Clinical Experience With HLA-B7 Plasmid DNA/Lipid Complex in Advanced Squamous Cell Carcinoma of the Head and Neck

Lyon L. Gleich, MD; Jack L. Gluckman, MD; John Nemunaitis, MD; James Y. Suen, MD; Ehab Hanna, MD; Gregory T. Wolf, MD; Marc D. Coltrera, MD; Douglas B. Villaret, MD; Lawrence Wagman, MD; Dan Castro, MD, PhD; Markus Gapany, MD; William Carroll, MD; Deirdre Gillespie, MD; Linda M. Selk, BA

Objective: To investigate the safety and efficacy of alloantigen plasmid DNA therapy in patients with advanced head and neck squamous cell carcinoma using Allovectin-7 (Vical Inc, San Diego, Calif), a DNA/lipid complex designed to express the class I major histocompatibility complex antigen HLA-B7.

Design: Multi-institutional prospective trial.

Setting: Academic medical setting.

Patients: A total of 69 patients were enrolled in 3 sequential clinical trials: a single-center phase 1 trial and 2 multicenter phase 2 trials. Eligibility criteria included unresectable squamous cell carcinoma that failed conventional therapy, Karnofsky performance status score of 70 or greater, and no concurrent anticancer or immunosuppressive therapies.

Intervention: Patients received 2 biweekly intratumoral injections of 10 µg (phase 1 and first phase 2 trials) or 100 µg (second phase 2 trial) of Allovectin-7 followed by 4 weeks of observation. Patients with stable or responding disease after the observation period were given a second treatment cycle identical to the first.

Main Outcome Measures: Patients were assessed for toxic effects, and tumor size was measured after cycles 1 (at 6 weeks) and 2 (at 16 weeks).

Results: Allovectin-7 treatment was well tolerated, with no grade 3 or 4 drug-related toxic effects. Of 69 patients treated, 23 (33%) had stable disease or a partial response after the first cycle of treatment and proceeded to the second cycle. After the second cycle, 6 patients had stable disease, 4 had a partial response, and 1 had a complete response. Responses persisted for 21 to 106 weeks.

Conclusions: Intratumoral plasmid DNA immunotherapy for head and neck cancer with Allovectin-7 is safe, and further investigations are planned in patients with less advanced disease, where it could potentially improve patient survival and reduce the need for radical high-morbidity treatments.


Despite improvements in surgical techniques and advances in radiation therapy and chemotherapy, cure rates for head and neck cancer are stagnant. Alternative methods of treatment are needed to reduce morbidity and increase survival. DNA-based therapy is therefore being studied for head and neck cancer. Head and neck tumors are excellent targets for DNA-based therapy because the tumors are readily accessible for direct and repeated intratumoral administration of drugs, the tumors can be objectively monitored, and there is a need for new and better treatments.

A major limitation of DNA-based therapy for cancer treatment is the lack of vectors that can deliver the gene product to every cancer cell. This might be a significant limitation if the gene being delivered is targeted to replace an absent tumor suppressor gene. In contrast, if the gene that is being delivered can stimulate elimination of cells that are not transfected with the gene, the lack of ideal vectors might not be an issue. Therefore, we investigated DNA-based therapy using the class I major histocompatibility complex (MHC) HLA-B7 as an alloantigen to stimulate an antitumor immune response. The initial results from the first 9 patients with advanced head and neck cancer were reported previously. This study reports the results obtained from 69 patients with head and neck cancer treated at 9 institutions.

The basis for this approach is that cancers, including head and neck cancers, frequently have decreased expression of class I MHC proteins. Class I MHC expression is needed for presentation of tumor-associated antigens. Increasing class I MHC expression through DNA-based therapy can initiate a tumor-specific immunologic re-
PATIENTS AND METHODS

A total of 69 patients have been treated with Allovectin-7 for squamous cell carcinoma of the head and neck. The initial 10 patients were treated at the University of Cincinnati in a single-institution study. The study was then expanded to 2 sequential multi-institutional trials with the following institutions participating: University of Cincinnati, Cincinnati, Ohio; Baylor University/US Oncology Inc, Dallas, Tex; University of Arkansas, Little Rock; University of Michigan, Ann Arbor; University of Washington, Seattle; City of Hope National Medical Center, Duarte, Calif; University of California at Los Angeles; University of Alabama, Birmingham; and University of Minnesota, Minneapolis. The DNA-based therapy followed a protocol that was approved by the Recombinant DNA Advisory Committee of the National Institutes of Health, the Food and Drug Administration, and each participating institution’s institutional review board and institutional biosafety committee. Patients were considered eligible if they had received complete standard therapy for squamous cell carcinoma of the head and neck and the tumor had persisted or recurred and could not be resected. All patients had therefore received radiotherapy and surgery if possible and had persistent or recurrent cancer. Chemotherapy was offered for palliation to all patients. None of the patients had chemotherapy within 6 weeks or radiotherapy within 4 weeks of DNA-based therapy.

Additional eligibility criteria included age of at least 18 years, Karnofsky performance status score of 70 or greater, estimated life expectancy of more than 16 weeks, willingness to use contraception during the study, and ability to give informed consent. Laboratory criteria for inclusion were a white blood cell count greater than 3.0 \( \times 10^9 \) /L, platelet count greater than 100 \( \times 10^9 \) /L, hemoglobin value of 90 g/L or greater, prothrombin time and partial thromboplastin time of no more than 1 second above the reference value, creatinine level of no more than 7 \( \mu \)mol/L (0.1 mg/dL) above the reference value, normal direct serum bilirubin level, and negative pregnancy test result in women of childbearing age. A chemistry panel including electrolyte and liver enzyme levels was also obtained. HLA-B7 was required to be negative when the study was initiated, but after disease responses were seen in patients with melanoma that were HLA-B7 positive it was left to the individual investigators to determine whether HLA-B7-positive patients would be included at their institutions.

Exclusion criteria were any of the following: active autoimmune disease, active infection requiring parenteral antibiotic therapy, uncontrolled diabetes mellitus, uncontrolled hypertension or New York Heart Association class III or IV heart disease, significant psychiatric disorders, or brain metastases. Patients could not receive any concurrent anticancer drug therapies, immunosuppressive drugs, or any other experimental therapies. Patients were ineligible if they had received corticosteroids within 3 weeks of DNA-based therapy.

DNA-BASED THERAPY ADMINISTRATION

Before treatment, all patients were assessed for eligibility, and informed consent was obtained. Medical history and physical examination results were recorded, and the tumors were

RESULTS

PATIENT DEMOGRAPHICS

Sixty HLA-B7–negative patients were treated (37 men and 23 women; mean age, 61.4 years; age range, 23–91 years).

Nine HLA-B7–positive patients were treated, and their data are discussed later. All patients had received previous radiation therapy except for 1 in the multi-institutional trial whose tumor did not respond after cycle 1. Fifty-four of the 60 patients had previous attempts at surgical resection of their tumors. Chemotherapy had been administered to 25 patients, and 5 had received previous experimental treatments. Sites of recurrent or persistent disease were as follows: neck nodes or soft tissue in 38 patients, oral cavity and/or oropharynx in 17, facial or parotid nodes in 2, sinonasal in 2, and an adrenal metastasis in 1.

Tumors ranged in size from 11 \( \times 10 \) to 120 \( \times 10 \) mm. Mean tumor size was 1795 mm\(^2\). Mean tumor size of the 10 tumors treated in the single-institution study was 2270 mm\(^2\), of the 28 tumors treated in the multi-institutional study at low dose was 1608 mm\(^2\), and of the 22 tumors treated in the multi-institutional study at high dose was 1688 mm\(^2\).

SAFETY

There were no serious adverse events related to use of Allovectin-7. Adverse events that were seen and were judged to be potentially related to Allovectin-7 therapy included fatigue in 3 patients, fever in 2, nausea in 1, aches in 1, and night sweats in 1. One patient experienced a serious adverse event of hypotension (vagal response) that was judged to be related to the injection procedure. Additional adverse events that were seen and were judged to be potentially related to the procedure of intratumoral injection included injection site pain in 7 pa-
measured by visual and palpable examination. When possible, computed tomographic scans were used to obtain measurements of the tumor size. The 2 largest perpendicular diameters were recorded. In the office setting, the tumor was injected with Allovectin-7 in 1 mL of isotonic sodium chloride solution. Gentle aspiration was used during injection to prevent intravascular injections. For larger tumors, multiple injection sites within the tumor were permitted at the investigator's discretion. The 10 patients treated in the single-institution study and the 28 patients treated in the first multi-institutional study received 10 µg of Allovectin-7 at each treatment session, hence referred to as low dose. The 31 patients treated in the second multi-institutional study received 100 µg of Allovectin-7 at each treatment session, hence referred to as high dose. Vital signs were measured 2 hours after injection, and patients were then discharged from the office. Two weeks after the first injection, a second identical injection was given if there were no adverse events. Completion of these 2 injections constituted cycle 1 of treatment.

Patients returned after the second injection, at which time the history and physical examination were repeated. The tumor was again measured, and a computed tomographic scan was performed when possible. For the multi-institutional study, patients were considered evaluable if they had an evaluation 6 weeks after beginning therapy. If there was progressive disease, the patient received no further treatment with Allovectin-7. If there was no evidence of progressive disease after a 4-week treatment break, a second identical cycle of treatment, consisting of 2 injections, was administered. Responding patients therefore received a total of 4 injections. Sixteen weeks after starting treatment the tumor site was evaluated, and, when possible, a computed tomographic scan and biopsy of the tumor site were done.

**RESPONSE CRITERIA**

All patients were assessed for toxic effects at each visit according to the National Cancer Institute's Common Toxicity Criteria. Response was evaluated by clinical evaluation, computed tomographic scanning, and histologic evaluation. The same method was repeatedly used for each patient to maintain consistency. A clinical complete response was defined as disappearance of all clinical and radiographic evidence of active tumor for a minimum of 4 weeks. The patient must also be free of all symptoms of cancer. To have a pathologic complete response the biopsy result must reveal no tumor cells. A partial response was defined as a 50% or greater decrease in the sum of the products of all diameters of measurable lesions. These reductions in tumor size must endure for a minimum of 4 weeks. No simultaneous increase in the size of any lesion or the appearance of new lesions can occur. Stable disease was defined as a less than 50% decrease in the sum of the products of all diameters of measurable lesions or an increase in the tumor mass of less than 25% in the absence of the development of new lesions. Progressive disease was defined as the appearance of a new lesion, an increase in the tumor mass of 25% or greater in the sum of the products of the diameters of measurable lesions, or worsening of tumor-related symptoms. Survival was measured from the first day of DNA-based therapy.

**TUMOR RESPONSE**

After completion of the first cycle of treatment, progressive disease was seen in 40 of the 60 patients. These 40 patients were removed from the study (3 of these patients inappropriately received the second cycle of treatment and their disease continued to progress). Of the 40 patients whose disease progressed, 10 were participating in the multi-institutional study and were not seen at their scheduled week 6 evaluation, generally because of early progression of disease, and are therefore technically invaluable. When patients were evaluated on an intent-to-treat basis, 40 of the 60 had progressive disease.

After the first cycle of treatment, 20 (33%) of the 60 tumors were either stable or smaller. Six weeks after starting treatment, 6 patients had partial responses to therapy and 14 had stable disease (Table 1). All 20 of these patients went on to the second cycle of treatment (Table 2).

After completing the second cycle of treatment, these 20 patients were reassessed 16 weeks after the first injection. Eleven patients (18%) did not have disease progression by 16 weeks, with 5 having a partial or complete response and 6 having stable disease (Table 3). Of the 6 patients who had a partial response after the first cycle of treatment, only 1 had progression by 16 weeks. Four of these 6 patients had been treated in the initial single-institution trial. Two of those 4 patients had complete clinical responses, but all had persistent cancer on biopsy. The partial responses persisted 20, 21, 36, and 79 weeks in the 4 initial responding patients. The patient in the low-dose multi-institutional study who had a partial response at week 6 went on to have a complete clinical response by the end of the study (week 16) but never underwent biopsy and died of an unknown cause 35 weeks after beginning DNA-based therapy. The patient in the high-dose multi-institutional study who had a partial response began to experience disease progression during the second cycle of therapy.

Fourteen patients, all in the multi-institutional study, went on to the second cycle of therapy because of stable disease after cycle 1. Of these 14 patients, 10 received low-dose and 4 received high-dose Allovectin-7. Of the 10 low-dose patients, 5 had progressive disease at week 16 and 5 had stable disease. Of the 4 high-dose patients, 2 had progressive disease at week 16, 1 died at week 7 of a stroke, and 1 had stable disease. Six patients, therefore, had stable disease at week 16. Progression was later noted in 5 of these patients at weeks 20, 26, 26, 29, and 34. One patient has been followed up 18 weeks from starting treatment and the disease has remained stable.

In addition, a limited number of patients (n=9) who were seropositive for HLA-B7 were allowed to be enrolled, at the individual institution's discretion, in the high-dose multi-institutional trial. Three of these patients had stable disease at 6 weeks (Table 2, patients 21-23), but...
all 9 had progressive disease by week 16. These 9 patients are not included in the cohort analysis.

Comparisons were made between the potentially responding patients and those with progressive disease. All 20 patients whose disease had not progressed by week 6 had received previous radiation therapy. Sixteen of the 20 patients who responded had undergone previous surgical resection compared with 38 of the 40 patients whose disease progressed. Chemotherapy had been administered to only 3 of the 20 responding patients compared with 22 of the 40 patients whose disease progressed. Average tumor size of the 20 responding patients before treatment was 1503 mm² compared with 1990 mm² in the 40 patients whose disease progressed. The 11 patients whose disease had not progressed by week 16 were compared, and only 2 of these patients had previous chemotherapy, and average tumor size before treatment was 1625 mm².

**PATIENT SURVIVAL**

Of the 20 patients who appropriately received 2 cycles of treatment, 14 have died. Mean time from the first injection to death was 43 weeks in these 14 patients. Three of the 6 patients who remain alive have been followed up for more than 2 years since receiving DNA-based therapy; 2 underwent additional surgery and 1 underwent chemotherapy. The other 3 living patients have been followed up for less than 9 months.

Of the 40 patients who had progressive disease, 30 were evaluable and were monitored for survival. Twenty-four of these patients have died. Mean time from the first injection to death was 23 weeks. Of the 6 patients remaining alive, only 1 is alive more than 2 years after DNA-based therapy, and the other 5 have been followed up less than 6 months from starting treatment.

This series of clinical trials of alloantigen DNA-based therapy with Allovectin-7 for head and neck squamous

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**Table 1. Tumor Response After Cycle 1 (Week 6)**

<table>
<thead>
<tr>
<th>Patients, No.</th>
<th>Progressive Disease</th>
<th>Stable Disease</th>
<th>Partial Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single-institution study</td>
<td>10</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Multi-institutional study, low dose</td>
<td>28</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Multi-institutional study, high dose</td>
<td>22</td>
<td>17</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>40</td>
<td>14</td>
</tr>
</tbody>
</table>

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**Table 2. Allovectin-7 DNA-Based Therapy: Tumor Responses in 23 Responding Patients**

<table>
<thead>
<tr>
<th>Patient No./ Sex/Age, y</th>
<th>PS</th>
<th>Previous Treatment</th>
<th>Tumor Site</th>
<th>Tumor Size, mm, Week 6</th>
<th>Tumor Size, mm, Week 16</th>
<th>Weeks to Progression</th>
<th>Death†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/69 90 Y Y N</td>
<td>Surg xRT CTX</td>
<td>Oropharynx</td>
<td>30 x 30</td>
<td>25 x 20 PR</td>
<td>0 PR</td>
<td>79 106</td>
<td></td>
</tr>
<tr>
<td>2/M/77 90 N Y N Neck</td>
<td></td>
<td></td>
<td>80 x 60</td>
<td>60 x 40 PR</td>
<td>60 x 40 PR</td>
<td>21 21</td>
<td></td>
</tr>
<tr>
<td>3/F/68 90 Y Y Neck Oropharynx</td>
<td>30 x 25</td>
<td>10 x 10 PR</td>
<td>14 x 14 PR</td>
<td>36 48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/M/58 90 Y N Oropharynx</td>
<td>60 x 50</td>
<td>50 x 40 PR</td>
<td>30 x 20 PR</td>
<td>20 Alive 2 y</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Low-Dose Multi-institutional Protocol**

| 5/M/68 90 Y Y N Oropharynx | 20 x 20 | 20 x 14 SD | Prog | 15 16 |
| 6/F/74 90 Y Y N Sinus | 22 x 18 | 18 x 16 SD | 18 x 16 SD | 20 45 |
| 7/M/62 70 Y Y N Preauricular | 55 x 45 | 44 x 44 SD | 50 x 40 SD | 29 42 |
| 8/M/76 90 Y Y N Neck | 30 x 25 | 25 x 25 SD | 30 x 30 SD | 26 44 |
| 9/F/84 90 Y Y N Oropharynx | 40 x 20 | 40 x 20 SD | 46 x 38 Prog | 16 55 |
| 10/F/77 70 N Y N Oropharynx | 20 x 20 | 20 x 20 SD | 20 x 20 SD | 26 36 |
| 11/M/56 90 Y Y Y Neck | 55 x 40 | 60 x 40 SD | 55 x 48 SD | 34 Alive 2 y |
| 12/M/75 80 N Y Y Neck | 40 x 30 | 5 x 5 PR | 0 CR | Unknown 35 |
| 13/F/60 70 Y Y N Neck | 55 x 38 | 40 x 25 SD | 81 x 50 Prog | 13 28 |
| 14/M/47 90 Y Y N Neck | 85 x 35 | 85 x 40 SD | 100 x 42 Prog | 8 Alive 2 y |
| 15/F/71 80 Y Y N Oropharynx | 30 x 20 | 35 x 20 SD | Prog | 8 82 |

**High-Dose Multi-institutional Protocol**

| 16/M/59 80 Y Y N Stoma | 22 x 20 | Minimal PR | Prog | 7 34 |
| 17/M/65 90 Y Y N Neck | 50 x 20 | 60 x 20 SD | 50 x 20 SD | None > 18 Alive |
| 18/F/68 70 Y Y N Oral | 38 x 36 | 35 x 30 SD | Prog | 12 Alive |
| 19/F/72 80 N Y Y Sinus | 50 x 30 | 40 x 25 SD | Prog | 8 Alive |
| 20/M/84 70 Y Y N Oral | 60 x 38 | 60 x 35 SD | Stroke | 7 7 |

**High-Dose Multi-institutional Protocol and HLA-B7 Positive**

| 21/M/66 90 Y Y Y Supraglottic | 50 x 40 | 60 x 50 SD | Prog | 16 Alive |
| 22/M/78 80 Y Y Y Neck | 100 x 70 | 80 x 40 SD | Prog | 12 12 |
| 23/M/73 80 Y Y Y Parotid | 60 x 30 | 60 x 35 SD | Prog | 12 Alive |

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*PS indicates Karnofsky performance status score; Surg, surgery; xRT, radiation therapy; CTX, chemotherapy; Y, yes; N, no; CR, complete response; PR, partial response; SD, stable disease; and Prog, progressive disease.

†Patients listed as alive have all been followed up for less than 20 weeks from their first injection and remain alive but have active cancer.
cell carcinoma aimed to determine whether this treatment was safe and efficacious using a group of patients with highly advanced disease. The data clearly support the safety of this approach. This form of DNA-based therapy was administered with a cationic lipid mixture, reducing the risk of any potential toxic effects from viral vectors, and was well tolerated. The data also demonstrate that this approach has potential efficacy for head and neck cancer. Potential tumor responses were seen in 20 of the 60 patients 6 weeks after the first injection, and persistent responses lasting 16 weeks or longer were seen in 11 patients.

Increased survival was seen in patients who responded after 1 cycle of treatment. More of the responding patients remained alive 1 year after treatment, and, in patients who died, an increased average time to death of 43 weeks was seen compared with 23 weeks in patients who had disease progression after cycle 1. These results imply that Allovectin-7 therapy possibly offers significant survival benefit, but it must be remembered that patients who were considered treatment failures were based on disease progression, the pace of which is unpredictable.

It remains unclear why some patients’ tumors responded after this treatment and others did not. Tumor size was a factor, but there were very large tumors that did respond and small tumors that did not. All of the patients in these trials had significant tumor burdens and previous therapy. Raising the dose did not increase the tumor response rate.

There was an association between not having previous chemotherapy and responding after alloantigen DNA-based therapy. It is possible that patients who received previous chemotherapy, in addition to their other therapy, were in some way more debilitated and therefore did not respond, or this might be a coincidence given the limited number of patients.

The exact mechanism by which tumor regression occurs after Allovectin-7 therapy still is unclear. Nine patients who were HLA-B7 positive, in addition to the 60 HLA-B7–negative patients, were treated, but none had tumor responses at 16 weeks, suggesting that the alloantigen, being foreign, augments the response. Data support that this alloantigenic stimulation induces apoptosis.

There are, however, no clear predictors of response to Allovectin-7 use in this patient population.

In these patients with highly advanced cancer, evidence of tumor regression due to Allovectin-7 therapy was seen in some patients with minimal toxic effects. This supports the investigation of this approach in patients with less advanced tumors. Because Allovectin-7 uses the immune system to cause tumor regression, it would be expected that patients who are more robust, who have not had previous therapy, and who have smaller tumor burdens would have a better response to therapy. Therefore, testing of Allovectin-7 as an adjunct to treatment in patients with earlier-stage head and neck cancer is planned.

Accepted for publication January 24, 2001.

From the Departments of Otolaryngology–Head and Neck Surgery, University of Cincinnati, Cincinnati, Ohio (Drs Gleich and Gluckman), Baylor University/US Oncology Inc, Dallas, Tex (Dr Nemunaitis), University of Arkansas, Little Rock (Drs Suen and Hanna), University of Michigan, Ann Arbor (Dr Wolf), University of Washington, Seattle (Drs Coltrera and Villaret), University of California at Los Angeles (Dr Castro), University of Minnesota, Minneapolis (Dr Gapany), and University of Alabama, Birmingham (Dr Carroll); the Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, Calif (Dr Wgman); and Vical Inc, San Diego, Calif (Dr Gillespie and Ms Selk).

Presented at the annual meeting of the American Head and Neck Society, Fifth International Conference on Head and Neck Cancer, San Francisco, Calif, August 1, 2000.

Corresponding author and reprints: Lyon L. Gleich, MD, Department of Otolaryngology–Head and Neck Surgery, University of Cincinnati Medical Center, PO Box 670528, 213 Bethesda Ave, Cincinnati, OH 45267-0528 (e-mail: lyon.gleich@uc.edu).

REFERENCES