Alloantigen Gene Therapy for Squamous Cell Carcinoma of the Head and Neck

Results of a Phase 1 Trial

Lyon L. Gleich, MD; Jack L. Gluckman, MD; Shanna Armstrong, RN; Paul W. Biddinger, MD; Mary Ann Miller, BS; Kamala Balakrishnan, MD; Keith M. Wilson, MD; Harold I. Saavedra, PhD; Peter J. Stambrook, PhD

Objective: To determine the safety and efficacy of an immunogenic gene therapy using a drug designed to produce expression of a foreign class I major histocompatibility complex protein in patients with head and neck cancer.

Design: Phase 1 prospective clinical trial.

Setting: Academic medical setting.

Patients: Nine patients with advanced head and neck squamous cell carcinoma who had failed conventional therapy and did not express HLA-B7, a class I major histocompatibility complex protein.

Intervention: Patients were treated with Allovectin-7 (Vical Inc, San Diego, Calif) by direct intratumoral injection. Allovectin-7 contains a plasmid complementary DNA complexed with a cationic lipid, which results in expression of HLA-B7.

Main Outcome Measures: Patients were assessed for any toxic effects and for any change in tumor volume. Biopsy specimens obtained before and after therapy were evaluated by immunohistochemistry to detect HLA-B7 expression and with the terminal deoxynucleotide transferase-mediated deoxyuridine triphosphate–biotin nick end labeling (TUNEL) assay to detect any induction of apoptosis.

Results: There were no toxic effects of the gene therapy. In 4 of these 9 patients there was a partial response to treatment, evidenced by a gradual reduction in tumor size. One patient has remained alive for more than 17 months since commencing treatment, with no clinical evidence of disease but with persistent histological evidence of cancer. Analysis of the biopsy specimens from 2 of the patients who responded to therapy demonstrated HLA-B7 expression. The TUNEL assay demonstrated extensive apoptosis in both of these patients, suggesting that this may be the mechanism of tumor reduction.

Conclusions: These data demonstrate the potential efficacy and lack of toxicity of this form of alloantigen gene therapy. A multi-institutional study has been initiated to expand on these findings.


From the Departments of Otolaryngology (Drs Gleich, Gluckman, and Wilson and Ms Armstrong), Pathology and Laboratory Medicine (Drs Biddinger and Balakrishnan and Ms Miller), and Cell Biology, Neurobiology, and Anatomy (Drs Saavedra and Stambrook), University of Cincinnati Medical Center, Cincinnati, Ohio.
PATIENTS AND METHODS

ELIGIBILITY

Treatment followed a protocol that was approved by the University of Cincinnati Medical Center Institutional Review Board, the Recombinant DNA Advisory Committee of the National Institutes of Health, and the Food and Drug Administration. Patients were eligible if they had received complete standard therapy for a 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 gene therapy. Additional eligibility criteria included age older than 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 as follows: HLA-B7 negative, white blood cell count greater than $3 \times 10^9/L$, 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 7.1 µmol/L above the reference value, normal direct serum bilirubin level, and negative pregnancy test in women of child-bearing age. A chemistry panel including electrolyte and liver enzyme levels was also obtained. Exclusion criteria were any of the following: active autoimmune disease, active infection requiring parenteral antibiotics, uncontrolled diabetes mellitus, uncontrolled hypertension of 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 prior to gene therapy.

PATIENT POPULATION

Nine patients, 6 men and 3 women, with a median age of 63 years (range, 41-77 years; mean, 63.1 years), with biopsy-confirmed recurrent or persistent squamous cell carcinoma of the head and neck following initial treatment were entered into the study (Table). All the patients had received radiotherapy. In 3 patients, radiotherapy was administered as the primary treatment modality. Eight patients had undergone surgical resections with curative intent; in 4 patients, multiple resections had been performed. Chemotherapy had been administered in 2 patients and 1 patient received photodynamic therapy. The sites of recurrent or persistent disease were oral cavity and/or oropharynx in 4 patients, at the tracheostoma in 1 patient, and in neck nodes in 4 patients. One patient also had radiographic evidence of lung metastases. The cancer was deemed unresectable in all of the patients because of carotid involvement, prevertebral muscle invasion, or patient refusal of a highly morbid procedure. All the patients were offered chemotherapy for palliation or pain medication alone as an alternative to gene therapy.

GENE THERAPY ADMINISTRATION

Before treatment, all patients were assessed for eligibility and informed consent was obtained. The medical history and physical examination results were recorded and the tumors measured by visual and palpable examination. If the entire tumor was not measurable on routine examination, either computed tomography (CT) or magnetic resonance imaging (MRI) was used to size the tumor. In the office setting, the tumor was injected with 10 mg of Allovectin-7 dissolved in 1 mL of isotonic sodium chloride containing 1% glycerin and 0.01% vitamin E. Gentle aspiration was used during injection to prevent vascular injection. Vital signs were measured 2 hours following injection and the patients were then discharged from the office. During the next 14 days the chemistry survey was repeated. Two weeks following 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.

The patients returned 1 week following the second injection at which time the history and physical examination were repeated. The tumor was remeasured, with CT or MRI scans if necessary. If there was progressive disease, another biopsy of the tumor was performed and the patient received no further treatment with Allovectin-7. If there was no evidence of progressive disease, the patient returned 3 weeks later, completing a 4-week treatment break, and received a second identical cycle of treatment. Responding patients therefore received a total of 4 injections. Sixteen weeks after starting treatment, a biopsy was performed on the tumor site. The tumor biopsy specimens were less than 5 mm³ and were processed for histological evaluation as a single paraffin block.

RESPONSE CRITERIA

All patients were assessed for toxic effects at each visit according to the National Cancer Institute’s Common Toxicity Criteria.28 Response was evaluated by clinical examination if possible and by CT or MRI scan if necessary. The same method was repeatedly used for each patient to maintain consistency. A complete response was defined as disappearance of all clinical and radiographic evidence of active tumor for a minimum of 4 weeks. The patient must be free of all symptoms of cancer with a negative biopsy result. 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 may 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%.
25% in the absence of the development of new lesions. Progressive disease was defined as the appearance of a new lesion, 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 gene therapy injection.

IMMUNOHISTOCOMPATIBILITY

Testing for the HLA-A and HLA-B class I antigens was performed by the microlymphotoxicity dye exclusion method using Ficoll-Hypaque separated cells and commercial trays. Each HLA-A and HLA-B antigen was defined by at least 2 sera that were functionally monospecific. A full typing of HLA-A and HLA-B antigens was performed to define clearly the HLA-B7 antigen as cross-reacting antigens were represented in the tray format. The anti HLA-B7 antibody reagent serum was obtained from a multiparous woman and screened against a 60-cell panel of HLA-typed cells for confirmation.

IMMUNOHISTOCHEMICAL STAINING

HLA-B7 expression was evaluated with the same antibody that was used for the immunohistocompatibility analysis. Immunohistochemistry was performed on 4-µm-thick sections that were pretreated with trypsin using an indirect biotin-avidin method and an automatic immunostainer (Ventana 320, Ventana Medical Systems, Tucson, Ariz). 3’3’-Diaminobenzidine hydrochloride (DAB) was used as the chromogen and hematoxylin as the counterstain. The primary antibody was diluted 1:600. The secondary antibody was replaced with a biotin-conjugated affinity-purified goat anti–human IgG (Jackson Laboratories, West Grove, Pa) diluted 1:600 to account for the use of a human primary antibody. Negative controls were prepared by substituting the primary antibody with nonimmune IgG and IgM (Sigma Inc, St Louis, Mo). Sections from a squamous cell carcinoma specimen from a known HLA-B7–expressing patient were used as a positive control.

APOPTOSIS ASSAY

The terminal deoxynucleotide transferase–mediated deoxyuridine triphosphate–biotin nick end labeling (TUNEL) assay was performed using an in situ apoptosis detection kit (ApopTag Plus, Oncor Inc, Gaithersburg, Md), which is a modification of a previously described technique. The fluorophore incorporated into the antibody used for labeling generates an intense signal at 523 nm when excited by light of 494 nm. A propidium iodine counterstain was used.

Figure 1. Schematic of the Allovectin-7 (Vical Inc, San Diego, Calif) plasmid, VCL-1005, which contains the HLA-B7 complementary DNA (cDNA) sequence. Each of the component parts of the plasmid and their respective functions are designated by letters: a, respiratory syncytial virus promoter; b, HLA-B7 heavy chain cDNA; c, CITE sequence; d, β2-microglobulin cDNA; e, bovine growth hormone transcription terminator and polyadenylation signal; f, kanamycin resistance gene; and g, pBR322. The outer thick arrows represent transcribed regions and the inner thin arrows represent translation units.

administration of ganciclovir results in death of tk-transduced tumor cells. Both proximal and distant non-transduced cells are eliminated by a bystander effect consistent with an antitumor response.

A similar rationale has been suggested by Plautz et al, who proposed that ectopic expression of an alloantigen in tumor cells might elicit a broad, specific, antitumor response. Tumor cells frequently have diminished or absent class I major histocompatibility complex (MHC) proteins, which limits the ability of these cells to present antigens to cytotoxic T cells. If these cell lines are transduced with low levels of class I MHC antigens, they become less oncogenic. This approach was applied to a murine tumor model, and led to the development of Allovectin-7 (Vical Inc, San Diego, Calif) for clinical investigations.

Allovectin-7 contains a VCL-1005, 4695–base pair plasmid DNA that encodes the HLA-B7 heavy chain and β2-microglobulin. The β2-microglobulin allows for the expression and stabilization of the complete HLA-B7 class I MHC on the cell surface. The plasmid has a respiratory syncytial virus promoter to drive expression of both complementary DNAs (cDNAs). The cDNAs for HLA-B7 and β2-microglobulin are separated by CITE, an internal ribosomal entry site that permits coexpression of both genes from a single promoter. The plasmid DNA is complexed with DMRIE/DOPE (1,2-dimyristoylphosphatidyl-3-dimethylammonium bromide/dioleoyl phosphatidyl ethanolamine), a cationic lipid mixture that facilitates cellular uptake of the DNA.

Studies with Allovectin-7 in rodents and primates have demonstrated no significant toxic effects.
tumoral injection of Allovectin-7 in 5 patients with melanoma demonstrated successful gene transfer, an immune response in 2 patients, a partial remission in 1 patient, and no adverse events attributable to the drug.21 In aggregate, these studies formed the basis for a clinical trial to test the safety and antitumor efficacy of foreign surface antigen–mediated gene therapy for advanced squamous cell carcinoma of the head and neck.

RESULTS

All the gene therapy injections were administered in the office setting without any drug-related adverse events. One patient (patient 3) developed increasing dysphagia during therapy, which was attributed to tumor growth. A gastrostomy was placed. Another patient (patient 4) developed increasing airway obstruction from progressive disease 4 weeks after completing gene therapy. A tracheostomy was performed. There were no adverse events related to the gene therapy or its administration.

All the patients were evaluated at 1 week following the completion of the first cycle of therapy (Table). In 5 patients (patients 1, 3, 4, 7, and 8), there was evidence of progressive disease, manifested by increased tumor size in 4 patients and a decrease in performance status in 1 patient. In 4 of these patients, there was redness or tumor necrosis at the injection site, but the overall tumor continued to progress and treatment was therefore discontinued. Three of these patients have died of their cancer.
Four patients (patients 2, 5, 6, and 9) had a partial response following the first cycle of therapy, and went on to receive the second cycle of therapy. In 2 patients, the tumor has completely regressed on clinical examination. These tumors had slowly decreased in size with no gross evidence of necrosis. However, in all patients who completed treatment, the posttreatment biopsy specimen revealed histological evidence of squamous cell carcinoma. They are therefore classified as partial responders. These patients have been observed for up to 63 weeks from the time of the last drug injection and no overt tumor growth has been seen. One patient (patient 5) died 21 weeks after commencing treatment. He had a large necrotic neck mass that was intimately associated with the carotid artery. The mass decreased by approximately 50% during treatment and changed minimally in size thereafter, but remained necrotic and eventually resulted in a fatal carotid rupture.

One patient (patient 2) has survived 72 weeks since the first drug administration. A biopsy specimen of the treated tumor site taken at 10 months likewise revealed persistent squamous carcinoma cells. The absence of any tumor mass in his oropharynx for 72 weeks by clinical and radiologic evaluation suggests that these persistent cancer cells are either not rapidly dividing or are undergoing rapid cell death.

All the posttreatment biopsy specimens demonstrated lymphocytic infiltration of the remaining tumor cells (Figure 2). This was not significantly different, however, from the pretreatment biopsy specimens in which...
lymphocytic infiltration was also a common finding. Five of the tumors had significant regions of necrosis. Of interest, only 1 of these necrotic-appearing tumors responded to treatment (patient 5).

Immunohistochemistry was performed on all the biopsy specimens obtained before and after gene therapy. In many of the specimens the results were difficult to interpret due to necrosis. The specimens from patients 2 and 6 were the most informative, as both patients responded to therapy and had tumors with minimal necrosis. In both patients, the pretreatment biopsy specimen showed no evidence of HLA-B7 protein. The individual sections prepared from the post-treatment specimens from the original tumor site in both patients demonstrated focal weak staining for HLA-B7 in 10% or less of the remaining squamous carcinoma cells (Figure 3). These specimens were obtained 16 weeks after starting treatment. Patient 2 underwent another biopsy 6 months later, and the tumor site continued to demonstrate squamous carcinoma cells with an inflammatory infiltrate of neutrophils and lymphocytes. HLA-B7, however, was no longer demonstrable by immunohistochemistry.

The TUNEL assay was performed as a measure of apoptosis. Tumor necrosis can produce diffuse staining with this assay, and many of the specimens were therefore not interpretable. The specimens from patients 2 and 6 were the most informative, as both patients responded to therapy and had tumors with minimal necrosis. Comparison of pretreatment specimens with post-treatment specimens demonstrated a marked increase in apoptotic cells (Figure 4). Comparison of these sections with serial sections stained with hematoxylin and eosin suggested that these apoptotic cells were within the residual squamous cell carcinoma.

**COMMENT**

The injection of a cDNA “drug” that induces expression of a foreign class I MHC resulted in a cytoreductive response in 4 of 9 patients. One patient’s tumor response has persisted for more than 1 year. This is a remarkable
finding, as these patients had been maximally treated for advanced head and neck cancer prior to gene therapy. Injection of this cDNA and cationic lipid complex elicited no observable toxic effects. The procedure was performed in the office setting with minimal patient discomfort. Considerable care was taken to avoid injection into great vessels or nerves. This treatment therefore is minimally toxic and potentially tumor reductive.

This gene therapy resulted in tumor regression in 4 patients without any detectable toxic effects in any of the treated patients. Although the patient sample is small, the proportion of patients who responded is remarkably high given that these patients had already failed conventional therapy and spontaneous remission of head and neck squamous cell carcinoma does not occur. This treatment, therefore, has significant potential in the treatment of head and neck squamous cell carcinoma. These patients had undergone prior therapy with surgery and radiation and yet 4 remained responsive to gene therapy, suggesting that a response is still possibly induced after significant local fibrosis and immunosuppression. All patients had highly advanced disease, but were selected for treatment based on a high performance status. These results, therefore, cannot be applied to patients with advanced disease who are debilitated.

The exact mechanism by which tumor regression is effected is unresolved. Expression of HLA-B7 protein was demonstrated in 2 of the patients who responded to therapy, but the immunologic and cellular responses that caused tumor regression remain unclear. The TUNEL assay demonstrated that some of the tumor cells were dying by a mechanism consistent with apoptosis, suggesting that the gene therapy may activate or restore such pathways. This is intriguing since a hallmark of cancer cells is their inability to undergo programmed cell death. The possibility remains that the TUNEL assay detected apoptotic immune cells at the cancer site; however, based on histological examination of serial sections of the biopsy specimens, this possibility is unlikely. Thus, the mechanism of this apparent immune-mediated therapy needs further study. The involvement of cytokines as mediators of this antitumor response and their potential to augment tumor regression is also worthy of further investigation.

In a recent study in which 17 patients were treated with Allovecin-7 for metastatic melanoma, 7 had a greater than 25% reduction in tumor volume. In contrast, Allovecin-7 treatment for metastatic renal cell and colorectal carcinoma failed to cause tumor regression. The failure of patients with renal cell and colorectal carcinoma, as well as some patients with melanoma and head and neck squamous cell carcinoma, to achieve cytodestructive responses may be due to an inability of these tumors to adequately express the MHC antigen, or to an impairment of the immune system. An appreciation of the cell types and cytokines that participate in the antitumor response may help determine why some patients respond to treatment and others do not.

This form of gene therapy offers significant advantages to many other forms of gene therapy under development. Many gene-based therapies aim to replace defective genes, thereby restoring normal cellular function. Tumorigenesis, however, is not a consequence of a single genetic alteration, but is the result of the accumulation of multiple mutations. If replacement of defective genes is to succeed in causing a lasting tumor response, not only are better methods of transfection needed but also a library of genes that can be tailored to each patient and tumor genotype. A further complicating factor is the inherent genomic instability characteristic of most tumors that can result in subsequent loss of replacement genes. Cancer gene therapy strategies designed to express an alloantigen and elicit an immune response avoids the limitations of gene replacement approaches, appears to have low toxicity, and is worthy of further study.

Significant potential clinical application exists for this treatment, including its use for patients with head and neck cancer who have failed radiotherapy and whose tumors are otherwise unresectable. A phase 2 multi-institutional study with similar inclusion criteria has been...
initiated to confirm the results obtained with this phase 1 study and to increase the experimental population base. If the phase 2 results confirm our data, this potentially cytoreductive, minimally toxic therapy should not be used only when all other treatment modalities have failed. The results of the phase 2 trial will determine whether this gene therapy should be tested in patients with less advanced cancer, where it can be applied as an adjunctive therapy. In these patients with less debilitating disease, the response rate may be even greater. The overall aim would thereby be to improve patient survival while reducing the need for radical treatments associated with high morbidity.

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

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