$  plaque-forming units (pfu). Although previous trials of direct, stereotactic injection of replicating, tumor-selective herpes simplex viruses into the malignant gliomas of patients were reported [24–26], this study provides the first demonstration that injections of a replicating, tumor-selective virus into brain tissue, adjacent to a freshly excised glioma, are well tolerated. This provides justification for additional studies of such modalities in patients with malignant gliomas.

## RESULTS

Twenty-four patients were enrolled in the study between January 2000 and May 2002. All twenty-four patients were treated and all included in the intent-to-treat population. Patient characteristics are shown in Table 1.

A summary of the adverse events that occurred during the treatment period is shown in Table 2. Ten of the 24 patients experienced one or more adverse events. None of the adverse events were judged as possibly related to ONYX-015 treatment, since other etiologies provided more likely explanations. This judgment was made by the clinicians involved in the care of these patients and was reviewed by the Data Safety Monitoring Board after each enrollment into each cohort was completed. This judgment was based on the likelihood that additional etiologies for the adverse events were more likely than the injection of ONYX-015. For instance, dyslexia, dyscalculia, and dysgraphia in the postoperative period after resection of a tumor near eloquent cortex responsible for such function was more likely due to the trauma of surgery than to ONYX-015 injection.

One patient in the high-dose ( $10^{10}$ ) cohort, whose on-study histology was anaplastic astrocytoma, has not progressed (stable disease). The remaining 23 patients have progressed. However, it should be noted that 1 of the patients who had been declared to have progressed because of increased enhancement on the MRI scan was found to have decreased enhancement on a subsequent scan (Fig. 1). This episode of increased enhancement with subsequent decreased enhancement occurred again a few months later, as detailed in the legend to Fig. 1. Median time to progression of disease after ONYX for all patients was 46 days with a range of 13 to 452 + days.

**TABLE 1:** Baseline demographic and clinical characteristics for all patients and stratified by on-study diagnosis

	All patients ( <i>n</i> = 24)	GBM ( <i>n</i> = 17)	Other (AA, AO) ( <i>n</i> = 7)
Sex, male	17 (71)	11 (65)	6 (86)
Race, white	24 (100)	17 (100)	7 (100)
Age, years	52 (35–70)	55 (37–70)	38 (35–61)
Karnofsky performance status	90 (60–100)	90 (60–100)	90 (70–100)
Prior chemotherapy <sup>a</sup>	22 (92)	16 (94)	6 (86)
Anticonvulsant therapy	18 (75)	11 (65)	7 (100)
Original diagnosis histology <sup>b</sup>			
Glioma	1 (4)	1 (6)	0 (0)
Grade 2 glioma	2 (8)	0 (0)	2 (29)
Anaplastic astrocytoma	5 (21)	0 (0)	5 (71)
Glioblastoma multiforme	16 (67)	16 (94)	0 (0)

Median (range) or *N* (%) is shown.

<sup>a</sup> All patients had prior surgery and XRT.

<sup>b</sup> Refers to the histology at initial presentation of the patient with disease. Patients then underwent conventional, standard treatment. At re-presentation with a recurrence, this histology was either the same or had progressed to a more malignant grading (the histology at recurrence is textually described under Results).

**TABLE 2:** Serious adverse events

Adverse event	Grade <sup>a</sup>	Number of patients
Neuropathy-motor	3/4	2
Dyslexia, dyscalculia, dysgraphia	3	1
Headache	3	1
Diarrhea	3	2
Confusion	3	2
Hypertension	3	1
Decreased LOC	3	1
Hyponatremia	3	1
Abnormal PT	3	1
Thrombosis/embolism	3	1
Febrile neutropenia	3/4	1
Fever	3	1
Nausea	3	1
Vomiting	3	1
Fatigue	4	1
Abnormal SGPT	4	1
Ataxia	4	1
Hydrocephalus	3	1

Ten of the 24 patients experienced one or more serious adverse events.

<sup>a</sup> The relationship to treatment was coded as unlikely for all serious adverse events.

3/6 AA, and 1/6 anaplastic oligodendroglioma (AO) for the 10<sup>10</sup> pfu cohort. For the 3 surviving patients, 2 patients had histologies of AA and the other had AO. The Kaplan–Meier survival curve for GBM and non-GBM (AA and AO) patients is shown in Fig. 2. Median survival for the GBM patients was 4.9 months, and for AA and AO patients it was 11.3 months.

We obtained serum from all of the patients prior to the introduction of ONYX-015 and again on day 42 to assess for antibodies to adenovirus. Of the 24 patients, only 2 patients tested positive for adenovirus antibodies before inoculation. After delivery of ONYX-015, 2 patients seroconverted from negative to positive for adenovirus antibodies. One patient belonged to the 10<sup>9</sup> cohort and the other patient belonged to the 10<sup>10</sup> cohort.

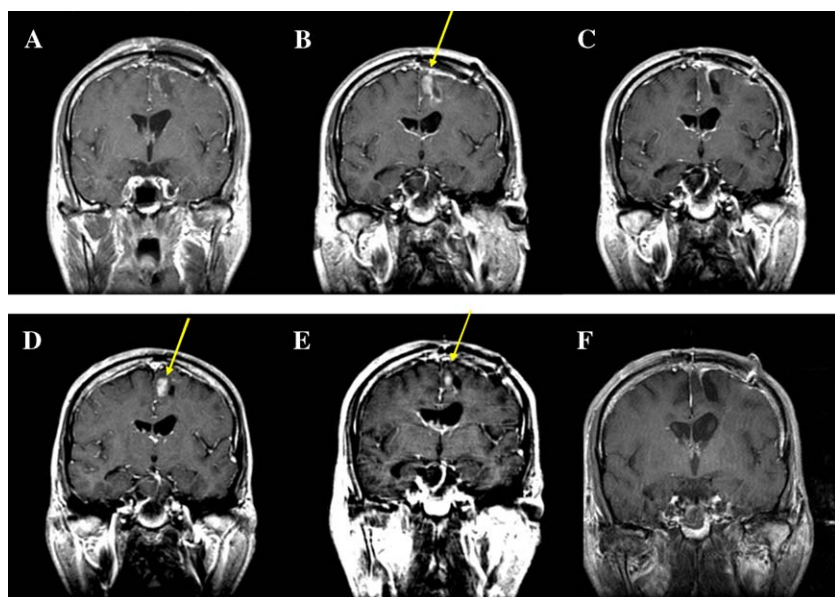
One patient in the 10<sup>10</sup> cohort (maximum dose) and one patient in the 10<sup>7</sup> cohort had a recurrent mass 3 months after inoculation with ONYX-015. On MRI scans these masses were manifested as increases in gadolinium enhancement (Fig. 3). Because of their good performance status, these patients thus underwent reoperation for resection. On histologic examination of the recurrent tumor, profound lymphocytic and plasmacytoid cellular infiltrates in perivascular locations were noted within the tumor but not in the surrounding brain parenchyma in both cases (Fig. 4).

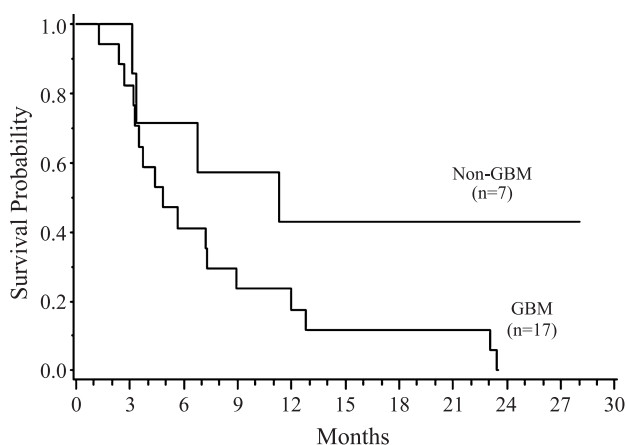
**DISCUSSION**

The primary objective of this study was to determine if injection of an oncolytic virus (i.e., a virus that can still replicate in a relatively selective fashion in tumor cells) into human brain that surrounds a resected malignant

Twenty patients have died due to tumor progression, 1 died of non-tumor-related events (ruptured intestine), and 3 remain alive. Of the survivors, 1 belongs to the 10<sup>9</sup> pfu cohort, and 2 belong to the 10<sup>10</sup> pfu cohort. Median survival time for all patients was 6.2 months (range 1.3–28.0 months). On-study histology for the patients by cohort was 6/6 glioblastoma multiforme (GBM) for the 10<sup>7</sup> pfu cohort, 4/6 GBM and 2/6 anaplastic astrocytoma (AA) for the 10<sup>8</sup> pfu cohort, 5/6 GBM and 1/6 AA for the 10<sup>9</sup> pfu cohort, and 2/6 GBM,

**FIG. 1.** Serial coronal MRI scans with gadolinium enhancement in a patient treated with ONYX-015. This patient was initially treated with resection of recurrent glioma and injection of ONYX-015 in August 2001. (A) The patient’s immediate postoperative scan showed minimal evidence of enhancement at the margin. However, 2 months later (10/08/2001), evidence for increased enhancement at this margin (B, yellow arrow) was observed, reflecting increased blood-brain barrier breakdown, possible postoperative gliotic reaction, and/or possible recurrence. However, on subsequent monthly scans, such enhancement gradually decreased, eventually returning to baseline levels on 2/18/2002 (C). Yet again, on 8/6/2002 (D) and 8/8/2002 (E), enhancement in the same region returned (yellow arrows). (F) Again, by 1/28/2003, such enhancement returned to baseline levels.



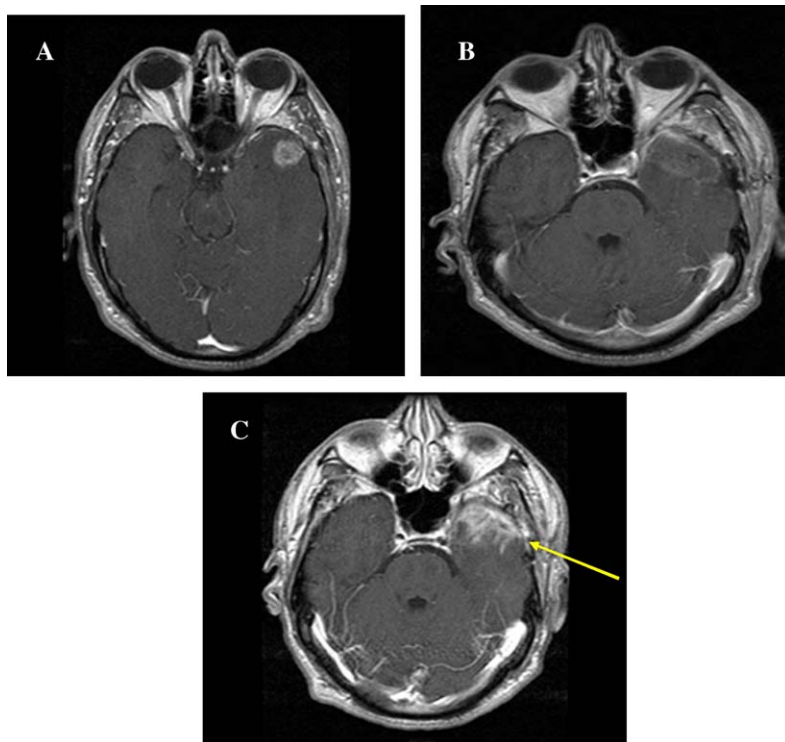


**FIG. 2.** Kaplan–Meier survival curve for patients treated with ONYX-015 ( $n = 24$ ). Kaplan–Meier survival curves for GBM ( $n = 17$ ) and other histology ( $n = 7$ ) patients treated with ONYX-015.

glioma would be tolerated. We determined that the treatment was well tolerated by all patients even at the highest dose of ONYX-015 that was available.

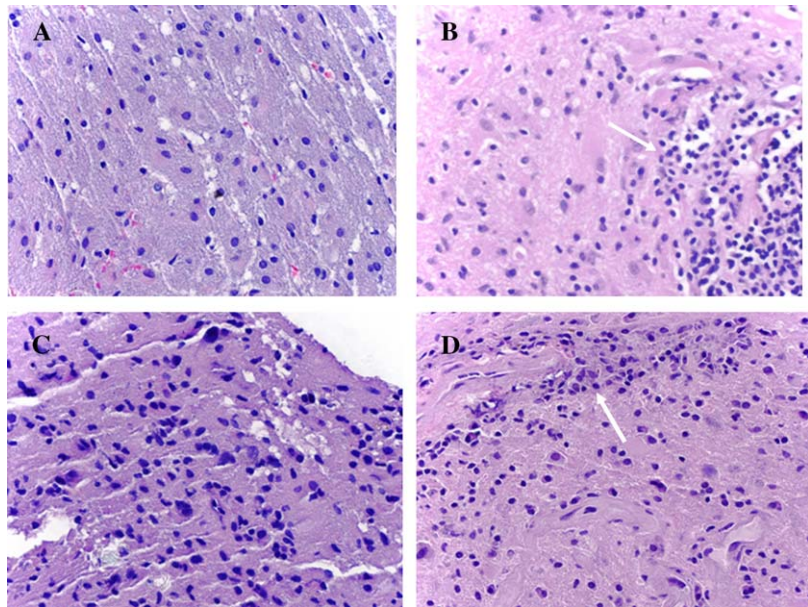
This study was conducted as a phase I dose-escalation trial in six different institutions that belonged to the New Approaches to Brain Tumor Therapy (NABTT) CNS Consortium. The major novelty in this trial is that for the first time a tumor-selective virus that maintains replication was injected into brain that surrounds a surgically

resected glioma. Previous trials with another oncolytic virus (based on the HSV-1 viral mutant designated G207 and another HSV-1 mutant designated 1716) have tested the viral vector via stereotactic intratumoral administration [24,26]. Other trials have involved injections of a replication-defective adenovirus vector, and not of a replication-conditional virus such as ONYX-015, to deliver p53 or the herpes simplex virus thymidine kinase gene [27–30]. Although these trials did not see evidence of toxicity until doses of  $10^{12}$  viral particles, a very appropriate concern relates to the possibility that an oncolytic virus may still provoke clinically significant damage to brain if its action was not confined to a tumor. In fact, reports have shown evidence of cerebral inflammation in animals whose brains were inoculated with replication-defective adenoviral vectors [31–34]. It has been suggested that this may represent an immune-mediated reaction to adenoviral gene products/proteins and/or antigens released from dying tumor cells. By injecting this oncolytic adenovirus directly into human brain tissue that surrounded a resected glioma, we have demonstrated that ONYX-015 is unlikely to cause clinically significant disease in humans at doses up to  $10^{10}$  pfu. We did not detect clinical or radiologic evidence of neurologic or systemic injury and the maximum tolerated dose was not reached at  $10^{10}$  pfu. However, in two patients whose recurrent tumors were available for analyses we did find evidence of lymphocytic and plasma cell infiltrates that would be relatively unusual for this patient population



**FIG. 3.** Serial MRIs with representative findings of trial. In (A), a left temporal lobe recurrence of a malignant glioma was observed and confirmed intraoperatively. (B and C) Immediate postoperative scans (following resection and margin injection of  $10^{10}$  pfu of ONYX-015) reveal gross total resection of tumor. Approximately 3 months later, probable recurrence of glioma was visualized in resection cavity infiltrating the brain. Histological findings from the resection of this tumor are presented in Figs. 4A and 4B.

**FIG. 4.** Histological findings of gliomas resected months after ONYX-015 injection. In (A), the histological findings of the recurrent glioma from the patient presented in Fig. 3 are shown before ONYX-015 injection, with characteristics consistent of malignant glioma. Approximately 3 months later, a new recurrence was resected. (B) The extensive lymphocytic and plasmacytoid cell infiltrate in this recurrence are shown. Similarly, (C) represents the histological picture of a recurrent malignant glioma before injection of  $10^7$  pfu of ONYX-015. Approximately 3 months later, this tumor was judged by gadolinium enhancement to have recurred and reexcision was performed. (D, white arrow) The presence of numerous perivascular lymphocytic and plasma cell infiltrates is indicated.



and that would be consistent with the aforementioned animal studies.

No definite anti-tumor efficacy could be demonstrated in this trial. All but one patient experienced progression of disease, as determined by >25% increase in gadolinium enhancement. One patient showed evidence for increased enhancement approximately 1 year after treatment, which decreased on a subsequent scan. This episode of increased enhancement, which then decreased, had also occurred postoperatively. The postoperative changes in enhancement could be attributed to postoperative gliotic reactions, but the changes that occurred 1 year later would be more difficult to explain on this basis. Although no histologic data are available, one can speculate that it may represent an episode of transient breakdown of the blood-brain barrier due to a waxing and waning inflammatory reaction to recurrent tumor and/or residual replicative virus, in agreement with the histological findings of the recurrent tumors described above.

The finding that such an inflammatory event may occur within a glioma that is recurring several weeks after ONYX-015 injection calls into question the current criterion of using an increase in gadolinium enhancement to decide whether there is progression of a glioma treated with such biologic agents. Although it still remains likely that observed >25% increases in gadolinium enhancement were due to tumor progression, we cannot exclude that a very localized inflammatory reaction within the injected tumor bed could have been partially responsible for such radiologic images.

Only two patients displayed evidence of seroconversion. Such a low number was likely due to the relatively immunocompromised state of this patient population,

which is on steroid medication and has been treated with radiation and chemotherapy, and to the injection within the brain, an organ that is relatively immunoprivileged.

Although survival and time to progression evidence were relatively encouraging for four patients, three of these had diagnoses other than glioblastoma multiforme. It is known that patients with anaplastic astrocytomas and oligodendroglioma display more favorable average survival rates than patients with glioblastoma multiforme and thus the result of this trial should be judged in this context. It was encouraging, though, that one of the patients with glioblastoma multiforme remained alive for over a year after treatment.

In conclusion, this trial has shown the relative safety of injection of ONYX-015 into brain surrounding a resected malignant glioma. Further studies to determine a maximum tolerated dose and potential for efficacy are warranted.

## PATIENTS AND METHODS

Approval of the trial was accomplished at each institution's Institutional Review Board, in accord with an assurance filed with and approved by the Department of Health and Human Services. Informed consent was obtained from each subject. The following NABTT institutions participated: Massachusetts General Hospital (Boston, MA, USA), Wake Forest University (Wake Forest, NC, USA), Henry Ford Hospital (Detroit, MI, USA), Emory University (Atlanta, GA, USA), Moffitt Cancer Center (Tampa, FL, USA), and University of Alabama (Birmingham, AL, USA).

### Inclusion and Exclusion Criteria

Patients were 18 years or older and had to have a histologically documented supratentorial malignant glioma (glioblastoma multiforme, anaplastic astrocytoma, or anaplastic oligodendroglioma) that had progressed after initial external beam radiation therapy. Radiation therapy had to have been between 5400 and 6700 cGy delivered in 180-to 200-cGy fractions. Patients had to have recovered from toxicity of prior therapy. An interval of at least 3 months had to have elapsed since the completion of the most recent course of radiation, while at least 3 weeks had to have elapsed since the completion of a non-nitrosourea-containing chemotherapy regimen and at least 6 weeks since the completion of a nitrosourea-containing chemotherapy. They may not have received more than two prior chemotherapy regimens. They had to have been eligible for resection of a portion of the recurrent tumor that was at least 1 cm in greatest dimension. There must have been no anticipated physical connection between the postresection tumor cavity and the cerebral ventricle. A Karnofsky performance status (KPS) of at least 60, a life expectancy of at least 3 months, and the ability to provide informed consent were required. Criteria for baseline organ function determined within 2 weeks of the start of treatment included the following: an absolute neutrophil count  $\geq 1500 \text{ mm}^3$ , platelet count  $\geq 100,000 \text{ mm}^3$ , creatinine  $\leq 1.7 \text{ mg/dl}$ , total bilirubin  $\leq 1.5 \text{ mg/dl}$ , transaminases  $\leq 4$  times above the limits of the institutional norm, PT and PTT  $\leq$  upper limit of normal, and CD4 lymphocyte count  $>200/\mu\text{l}$ . Finally, at the time of tumor resection, a frozen biopsy confirmation of malignant glioma was required.

Patients were excluded if they required immediate excision because of impending neurological decline or if a postsurgical connection between the resection cavity and the ventricular system was anticipated. Patients who had prior treatment of the tumor with gene therapy, brachytherapy, radiosurgery, or implants of polymers containing chemotherapeutic agents were excluded. Any patient with the presence of an immunosuppressive disorder (e.g., HIV infection) or iatrogenic immunosuppression (with the exception of corticosteroid use) was excluded. Patients with any active infection (defined as a clinically diagnosed viral, bacterial, or fungal infection that required active treatment and caused oral temperature  $>38.5^\circ\text{C}$  and/or clinically significant leukocytosis) were excluded. Likewise, any viral syndrome clinically diagnosed within 2 weeks prior to treatment on this protocol led to exclusion. To be included, patients had to have no concurrent malignancy except curatively treated basal or squamous cell carcinoma of the skin or carcinoma *in situ* of the cervix and breast. Patients with prior malignancies had to be disease-free for  $\geq 5$  years.

Known diagnosis of Li-Fraumeni syndrome or known germ-line defect in the p53 gene was grounds for exclusion. Pregnant or lactating females were not included. Women of child-bearing potential were required to practice birth control for the duration of the treatment. Men were advised to use barrier protection for the duration of treatment. This exclusion was based on the potential risks of adenoviral encephalitis to the fetus and newborn. Finally, patients with gliomatosis cerebri were excluded.

### Description of ONYX-015

ONYX-015 was manufactured under contract to Onyx Pharmaceuticals, Inc., by MAGENTA Corp. (currently BioReliance, Inc., Bethesda, MD, USA) in compliance with Good Manufacturing Practice Regulations. It was distributed to the individual trial sites by the Cancer Therapy Evaluation Program/National Cancer Institute. The purified virus was stored frozen below  $-60^\circ\text{C}$  in aliquots. Prior to release for clinical use, each lot was tested to ensure that it met the following criteria: (1) sterility, (2) bacterial endotoxin  $<10 \text{ EU/ml}$ , (3) general safety, (4) adenovirus titer in HEK293 cells for low-dose vials  $>2 \times 10^8 \text{ pfu/ml}$ , (5) adenovirus titer in HEK293 cells for high-dose vials  $>2 \times 10^{10} \text{ pfu/ml}$ , (6) E1B deletion confirmed by PCR assay, and (7) selective replication in p53-deficient cells confirmed.

The product was formulated as a sterile viral solution in Tris buffer (10 mM Tris, pH 7.41; 1 mM  $\text{MgCl}_2$ ; 150 mM NaCl; 10% glycerol). The product was supplied frozen in a single-use, plastic screw-cap vial. Prior to use, vials were thawed at room temperature. The appropriate dilution was admixed using aseptic procedures and transported on ice to the operating room in a sealed package. Injections were performed within a 4-h time frame after the virus was thawed. The injection protocol is described below (dose-escalation scheme). All injections were performed with a clinical lot, before the date of expiration. Although a standard for replication-defective vectors is to provide the viral particle to plaque-forming unit ratio and to provide doses as viral particles per milliliter, this was not a required release criterion for ONYX-015 and regulatory agencies did not require this release criterion.

### Treatment

**Pretreatment testing.** All pretreatment testing/evaluations were conducted within 2 weeks of treatment and consisted of: (1) a complete history (disease history and prior oncologic therapies); (2) a physical examination including vital signs (heart rate, respiratory rate, blood pressure, temperature, and pulse), height and weight, KPS, and neurologic exam; (3) laboratory exams including CBC with differential and platelet count, serum electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , bicarbonate), BUN, creatinine, glucose, total protein, calcium, phosphorous, magne-

sium, AST, ALT, total bilirubin, alkaline phosphatase, PT/PTT, CD3, CD4, CD8, total lymphocyte counts, serum antibody to group C adenovirus (total and neutralizing), serum pregnancy test within 72 h of treatment (for all premenopausal women with child-bearing potential), and urinalysis with microscopy; (4) an EKG (12 lead); (5) a chest X-ray (PA and Lat) (within 6 weeks); (6) an MRI of the brain with and without gadolinium.

**ONYX-015 administration, schedule, and dose**

**escalation.** Four cohorts of six patients each were treated. The number of patients per cohort was selected based on the estimate that between one-third and one-half of patients may have a mutation in the p53 tumor suppressor gene or a defect within the p53 tumor suppressor pathway [1]. Each patient received 10 injections (volume of 100 µl per injection) into the cavity wall after resection of the recurrent tumor. A period of 4 weeks (28 days) was allowed to elapse after treatment of the last patient at each dose level before escalation to the next higher dose level. At the end of each cohort study, the Data Safety Monitoring Committee (DSMC) evaluated administration methods, dosages, and overall treatment plan. The DSMC was composed of Drs. Chiocca and Barker (Massachusetts General Hospital), Dr. Stuart Grossman (Johns Hopkins University), Drs. James Zwiebel and Rick Kaplan (NCI/CTEP), Dr. Philip Gutin (Memorial Sloan-Kettering), and Dr. Michael Walker (NCI/NIH). The starting dose of ONYX-015 consisted of 10<sup>7</sup> pfu inoculated into the resected tumor cavity (as 10 single doses into 10 separate locations). Dose levels were successively escalated by a factor of 10 in subsequent cohorts. The selection of peritumoral injection sites was left to the discretion of the operating neurosurgeon. Injections were carried out using 25-gauge needles on tuberculin syringes. The maximum depth thus did not exceed 1 cm.

**Treatment schedule.** On the first day, the patient was admitted to the hospital. A craniotomy with resection of the recurrent tumor was then performed. After tumor resection was complete and an intraoperative diagnosis of malignant glioma was rendered, free-hand injections of 100 µl of ONYX-015 virus were performed by the neurosurgeon into each of 10 sites in the wall of the resection cavity. Separate tuberculin syringes were used for each injection (10 total injections). The choice of injection site was left to the judgment of the operating neurosurgeon, but the sites had to be separated by a least 1 cm. Injections were performed slowly, usually over a period of a few minutes, to avoid spillage. The depth of injection did not exceed 1 cm (length of the needle of the tuberculin syringe). The sites were selected by the neurosurgeon to avoid injections into adjacent motor or speech cortex or the cerebral ventricle or spillage into the subarachnoid space. After the injections, the wound was closed as per routine. Postoperatively, the patients were admitted to the Intensive Care Unit. Postoperative care followed standard neurosurgical practice and it included a brain MRI ± gadolinium at day 3, followed by one at day 14, one at day 42, and then every 6 weeks after. A postoperative serum sample for adenovirus antibodies was obtained on day 42.

**Toxicity Assessment**

A dose-limiting toxicity was defined as any one of the following: (1) NIH Common Toxicity Criteria (CTC; version 2.0) grade 4 toxicity for flu-like symptoms (fever, fatigue, myalgia) attributed to ONYX-015 or (2) CTC grade 3 toxicity for neurologic symptoms or for symptoms in other organ systems lasting longer than 5 days and attributed to ONYX-015. Because in the CTC (version 2.0) the grading for neurosurgical oncologic complications, such as cerebral edema, hydrocephalus, seizures, and hemorrhages, was not available or was unclear, we devised a supplemental table of neurosurgical toxicity criteria that was followed by the six participating institutions in addition to the CTC (Table 3).

**TABLE 3:** Supplemental table of toxicities for neurological/neurosurgical disease employed in trial

CNS toxicity	Grade of toxicity			
	Grade I	Grade II	Grade III	Grade IV
Hydrocephalus	Asymptomatic ventricular dilation	Ventriculomegaly with headache	Ventriculomegaly with severe headache, nausea, and vomiting	Ventriculomegaly requiring permanent CSF drainage
Meningitis	Asymptomatic diffuse meningeal enhancement on MRI	Mild signs of meningeal irritation (headache, photophobia)	Moderate signs of meningeal irritation (severe headache, photophobia, vomiting, nuchal rigidity)	As in Grade III with altered mental status (stupor or coma)
Edema	Asymptomatic edema on MRI	Focal edema on MRI with corresponding new focal neurological deficit	Diffuse edema on MRI with corresponding new neurological deficit	As in Grade III, but with altered mental status
Seizures	—	3 seizures or fewer per day	>3 seizures in 1 day	Status epilepticus

### Assessment of Response

Neurosurgical resections did not have to be gross total resections for ONYX-015 injection to occur. The scan done postoperatively was to assess the tumor configuration after injection and evaluate the extent of tumor resection. It was not used to determine response. However, it was this postoperative scan, not the scan obtained prior to surgery, that was used as the "baseline scan." The scan obtained at day 42 was compared with the postoperative scan to determine response. Responses to ONYX-015 were determined at this 42-day time and every 6 weeks thereafter. The area of contrast enhancement was determined on each slice and the total was then multiplied by slice thickness to obtain a total volume. Response criteria were: (1) Complete Response, complete disappearance of all tumor on MR/CT scan and not taking glucocorticoids, with a stable or improving neurologic exam for at least 6 weeks; (2) Partial Response, greater than or equal to 50% reduction in tumor size on volumetric MR/CT scan, on a stable or decreasing dose of glucocorticoids, with a stable or improving neurologic exam for at least 6 weeks; (3) Progressive Disease, progressive neurologic abnormalities not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity, electrolyte abnormalities, hyperglycemia) or a greater than 25% increase in the volume of the tumor by MR/CT scan; if neurologic status on a stable or increasing dose of steroids deteriorated or if new lesions appeared on serial MR/CT, the patient was removed from the study and became eligible for other therapies; (4) Stable Disease, a patient whose clinical status and MR/CT scan volumetrics did not meet the criteria for Partial Response or Progressive Disease.

### Endpoints

The main endpoint of the study was safety. Patients were evaluated for toxicity if they received at least one dose of ONYX-015. An adverse event was defined as any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use or procedure regardless of whether it was considered related to the medical procedure. The investigator documented his/her opinion of the relationship of the event to the study treatment (unrelated, unlikely, possible, probable, or definite). Serious adverse events were defined as an experience that was fatal or life-threatening, was disabling, or required inpatient care.

Efficacy of treatment was assessed as survival time and time to progression of disease, both measured from the first day of ONYX-015 treatment. As previously noted, progressive disease was defined as progressive neurologic abnormalities not explained by causes unrelated to tumor progression (e.g., anticonvulsant or corticosteroid toxicity, electrolyte abnormalities, hyperglycemia) or a greater than 25% increase in the volume of the tumor by MRI/CT

scan. Responses were determined at the 42-day time and every 6 weeks thereafter until documented tumor progression or another treatment was started.

### Statistical Considerations

Survival distributions were estimated using the product limit method. The analysis was intention-to-treat and included all eligible patients. SAS software version 9 (SAS Institute, Cary, NC, USA) was used to perform analyses.

### ACKNOWLEDGMENTS

This trial was supported through NCI/CTEP and NABTT grants.

RECEIVED FOR PUBLICATION MAY 4, 2004; ACCEPTED JULY 19, 2004.

### REFERENCES

- Kleihues, P., et al. (2002). The WHO classification of tumors of the nervous system. *J. Neuroopathol. Exp. Neurol.* **61**: 215–225; discussion 226–229.
- Prados, M. D., and Levin, V. (2000). Biology and treatment of malignant glioma. *Semin. Oncol.* **27**: 1–10.
- Prados, M. D. (2000). Future directions in the treatment of malignant gliomas with temozolomide. *Semin. Oncol.* **27**: 41–46.
- Yung, W. K. (2000). Temozolomide in malignant gliomas. *Semin. Oncol.* **27**: 27–34.
- Castro, M. G., et al. (2003). Current and future strategies for the treatment of malignant brain tumors. *Pharmacol. Ther.* **98**: 71–108.
- Chiocca, E. A. (2002). Oncolytic viruses. *Nat. Rev. Cancer* **2**: 938–950.
- Kim, D., Martuza, R. L., and Zwiebel, J. (2001). Replication-selective virotherapy for cancer: biological principles, risk management and future directions. *Nat. Med.* **7**: 781–787.
- Bischoff, J. R., et al. (1996). An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* **274**: 373–376.
- Edwards, S. J., et al. (2002). Evidence that replication of the antitumor adenovirus ONYX-015 is not controlled by the p53 and p14(ARF) tumor suppressor genes. *J. Virol.* **76**: 12483–12490.
- Nemunaitis, J., et al. (2003). Pilot trial of intravenous infusion of a replication-selective adenovirus (ONYX-015) in combination with chemotherapy or IL-2 treatment in refractory cancer patients. *Cancer Gene Ther.* **10**: 341–352.
- Hamid, O., et al. (2003). Phase II trial of intravenous CI-1042 in patients with metastatic colorectal cancer. *J. Clin. Oncol.* **21**: 1498–1504.
- Makower, D., et al. (2003). Phase II clinical trial of intrasplenic administration of the oncolytic adenovirus ONYX-015 in patients with hepatobiliary tumors with correlative p53 studies. *Clin. Cancer Res.* **9**: 693–702.
- Hecht, J. R., et al. (2003). A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. *Clin. Cancer Res.* **9**: 555–561.
- Warren, R. S., and Kim, D. H. (2002). Liver-directed viral therapy for cancer p53-targeted adenoviruses and beyond. *Surg. Oncol. Clin. North Am.* **11**: 571–588, vi.
- Reid, T., et al. (2002). Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints. *Cancer Res.* **62**: 6070–6079.
- Vasey, P. A., et al. (2002). Phase I trial of intraperitoneal injection of the E1B-55-kDa gene-deleted adenovirus ONYX-015 (dl1520) given on days 1 through 5 every 3 weeks in patients with recurrent/refractory epithelial ovarian cancer. *J. Clin. Oncol.* **20**: 1562–1569.
- Reid, T., et al. (2001). Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: a phase I trial. *Gene Ther.* **8**: 1618–1626.
- Nemunaitis, J., et al. (2001). Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: safety, feasibility and biological activity. *Gene Ther.* **8**: 746–759.
- Mulvihill, S., et al. (2001). Safety and feasibility of injection with an E1B-55 kDa gene-deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: a phase I trial. *Gene Ther.* **8**: 308–315.
- Nemunaitis, J., et al. (2001). Phase II trial of intratumoral administration of ONYX-015, a replication-selective adenovirus, in patients with refractory head and neck cancer. *J. Clin. Oncol.* **19**: 289–298.
- Lamont, J. P., Nemunaitis, J., Kuhn, J. A., Landers, S. A., and McCarty, T. M. (2000). A prospective phase II trial of ONYX-015 adenovirus and chemotherapy in recurrent squamous cell carcinoma of the head and neck (the Baylor experience). *Ann. Surg. Oncol.* **7**: 588–592.

22. Khuri, F. R., *et al.* (2000). A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer. *Nat. Med.* **6**: 879–885.
23. Ganly, I., *et al.* (2000). A phase I study of Onyx-015, an E1B attenuated adenovirus, administered intratumorally to patients with recurrent head and neck cancer. *Clin. Cancer Res.* **6**: 798–806.
24. Markert, J. M., *et al.* (2000). Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther.* **7**: 867–874.
25. Papanastassiou, V., *et al.* (2002). The potential for efficacy of the modified (ICP 34.5(-)) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. *Gene Ther.* **9**: 398–406.
26. Rampling, R., *et al.* (2000). Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther.* **7**: 859–866.
27. Trask, T. W., *et al.* (2000). Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. *Mol. Ther.* **1**: 195–203.
28. Smitt, P. S., Driessse, M., Wolbers, J., Kros, M., and Avezaat, C. (2003). Treatment of relapsed malignant glioma with an adenoviral vector containing the herpes simplex thymidine kinase gene followed by ganciclovir. *Mol. Ther.* **7**: 851–858.
29. Germano, I. M., Fable, J., Gultekin, S. H., and Silvers, A. (2003). Adenovirus/herpes simplex-thymidine kinase/ganciclovir complex: preliminary results of a phase I trial in patients with recurrent malignant gliomas. *J. Neurooncol.* **65**: 279–289.
30. Lang, F. F., *et al.* (2003). Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: biological and clinical results. *J. Clin. Oncol.* **21**: 2508–2518.
31. Dewey, R. A., *et al.* (1999). Chronic brain inflammation and persistent herpes simplex virus 1 thymidine kinase expression in survivors of syngeneic glioma treated by adenovirus-mediated gene therapy: implications for clinical trials. *Nat. Med.* **5**: 1256–1263.
32. Boviatsis, E. J., *et al.* (1994). Gene transfer into experimental brain tumors mediated by adenovirus, herpes simplex virus, and retrovirus vectors. *Hum. Gene Ther.* **5**: 183–191.
33. Byrnes, A. P., Rusby, J. E., Wood, M. J., and Charlton, H. M. (1995). Adenovirus gene transfer causes inflammation in the brain. *Neuroscience* **66**: 1015–1024.
34. Bhat, N. R., and Fan, F. (2002). Adenovirus infection induces microglial activation: involvement of mitogen-activated protein kinase pathways. *Brain Res.* **948**: 93–101.