

## Phase II Trial of Intratumoral Administration of ONYX-015, a Replication-Selective Adenovirus, in Patients With Refractory Head and Neck Cancer

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**Purpose:** To determine the safety, humoral immune response replication, and activity of multiple intratumoral injections of ONYX-015 (replication selective adenovirus) in patients with recurrent squamous cell carcinoma of the head and neck (SCCHN).

**Patients and Methods:** This phase II trial enrolled patients with SCCHN who had recurrence/relapse after prior conventional treatment. Patients received ONYX-015 at a dose of  $2 \times 10^{11}$  particles via intratumoral injection for either 5 consecutive days (standard) or twice daily for 2 consecutive weeks (hyperfractionated) during a 21-day cycle. Patients were monitored for tumor response, toxicity, and antibody formation.

**Results:** Forty patients (30 standard and 10 hyperfractionated) received 533 injections of ONYX-015. Standard treatment resulted in 14% partial to complete regression, 41% stable disease, and 45% progressive disease rates. Hyperfractionated treatment resulted in

10% complete response, 62% stable disease, and 29% progressive disease rates. Treatment-related toxicity included mild to moderate fever (67% overall) and injection site pain (47% on the standard regimen, 80% on the hyperfractionated regimen). Detectable circulating ONYX-015 genome suggestive of intratumoral replication was identified in 41% of tested patients on days 5 and 6 of cycle 1; 9% of patients had evidence of viral replication 10 days after injection during cycle 1, and no patients had evidence of replication  $\geq 22$  days after injection.

**Conclusion:** ONYX-015 can be safely administered via intratumoral injection to patients with recurrent/refractory SCCHN. ONYX-015 viremia is transient. Evidence of modest antitumoral activity is suggested.

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THE PROGNOSIS FOR recurrent squamous cell carcinoma of the head and neck (SCCHN) region is discouraging.<sup>1,2</sup> Local tumor progression leads to morbidity and even death in the majority of patients. Therefore, improved local and local-regional therapeutic approaches are needed. Treatment after failure of surgery and radiation therapy generally involves chemotherapy.<sup>1,2</sup> Approximately 30% to 40% of patients with recurrent head and neck cancer respond to combination chemotherapy, which generally includes cisplatin. The duration of response is short, and median survival is less than 6 months.<sup>1-9</sup> Furthermore, local expansion of disease during or after chemotherapy leads to devastating functional, economic, cosmetic, and psychologic effects to the patient. Because recurrence frequently occurs within a prior radiation field, further radiotherapy is not an option, and palliative surgery is generally associated with excess morbidity and additional cost, while not affecting survival. Second-line chemotherapy with other agents, such as paclitaxel, docetaxel, methotrexate, topotecan, or gemcitabine (alone or in combination), has been tested, but response rates remain poor,<sup>10-31</sup> and the duration of the response is less than a few months.<sup>9,32,33</sup> Therefore, novel approaches to the local control of chemotherapy resistant/refractory SCCHN are needed.

ONYX-015 (dl1520) is a replication-selective adenovirus.<sup>34</sup> Efficient adenovirus replication is dependent on the

expression of proteins that inactivate *p53*.<sup>35,36</sup> The normal *p53* gene product inhibits viral replication. ONYX-015 is an adenovirus that has been modified by deletion of the E1B 55-kd DNA fragment. The E1B-55-kd gene product inactivates *p53* in complex with E4ORF6.<sup>37</sup> It has been hypothesized that deletion of the E1B-55-kd region enables the *p53* protein to maintain its function, thereby inhibiting viral replication in cells with normal *p53* function; however, in cells that lack normal *p53* function, such as malignant cells, the E1B-55-kd gene product may be expendable and the cells should be susceptible to replication and killing after infection.

Initial reports<sup>38</sup> suggested that *p53* mutant tumor cells could be lysed in a replication-dependent fashion both in

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vitro and in vivo after exposure to ONYX-015.<sup>34,39</sup> In addition, several tumor lines containing a normal wild-type *p53* gene sequence were also found to be sensitive to the oncolytic activity of ONYX-015.<sup>39-41</sup> This finding is expected, since *p53* function can be lost through multiple mechanisms besides gene mutation (eg, *p53* protein binding degradation). Importantly, most groups found significantly less replicative capacity of ONYX-015 in weak normal cells compared with malignant cells,<sup>39,41,42</sup> which suggests a possible therapeutic index to ONYX-015 in the treatment of cancer.

Phase I investigation identified the toxicity of intratumoral injection of ONYX-015 to be limited to transient low-grade fever and injection site pain in one third of patients (S. Kaye, manuscript in preparation). Viral doses up to  $1 \times 10^{11}$  plaque-forming units (pfu) given daily once every 3 weeks, or  $1 \times 10^{10}$  pfu for 5 consecutive days every 3 weeks, were well-tolerated. No dose-limiting toxicity or maximum-tolerated dose was identified. Dose escalation, therefore, proceeded to the highest dose that could be practically manufactured. Additionally, multiday dosing with each dose administered to separate tumor quadrants seemed to be associated with a more effective induction of tumor necrosis over single-day dosing. Thus, we initiated a phase II investigation with ONYX-015 to be administered by intratumoral injection with multiple doses per cycles to patients with recurrent or refractory SCCHN.

## PATIENTS AND METHODS

### Enrollment Criteria

Patients were required to have histologically confirmed SCCHN (excluding nasopharyngeal) that had (1) recurred/relapsed after surgery and/or radiotherapy for the primary tumor and (2) had progressed on or within 8 weeks after completion of chemotherapy and/or radiotherapy (ie, tumors were refractory). Tumors could not be surgically curable. The tumor mass to be treated with ONYX-015 had to be adequately injectable (as defined below) and measurable (radiographically or by physical examination). Patients had to be older than 18 years old and had to have a Karnofsky performance status score of  $\geq 70$  and life expectancy of  $\geq 3$  months. Normal hematologic function and renal function were also required. A signed consent form (internal review board-approved) was required before enrollment. The *p53* gene status was not used as an enrollment criterion. Institutional review board approval of the protocol and consent form was required.

### Baseline Assessment

Baseline assessments were made before treatment. Baseline *p53* gene sequencing and immunohistochemistry were performed on paraffin-embedded or frozen ( $-70^\circ\text{C}$ ) tumor material used for diagnosis of recurrence (when available). Baseline blood tests were performed that included complete blood counts, CD3, CD4, and CD8 lymphocyte counts, electrolytes, blood urea nitrogen, creatinine, and liver function tests. In addition, baseline neutralizing antibody titers to ONYX-015 were determined (most adults have neutralizing antibodies to the adenovirus type 5 coat proteins that are present on ONYX-015). In

ctctggccgagaagtattccatagagcagctgaccactactggctgcaagTCGACCGGatctggaaggtgctgagatcaatgagaccgccac  
5' Primer Probe 3' Primer

**Fig 1. Schematic of the ONYX-015 detection amplicon.** Nucleotides 2,453 to 2,544 of ONYX-015 are shown (5' strand only). This is the amplicon amplified in the TaqMan assay. The capital letters represent the sequence of the *Puc*-derived insert in construction of this virus. The underlined regions correspond to the 5' primer, the TaqMan probe, and the 3' primer. The probe and the 3' primer are homologous to the 3' strand.

addition, flow cytometry was performed to determine circulating levels of CD3, CD4, and CD8 cells at baseline.

### ONYX-015

ONYX-015 (dl1520, also known as CI-1042) is a chimeric human group C adenovirus (Ad2 and Ad5) that does not express the 55-kd product of the *E1B* gene (Pfizer, Inc, Ann Arbor, MI, and Onyx Pharmaceuticals, Richmond, CA).<sup>37</sup> It contains a deletion between nucleotides 2,496 and 3,323 in the *E1B* region encoding the 55-kd protein. In addition, a C-to-T transition at position 2,022 in *E1B* generates a stop codon at the third codon position of the protein. These alterations eliminate expression of the *E1B*-55-kd gene in ONYX-015-infected cells. ONYX-015 was grown and titered on the human embryonic kidney cell line HEK293.

### Detection of ONYX-015 Adenovirus

The TaqMan assay is designed to amplify an amplicon of 92 nts (nts 2,453 to 2,544) that is specific for ONYX-015 (Fig 1). The specific detection of ONYX-015 is due to two factors: the amplicon overlaps the *E1B* region deletion (911 nts are missing from the wild-type sequence) and an 8-base pair *Puc*-derived linker insert is part of the TaqMan probe. The lower limit of quantitation for the assay is  $4.2 \times 10^4$  particles of ONYX-015 per mL of plasma. The lower limit of detection is  $1.05 \times 10^4$  particles of ONYX-015 per mL of plasma. This assay is specific for ONYX-015 DNA and does not detect wild-type adenovirus sequences. Polymerase chain reaction (PCR) cycling conditions are as follows: hold at  $-50^\circ\text{C}$ , 2 minutes; hold at  $-95^\circ\text{C}$ , 10 minutes; 40 cycles at  $-95^\circ\text{C}$ , 15 seconds; and  $-63^\circ\text{C}$ . The presence of PCR inhibitors in the sample is monitored using an independent PCR reaction.

Patient samples are spiked with exogenous DNA to monitor recovery in the extraction step and the presence of PCR inhibitors. A standard curve is prepared by serial diluting ONYX-015 virus from  $2 \times 10^9$  to  $1.05 \times 10^4$  vp/mL. Negative controls consist of a plasma control without virus and a type D adenovirus wild-type control. Viral DNA is extracted from patient samples, standard, and controls using a QIAamp DNA mini kit (Valencia, CA). The amount of ONYX-015 viral DNA is then quantitated by reverse transcription PCR using the above-described specific primer and probe.

### ONYX-015 Handling and Processing

ONYX-015 is formulated as a sterile viral solution in Tris buffer (Tris 10 mmol/L [pH 7.4],  $\text{MgCl}_2$  1 mmol/L, CaCl 150 mmol/L, and 10% glycerol). The solution is supplied frozen ( $-20^\circ\text{C}$ ) in single-use, plastic screw-cap vials. Each vial contains 0.5 mL of virus solution at a specified viral titer. Vialled virus solution was thawed and diluted to the appropriate titer for dosing and was then further diluted to a final volume equivalent to 30% of the volume of the tumor to be injected. All dilutions were made with D5W (Baxter D5W electrolyte no. 45). Tumor volume was estimated by taking the product of the maximal

tumor diameter, its perpendicular and estimated depth, and dividing by 2. Vials of ONYX-015 were opened and diluted immediately before injection in biologic safety cabinets at the patient treatment area. All waste items were disposed in biohazard containers and autoclaved or incinerated.

### *Treatment Regimen*

To ensure uniform dosing to the injected tumor in each patient, a single tumor was identified for ONYX-015 injection in each patient. If more than one injectable tumor was present, the most symptomatic and/or largest tumor mass was injected with ONYX-015. The tumor was mapped into five equally spaced and equally sized sections. Local anesthesia was applied to the skin as needed. The tumor was injected once a day (standard schedule) or twice a day (hyperfractionated schedule) with  $10^{10}$  particles into each of the five quadrants. The suspension volume of D5W saline used for ONYX-015 administration was normalized to 30% of the estimated volume of the tumor mass to be injected (see above). During each treatment session, one puncture of the skin was made at a site approximately 80% of the distance from the tumor center out to the tumor periphery. Six to eight needle tracts were made radially out from the puncture site; virus was administered equally along the length of the needle tracts (25-gauge needle). This approach was carried out each day from puncture sites that were equally spaced out and encompassed the entire tumor mass. The majority of the viral dose was administered at the tumor periphery and at the interface between normal tissue and tumor tissue.

In the initial phase of the study, tumor injections were performed once daily for 5 consecutive days (standard schedule); these injections were repeated every 3 weeks or until tumor progression. After documentation of safety with this regimen ( $n = 30$ ), a more aggressive injection regimen was tested in a subsequent 10 patients by administering a four-fold higher dose; identical injections were performed twice daily for 5 days during each of the first 2 weeks on study (hyperfractionated schedule). After a 1-week rest period, the hyperfractionated regimen was repeated. Patients' vital signs were taken 15 minutes before and after each treatment for a minimum of 30 minutes. Patients were eligible for repeat treatment cycles at the same dosage every 3 weeks if no grade 4 toxicity with the prior treatment cycle of ONYX-015 occurred and no progression of the injected tumor was observed. After this induction regimen, maintenance treatment cycles were given by the same schedule as was used in the initial patient cohort (every 3 weeks as described above). The injections were given in outpatient clinics, including Mary Crowley Medical Research Center at Baylor University Medical Center (Dallas, TX), Albany Regional Cancer Center (Albany, NY), US Oncology Research (Houston, TX), Beatson Oncology Centre (Glasgow, Scotland), Dana-Farber Cancer Institute (Boston, MA), University of Chicago (Chicago, IL), and M.D. Anderson Cancer Center (Houston, TX).

### *Tumor Assessments*

Tumor masses were measured serially by either physical examination or radiographic scanning (computed tomography or magnetic resonance imaging), whichever the principal investigator deemed most accurate for the measurement of the injected tumor mass. In general, very superficial lesions were measured by physical examination and deeper tumors were measured most accurately by radiographic scanning. Tumor measurements were determined either every 3 weeks (physical examination) or every 6 weeks (computed tomography/magnetic resonance imaging scans) while patients were on active study treatment; after treatment completion, patients' tumors were assessed

every 8 weeks or sooner if signs or symptoms of progression became evident. Radiographic scans were assessed by independent radiologists who were not investigators on the study.

The degree of necrosis induction within injected tumors was categorized as follows: complete regression, complete disappearance of measurable tumor; partial regression,  $\geq 50\%$  but less than 100% decrease in nonnecrotic cross-sectional tumor area; minor response, less than 50%,  $\geq 25\%$  decrease in nonnecrotic tumor area; stable disease, less than 25% decrease and less than 25% increase in nonnecrotic tumor area; progressive disease,  $\geq 25\%$  increase in tumor area versus the baseline area. Radiologists were blinded to the *p53* gene status and neutralizing antibody titer of the patients at the time of tumor assessment. Tumors were considered assessable for response at earliest assessment at any time after the first injection. All lesions (injected/noninjected) were followed to assess response.

### *p53 Gene Sequencing Determination*

Exons 5 through 9 of the tumor *p53* gene were sequenced completely during the first two thirds of the trial. Exons 2 through 11 were assessed by *p53* gene chip technology during the final one third of the trial. Since certain gene deletions can be missed by gene chip analysis (ie, a wild-type sequence is reported despite a functionally significant mutation), wild-type *p53* gene sequences by gene chip analysis underwent confirmatory sequencing to be validated.

### *Determination of Neutralizing Antibody Titers*

Patient and control samples were incubated at 55°C for 30 minutes to inactivate complement. Clinical plasma samples previously determined to produce high, mid-range, and negative titers were designated as plasma controls. Each dilution was mixed with adenovirus stock at a titer prequalified to produce 15 to 20 plaques per well of a 12-well dish in DMEM growth medium. The patient samples and controls were inoculated for 1 hour at room temperature and applied to 70% to 80% confluent JH293 cells in 12-well dishes. After 2 hours of incubation at 37°C, 5% CO<sub>2</sub> plasma-virus mix was removed and 2 mL of 1.5% agarose in DMEM was added to each well. Plates were read on day 7 after inoculation by counting the number of plaques per well. The titer of neutralizing antibody for each sample was reported as the dilution of plasma that reduced the number of plaques to 60% of the number of plaques in the virus control without antibody. Determinations of neutralizing antibody titers were made before cycle 1 (baseline), before cycle 2, and before cycle 3.

### *Additional Follow-Up After Treatment Initiation*

Neutralizing antibody titers were repeated every 4 weeks. Routine blood testing, including complete blood count and differential, electrolytes, blood urea nitrogen, creatinine, and liver function tests, was repeated every 3 weeks. Blood samples to determine circulating ONYX-015 after intratumoral injection at cycle 1 were determined on days 1 and 5.

## RESULTS

### *Treatment*

Forty patients were entered onto the trial from six sites (Mary Crowley Medical Research Center at Baylor University, M.D. Anderson Cancer Center, Beatson Cancer Institute, Albany Regional Cancer Center, Dana-Farber Cancer

Table 1. Patient Demographics

	ONYX-015 Standard Schedule (qd × 5/21-day cycle)		ONYX-015 Hyperfractionated Schedule (bid × 10/21-day cycle)	
	No. of Patients	%	No. of Patients	%
No. of patients registered	30		10	
Age, years				
Median	58		70	
Range	39-74		49-78	
Sex				
Male	23	77	8	80
Female	7	23	2	20
KPS score				
90-100	16	54	2	20
70-80	14	46	8	80
Prior therapy				
Surgery	21	70	7	70
Radiotherapy	28	93	10	100
Chemotherapy	19	63	6	60
≥ 2 modalities	27	90	8	80
Location of recurrence*				
Larynx	4	13	2	20
Tongue	2	7	0	0
Cervical area	17	57	1	10
Other	7	23	7	70
Tumor size, cm				
Maximum area				
Median	12.76		10.99	
Range	1.56-38.50		1.1-69	
Maximum diameter				
Median	3.4		3.85	
Range	1-8.4		0.9-7	
Baseline neutralizing < 1:20	14	47	2	22
Antibody levels ≥ 1:20	16	53	7	78
Baseline CD4 counts				
Median	334.22		294	
Range	79.24-1,318.05		188-798	
p53 gene status				
Mutant	12	40	6	60
Wild-type	11	37	2	20
Undetermined	7	23	2	20

Abbreviation: KPS, Karnofsky performance status.

\*Two patients had distant pulmonary metastases that were not injected.

Institute, the University of Chicago) between July 1997 and September 1998. The first 30 patients were enrolled onto the standard ONYX-015 schedule trial; the 10 patients enrolled subsequently received the hyperfractionated regimen. All patients registered received at least a single injection of ONYX-015 and were assessable for toxicity. Thirty-six patients were considered assessable for initial response. Two patients (one standard, one hyperfractionated) were not assessable due to death before response assessment (not treatment-related), and two patients (hyperfractionated) withdrew before response assessment. Characteristics of patients receiving the standard versus hyperfractionated schedule are listed in Table 1. As listed in Table 2, 70 cycles

(345 doses) were administered to 30 patients who received standard-schedule ONYX-015, and 27 cycles (188 doses) were administered to 10 patients who received the hyperfractionated schedule.

#### Tumor Response

Data on the response of injected tumors is listed in Table 3. Four patients (14%) who received the standard dosing schedule achieved a partial or complete regression of the injected tumor, 12 (41%) had stable disease, and 13 (45%) progressed. One (14%) of the hyperfractionated patients achieved a complete regression, four (58%) achieved stable disease, and two (29%) progressed. The median time to

**Table 2. Treatment Parameters**

	ONYX-015 Standard Schedule	ONYX-015 Hyperfractionated Schedule
No. of cycles per patient		
Median	2	2
Range	1-8	1-6
Total number of cycles	70	27
No. of injections	325	188
No. of days on study per patient		
Median	35.5	87.5
Range	13-175	40-254
Dilution volume, ml per patient per injection		
Median	6.05	5.85
Range	0.1-53.1	0.1-42.4

injected tumor progression, progression-free survival, and survival with the standard versus hyperfractionated approaches are listed in Table 3. No significant differences were observed between the two dosing regimens. There was no correlation between baseline tumor area, neutralizing antibody level, and response. A significant correlation was demonstrated between antitumoral activity (complete, partial, and minor responses) and presence of a *p53* gene mutation ( $P = .017$ ).

### Toxicity

Toxicity that occurred in more than 25% of patients is listed in Table 4. The majority of the toxic effects were of

**Table 3. Response of Injected Lesions**

	ONYX-015 Standard	ONYX-015 Hyperfractionated
Complete response	2 (7)	1*
Partial response	2 (7)	0
Minor response	2 (7)	1
Stable disease	10 (34)	3
Progressive disease	13 (45)	2
Not assessable	1†	3†,‡
Median progression-free survival§, days:		
Responders (PR, CR, MR, SD)	59	80
Nonresponders	21	16.5
Nonassessable	—	5
All	22	53
Median survival§, days:		
Responders (PR, CR)	125	152
Nonresponders	183	58
Nonassessable	25	—
All	126	75

Abbreviations: PR, partial response; CR, complete response; MR, minor response; SD, stable disease.

\*Complete histologic response at autopsy.

†Death not related to treatment occurred before tumor assessment.

‡Patient withdrew before response assessment.

§Derived by the Kaplan-Meier product-limit method.

mild or moderate intensity. Fatal toxicity not related to ONYX-015 occurred in three standard-treatment patients (10%) and one hyperfractionation-treated patient (10%). One fatality was related to hematemesis from an unrelated gastrointestinal ulcer, one was due to hemorrhage from local progression, one was due to bacterial-induced septic shock, and one was due to anoxia caused by airway obstruction from progressive disease. Fourteen serious adverse events were reported in the standard arm, and nine serious adverse events occurred in the hyperfractionated arm. One serious adverse event was categorized as “probably related” to study medication in the standard arm (hemorrhage at injection site). The following events were categorized as possibly related to ONYX-015 injection: pneumonia with no organisms identified ( $n = 1$ ), confusion (although concurrent hypocalcemia may have been related;  $n = 1$ ), and recurrent atrial flutter ( $n = 1$ ). The other 10 serious adverse events were either not related or the relationships were unable to be determined. Among patients who received hyperfractionated treatment, one patient developed injection site hemorrhage categorized as possibly related to ONYX-015 injection. Other events were categorized as not related or unable to be determined. Nine of 40 patients developed pneumonia not related to study treatment (six standard and three hyperfractionated). A specific cause of pneumonia was identified in three patients (two bacterial and one unrelated peptic ulcer perforation). The six other causes were thought to be related to aspiration associated with the cancer. The pneumonia lasted from 6 to 13 days. Six patients were retreated with ONYX-015 after resolution of pneumonia without recurrence. Reasons for study discontinuation are listed in Table 5.

### Systemic Distribution of ONYX-015

All 30 patients (29 of 30 in cycle 1) in the standard arm were tested for circulating ONYX-015 using PCR analysis 24 hours after the 5-day intratumoral injection series. Previous studies have shown rapid clearance of the ONYX-015 genome from the blood (approximately 6 hours); therefore, viremia  $\geq 24$  hours after the last injection is strong evidence for viral replication and shedding. Detectable levels of ONYX-015 were identified in 12 (41%) of 29 patients 24 hours after the last ONYX-015 injection (Table 6). In two patients (9%), the ONYX-015 genome was detected 10 days after injection in cycle 1. No samples were positive for circulating ONYX-015 genome 22 days after any injection in cycle 1 or any other cycle, and 15 days after any injection beyond cycle 1. Six (28%) of 21 patients had detectable circulating ONYX-015 genome in cycle 2 24 hours after intratumoral injection, and two of eight patients had detectable circulating ONYX-015 genome 24 hours

**Table 4. Toxicity Occurring in More Than 25% of Patients Possibly Related to Study Treatment**

Toxicity	Grade						Total	
	Mild	Moderate	Severe	Life Threatening	Fatal*	Unknown	No.	%
<b>Standard approach (n = 30)</b>								
Fever	9	12	0	0	0	1	22	73
Asthenia	5	5	5	0	0	0	15	50
Chills	7	6	2	0	0	0	15	50
Injection site pain	1	7	6	0	0	0	14	47
Headache	1	5	3	0	0	0	9	30
Nausea	1	4	4	0	0	0	9	30
Dyspnea	0	6	2	0	0	0	8	27
<b>Hyperfractionated approach (n = 10)</b>								
Fever	1	4	1	0	0	0	6	60
Injection site pain	6	2	0	0	0	0	8	80
Asthenia	1	4	1	0	0	0	6	60
Headache	1	2	0	0	0	0	3	30
Bacterial infection	1	2	0	0	0	0	3	30
Depression	2	1	0	0	0	0	3	30
Respiratory disorder	1	0	2	1	0	0	4	40
Pneumonia	0	2	1	0	0	0	3	30
Nausea	3	3	1	0	0	0	7	70
Anorexia	3	0	0	0	0	0	3	30
Hypotension	0	1	1	2	0	0	4	40
Hemorrhage	1	0	1	1	0	0	3	30
Tachycardia	2	0	1	0	0	0	3	30
Hyponatremia	0	3	0	1	0	0	4	40
Sweating	2	1	0	0	0	0	3	30
Anemia	0	1	2	0	0	0	3	30

\*In the standard approach group, death that occurred in three patients was related to hematemesis, hemorrhage, and bacterial sepsis and was not related to study medication. Among the patients treated with the hyperfractionated schedule, death occurred in one patient due to bronchial obstruction induced by progressive disease.

after injection in cycle 3. The two patients who had detectable ONYX-015 genome in cycle 3 achieved a minor response and a complete response. Otherwise no correlation between circulating genome and response was observed in patients with detectable genome in cycle 2 or patients with circulating genome in cycle 1. Patients entered onto the hyperfractionated treatment arm were not followed for systemic distribution of ONYX-015 genome.

**Table 5. Reasons for Study Discontinuation**

	Standard		Hyperfractionated	
	No. of Patients	%	No. of Patients	%
Disease progression at injected site	10	33	1	10
Disease progression at noninjected site	10	33	4	40
Patient decision	4	13	1	10
Unrelated medical condition(s)	1	3	0	0
Investigator's decision	1	3	2	20
Death	3	10	1	10
Other	1	3	1	10

### Neutralizing Antibody Titers

Sixteen patients who received standard ONYX-015 and seven patients who received hyperfractionated ONYX-015 were identified as having high (elevated > 1:20) neutralizing antibody titers at baseline (Table 1). Fifty-three percent

**Table 6. PCR-Detectable ONYX-015 Genome in Circulation After Intratumoral Injection**

Cycle	Days 6 and 7		Day 15		Day 22	
	+	-	+	-	+	-
+	12	17	2	19	0	22
2	6	15	0	17	0	18
3	2	6	0	7	0	6
4	0	2	0	2	0	2
5	0	1	0	1	0	0
6	0	1	0	0	0	0
7	1	0	0	1	0	1
8	0	1	0	1	0	1

Symbols: +, number of patients with positive detectable genome; -, number of patients with genome not detectable.

of standard-arm patients had antibody titers more than 1:20 at baseline, and 23 (96%) of 24 patients measured after cycle 1 had antibody titers above 1:20. All patients in the standard arm had neutralizing antibody titers above 1:20 after cycle 2. The median antibody titers at baseline (n = 30) was 51 (range, 0 to 1,798). After cycle 1 (n = 24), the median titer was 11,896 (range, 0 to 81,920). After cycle 2 (n = 14), the median titer was 12,363 (range, 225 to 71,425). Similar titers were seen in the hyperfractionation-treated patients. At baseline (n = 9), the median neutralizing titer was 1,074 (range, 0 to 8,847). This increased to 9,733 (range, 2,165 to 62,700) after cycle 2 (n = 5). There was no correlation of baseline titer levels to tumor response, time to local progression, progression-free survival duration, or overall survival.

### DISCUSSION

The results from these trials indicate that intratumoral injection of the replication-selective adenovirus ONYX-015 at a dose of  $1 \times 10^{10}$  pfu daily for 5 days of a 21-day cycle was well tolerated. Transient low-grade fever and injection site pain were the most frequent toxicities. These were manageable on an outpatient basis. Antitumor activity (as measured by  $\geq 50\%$  tumor destruction) was observed in approximately 14% of patients and did not seem different between the standard and hyperfractionation arms. Survival was also not different between the two arms; however, injection site pain occurred more frequently on the hyperfractionated regimen. Future proof of clinical benefit will be necessary to determine clinical utility. These data suggest that ONYX-015 has a favorable safety profile and modest efficacy in recurrent head and neck cancer as a single agent. Future testing in this patient population has, therefore, focused on combinations with standard agents, such as cisplatin-based chemotherapy.<sup>43</sup>

Replication-competent viruses have been tested as therapeutic agents for more than 100 years. Smallpox was eradicated with a replicating virus vaccine.<sup>44,45</sup> Exploration of the use of replicating viruses for the treatment of cancer was documented as early as 1912 when a woman with advanced cervical cancer achieved a response after injection with an attenuated rabies virus.<sup>46,47</sup> In 1950, the oncolytic activity of Egypt 101 virus was validated in vitro,<sup>48</sup> and clinical activity was suggested after intratumoral injection in cancer patients.<sup>49-51</sup> However, the antitumoral effects were transient ( $< 3$  months). Subsequent clinical investigation with mumps virus as a cancer therapy was reportedly associated with a 41% "response" rate in 90 treated patients.<sup>52</sup> However, a follow-up trial<sup>53</sup> involving 200 cancer patients in whom mumps virus was administered by a multiple intratumoral injection schedule revealed transient

tumor regression in only 26 patients. Toxicity was limited to transient fever and injection site pain. Another oncolytic virus, Newcastle disease virus (NDV),<sup>54-58</sup> showed selective replicative capacity in malignant cells. The mechanism of NDV selectivity may be related to elevated *myc* oncogene expression or differences in membrane permeability, as opposed to the *E1B-55-kd* deletion effect on *p53* with ONYX-015.<sup>58-60</sup> Additionally, consistent with what we observed with ONYX-015, tumor response was correlated with viral replication-induced oncolysis.<sup>57</sup> NDV was used to lyse tumor cells in vitro for the purpose of creating a viral oncolysate (virus and lysed tumor cells). Several trials in melanoma patients with limited-stage disease undergoing surgical resection followed by vaccination with the NDV viral oncolysate suggested improved survival compared with historical controls.<sup>61-64</sup> Similar results have been found in separate trials involving patients with colorectal carcinoma,<sup>65</sup> advanced renal cell carcinoma,<sup>66</sup> metastatic breast cancer, and ovarian cancer.<sup>67</sup> Influenza virus and vaccinia virus have also been studied as a viral oncolysate for tumor vaccine trials.<sup>68-70</sup> More recently, a variety of replication-selective viruses have been either engineered for replication selection (including human adenovirus, herpes virus, and vaccinia virus)<sup>71</sup> or shown to be replication-selective based on specific genetic tumor target (ie, activated *ras* for retrovirus).<sup>72-75</sup> Replication-selective, tumor-targeting bacteria such as *Salmonella typhimurium* have also shown encouraging preclinical activity.

A great deal of data have been accumulated suggesting that adenovirus serotype 5 is an effective oncolytic virus with a low toxicity profile to humans. DNA from thousands of human tumors have been analyzed for the presence of adenovirus DNA, and no integrated viral DNA has been isolated from any human tumor.<sup>72</sup> Eighty percent of adults have existing antibodies to adenovirus serotype 5, but less than 15% of exposed patients become clinically symptomatic.<sup>73</sup> The most common symptoms of an adenoviral serotype 5 infection are flu-like in nature and include cough, gastroenteritis, conjunctivitis, and, rarely, pneumonia. However, these symptoms are rarely seen even in immunocompromised patients.<sup>74</sup> Oral adenoviral vaccines were given to thousands of military recruits in the 1960s without adverse effects or increase in cancer.<sup>75</sup> Long- and short-term safety of intratumoral adenoviral injection has been shown in several animal cancer models,<sup>76-82</sup> and live adenovirus inocula were given intratumorally and intra-arterially to patients with cervical carcinoma at the National Cancer Institute in the 1950s.<sup>51</sup> Again, no significant toxicities, other than transient fever and malaise, were observed, even in subsets of patients treated with corticosteroids and in those in whom neutralizing adenovirus antibodies were not

present. Intravascular administration was also well tolerated in a small group of patients.<sup>83</sup> Adenoviral vectors with the E1 and E3 deletion containing the *Escherichia coli* cytosine deaminase gene have also been administered via intradermal injection to normal individuals in studies of toxicity and immune response at dose levels of  $10^6$ ,  $10^7$ , and  $10^8$  pfu.<sup>84</sup> No significant toxicity was observed.<sup>84</sup> This was consistent with clinical trial results in the same patient population of head and neck cancer patients described in this trial who received a nonreplicating adenoviral vector containing a wild-type *p53* gene.<sup>85,86</sup>

Given the safety and toxicity profile of ONYX-015, it seems reasonable to explore this virus in patients with earlier-stage disease<sup>87-90</sup> and possibly even to enhance sensitivity when combined with chemotherapy or radiation therapy.<sup>39</sup> Independent of the ONYX-015 replication-induced oncolysis, ONYX-015 *E1A* gene expression can activate the cell cycle and increase cellular sensitivity to chemotherapy or radiation therapy.

Use of ONYX-015 for local management of SCCHN and as adjuvant therapy after surgical resection of SCCHN and, possibly, other malignant tumors should also be considered but will require further investigation. Comparison of survival between responding and nonresponding patients will also need to be followed in the future, although differences observed in this trial were not significant. Pursuit of other schedules of intratumoral administration (ie, > 5 days/21-

day course) are unlikely to be of value, although justification of a systemic infusion schedule for ONYX-015 may be warranted since it has been shown to be safe and efficacious in animal cancer models.<sup>39</sup> Detection of ONYX-015 genome in plasma on the last day of ONYX-015 injection suggests that circulating virus, at low plasma concentration, is safe. Furthermore, persistent detection in two patients 10 days after the last injection suggests that a viral replicative process was ongoing, although it did not persist since none of the samples tested showed evidence of circulating viral genome more than 17 days after the last injection.

Future work with ONYX-015 and other replication-selective viruses will also explore the possibility of arming these viruses with exogenous genes, particularly if selective tumor replication is confirmed. Antitumor effects correlating with enhanced cytotoxic T-lymphocyte activity have been noted in vivo with replication-selective herpes simplex virus (G207) carrying an interleukin 12 gene,<sup>71</sup> for example. Over the next year, a number of these replication-selective agents are expected to enter clinical testing.

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