Vaccination With Irradiated, Autologous Melanoma Cells Engineered to Secrete Granulocyte-Macrophage Colony-Stimulating Factor by Adenoviral-Mediated Gene Transfer Augments Antitumor Immunity in Patients With Metastatic Melanoma

By Robert Soiffer, F. Stephen Hodi, Frank Haluska, Ken Jung, Silke Gillessen, Samuel Singer, Kenneth Tanabe, Rosemary Duda, Steven Mentzer, Michael Jaklitsch, Raphael Bueno, Shirley Clift, Steve Hardy, Donna Neuberg, Richard Mulligan, Iain Webb, Martin Mihm, and Glenn Dranoff

Purpose: **Vaccination with irradiated, autologous melanoma cells engineered to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) by retroviral-mediated gene transfer generates potent antitumor immunity in patients with metastatic melanoma. Further clinical development of this immunization scheme requires simplification of vaccine manufacture. We conducted a phase I clinical trial testing the biologic activity of vaccination with irradiated, autologous melanoma cells engineered to secrete GM-CSF by adenoviral-mediated gene transfer.**

Patients and Methods: **Excised metastases were processed to single cells, transduced with a replication-defective adenoviral vector encoding GM-CSF, irradiated, and cryopreserved. Individual vaccines were composed of 1×10^6, 4×10^6, or 1×10^7 tumor cells, depending on overall yield, and were injected intradermally and subcutaneously at weekly and biweekly intervals.**

Results: **Vaccines were successfully manufactured for 34 (97%) of 35 patients. The average GM-CSF secretion was 745 ng/10^6 cells/24 hours. Toxicities were restricted to grade 1 to 2 local skin reactions. Eight patients were withdrawn early because of rapid disease progression. Vaccination elicited dense dendritic cell, macrophage, granulocyte, and lymphocyte infiltrates at injection sites in 19 of 26 assessable patients. Immunization stimulated the development of delayed-type hypersensitivity reactions to irradiated, dissociated, autologous, nontransduced tumor cells in 17 of 25 patients. Metastatic lesions that were resected after vaccination showed brisk or focal T-lymphocyte and plasma cell infiltrates with tumor necrosis in 10 of 16 patients. One complete, one partial, and one mixed response were noted. Ten patients (29%) are alive, with a minimum follow-up of 36 months; four of these patients have no evidence of disease.**

Conclusion: **Vaccination with irradiated, autologous melanoma cells engineered to secrete GM-CSF by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma.**

J Clin Oncol 21:3343-3350.

THERE IS convincing evidence that malignant melanoma frequently stimulates humoral and cellular responses. Serologic-based cloning strategies have identified an impressive repertoire of tumor-associated targets, including melanosomal proteins, cancer-testis antigens, and mutated gene products.[1-3] $CD4^+$ and $CD8^+$ T cells that recognize melanoma cells can be detected in the blood, lymph nodes, or tumors of many patients.[4-8] The host reaction to early-stage melanoma may influence clinical outcome. Brisk T-lymphocyte infiltrates in the vertical growth phase are strongly associated with a reduced incidence of recurrent disease and mortality.[9,10] Dense T-cell reactions in tumors that metastasize to regional lymph nodes similarly predict improved survival when compared with lesions that fail to elicit infiltrates.[11]

Notwithstanding these provocative data, most patients fail to develop antimelanoma responses that are sufficiently potent to prevent lethal tumor progression. The understanding that tumor cells generally stimulate poor antigen presentation has motivated the design of several new strategies to increase antitumor immunity,[12,13] and many of these strategies are under active study in melanoma.[14-30] Among the approaches using gene transfer, we demonstrated that vaccination with irradiated tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) generates potent, specific, and long-lasting antitumor immunity in multiple murine models, including the B16 melanoma.[31] Vaccination involves enhanced tumor antigen presentation by recruited dendritic cells and macrophages; the coordinated functions of $CD4^+$ and $CD8^+$ T

From the Departments of Medical Oncology, Surgery, and Biostatistics, Dana-Farber Cancer Institute; Departments of Medicine and Surgery, Brigham and Women's Hospital; Departments of Medicine, Surgery, Biostatistics, Genetics, and Pathology, Harvard Medical School; Department of Medicine, Division of Hematology-Oncology, and Departments of Surgery and Pathology, Massachusetts General Hospital; Department of Surgery, Beth Israel Deaconess Medical Center; and Children's Hospital, Boston, MA; and Cell Genesys, Foster City, CA.

Submitted July 1, 2002; accepted June 11, 2003.

Supported by grant no. CA74886 from the National Institutes of Health, Bethesda, MD; the Cancer Research Institute, New York, NY; the Leukemia and Lymphoma Society, White Plains, NY; and Cell Genesys, Foster City, CA.

Address reprint requests to Glenn Dranoff, MD, Dana-Farber Cancer Institute, Dana 510E, 44 Binney St, Boston, MA 02115; e-mail: glenn_dranoff@dfci.harvard.edu.

0732-183X/03/2117-3343/$20.00

cells, CD1d-restricted natural killer-T cells, and antibodies mediate protective immunity.[31-34]

We previously reported a phase I trial of vaccination with irradiated, autologous melanoma cells engineered to secrete GM-CSF by retroviral-mediated gene transfer in patients with metastatic melanoma.[35] Vaccination sites showed brisk infiltrates of dendritic cells, macrophages, eosinophils, and lymphocytes in all 21 assessable patients. Although metastatic lesions resected before vaccination disclosed minimal immune infiltrates, metastatic lesions resected after vaccination revealed dense infiltrates of $CD4^+$ and $CD8^+$ T lymphocytes and plasma cells with extensive tumor destruction, fibrosis, and edema in 11 of 16 examined patients.

Although these findings illustrate the biologic activity of GM-CSF–secreting autologous melanoma cells, the use of conventional retroviral vectors for vaccine manufacture may impede further clinical development. Murine leukemia virus–derived vectors require replicating target cells for efficient gene transfer and are potential carcinogens.[36] Therefore, short-term tumor cultures must be prepared from each patient sample, and the transduced cells must undergo extensive safety testing before clinical administration. However, adenoviral vectors are able to transduce resting target cells and show only minimal toxicities with ex vivo applications,[37] properties that render these vectors an attractive alternative for vaccine production. In this article, we report a phase I clinical trial that demonstrates the feasibility, safety, and immunogenicity of vaccination with irradiated, autologous melanoma cells engineered to secrete GM-CSF by adenoviral-mediated gene transfer.

PATIENTS AND METHODS

Patients

This phase I Dana-Farber Partners Cancer Care clinical protocol received approval from the local institutional review board and biosafety committees, the National Institutes of Health Recombinant DNA Advisory Committee, and the Food and Drug Administration. Patients were eligible for enrollment if they had metastatic melanoma, an Eastern Cooperative Oncology Group performance status of 0 or 1, an estimated life expectancy $\geq$ 6 months, age $\geq$ 18 years, and had signed informed consent, and were $\geq$ 4 weeks from chemotherapy, radiotherapy, immunotherapy, or corticosteroid therapy and more than 6 months from bone marrow or peripheral-blood stem-cell transplantation. Patients were excluded if they were pregnant or nursing, human immunodeficiency virus positive, or had uncontrolled active infection. Enrolled patients received staging scans and routine hematology and chemistry testing.

Vaccine Production

Metastases were excised, placed into sterile media, and brought to the Connell-O'Reilly Gene Transfer Laboratory (a dedicated biosafety level 2 facility for human gene transfer experiments) at the Dana-Farber Cancer Institute (Boston, MA). The masses were cut into small pieces and digested to single cells with mechanical agitation and collagenase. Two $\times$ 10^6 tumor cells were irradiated (100 Gy) and cryopreserved (90% fetal calf serum and 10% dimethyl sulfoxide) in 1 $\times$ 10^6 cell aliquots for delayed-type hypersensitivity testing. The remaining tumor cells were transduced overnight at 37°C (in alpha-minimal essential medium, 10% fetal calf serum, and gentamicin) with a replication-defective adenoviral vector encoding human GM-CSF (Ad-GM; Cell Genesys, Foster City, CA) at a multiplicity of infection of 10. Preliminary studies with Ad-Lac Z (the same vector backbone with a beta-galactosidase cDNA insert) demonstrated that at least 50% of the tumor cells were transduced under these conditions (data not shown). In four patients, additional vaccines were manufactured using cells obtained from short-term, autologous tumor cultures.

Ad-GM contains a GM-CSF expression cassette in the E1 region of adenovirus type 5 and a second deletion in the E3 region.[38] The GM-CSF expression cassette contains the cytomegalovirus immediate early promoter/enhancer,[39] a shortened human beta-globin second intron, the human GM-CSF gene,[40] and the beta-globin polyadenylation signal and 3′ untranslated region. The integrity of the viral vector was confirmed by restriction analysis. High titer stocks of Ad-GM underwent extensive testing and certification before Food and Drug Administration approval for clinical use.

After overnight transduction, the tumor cells were extensively washed and irradiated (100 Gy). For 48 hours, 1 $\times$ 10^6 cells were cultured. The supernatants were collected, and GM-CSF levels were measured by an enzyme-linked immunosorbent assay (ELISA; Endogen EH-GMCSF, Woburn, MA) according to the manufacturer's protocol. Preliminary studies showed that GM-CSF secretion was not significantly influenced by irradiation (data not shown). Based on the overall viable tumor cell yield, vaccine aliquots were cryopreserved as follows: $\leq$ 3 $\times$ 10^7 total, 1 $\times$ 10^6 aliquots (dose level 1); 3 $\times$ 10^7 to 1 $\times$ 10^8 total, 4 $\times$ 10^6 aliquots (dose level 2); $\geq$ 1 $\times$ 10^8 total, 1 $\times$ 10^7 aliquots (dose level 3). Samples of transduced and nontransduced tumor cells were tested for sterility, endotoxin, and *Mycoplasma pneumoniae*. For clinical administration, cryopreserved cells were thawed, washed extensively, and resuspended in 1 mL of sterile saline for the vaccines and 0.5 mL of sterile saline for the nontransduced cells for delayed-type hypersensitivity testing.

Vaccination and Evaluation

Vaccines were injected intradermally (0.5 mL) and subcutaneously (0.5 mL) into normal skin on the limbs and abdomen on a rotating basis. The treatment schema consisted of immunizations weekly for 3 weeks and then every other week until the vaccine supply was exhausted or the patient was removed from the study. A minimum of six vaccinations was required for a patient to be considered assessable for biologic activity. Patients were restaged at week 10 and then at 4-month intervals or when clinically indicated. Responding, stable, or indolent patients were eligible for additional rounds of vaccination.

Irradiated, dissociated, nontransduced tumor cells were injected intradermally (0.5 mL) into normal skin at the time of beginning vaccination and then with the fifth vaccination to evaluate delayed-type hypersensitivity. Punch biopsies were obtained 2 to 3 days after injections. When possible, distant metastases were biopsied after vaccination to assess immune infiltrates. For pathologic examination, tissues were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Immunohistochemistry was performed using standard techniques with monoclonal antibodies to CD4, CD8, CD20, and immunoglobulin kappa. Dendritic cells were identified by CD1a staining (four patients) and/or by a characteristic morphology in hematoxylin and eosin sections that was previously reported[41] (ovoid or dendritic shape with prominent pale-gray cytoplasm; oval, sometimes indented nucleus with clear nucleoplasm; and single, small blue nucleolus often apposed to a delicate nuclear membrane). Delayed-type hypersensitivity reactions were considered strong when the following conditions were present: mononuclear cells admixed with eosinophils and basophils accumulated around blood vessels; endothelial cells were swollen or partially necrotic, often with vessel luminal occlusion; and dermal edema and fibrin exudation were present. Tumor infiltrates were considered significant when they occupied at least 30% of multiple high-power microscopic fields.

Antiadenoviral antibodies were measured by an ELISA. Ninety-six well ELISA plates (COSTAR, Acton, MA) were coated with intact Ad-Lac Z particles or viral lysates (prepared with aminocaproic acid, soybean trypsin inhibitor, leupeptin, pepstatin, and 0.5% Nonidet P-40) in a carbonate buffer. The wells were blocked overnight at 4°C with 2% nonfat dried milk/phosphate-buffered saline, washed, and incubated in duplicate with 100 μL of patient sera diluted 1:500 in 2% nonfat dried milk/phosphate-buffered saline overnight at 4°C. A goat antihuman immunoglobulin G conjugated to horseradish peroxidase (Zymed, South San Francisco, CA) was added at

Table 1. Patient Characteristics

Characteristic		No. of Patients (N = 35)
Sex		
Male		22
Female		13
Age, years		
Median	55	
Range	23-77	
Interval, diagnosis to vaccination, months		
Median	36	
Range	3-324	
Site of metastases		
Subcutaneous/soft tissue		21
Lung		14
Lymph node		11
Liver		2
Bone		2
Spleen		2
Omentum		1
Uterus		1
Parotid gland		1
Adrenal gland		1
Gingiva		1
Prior therapy		
Surgery		17
Interferon		10
Chemotherapy		8
Radiation		8
Interleukin-2		5
Limb perfusion		2
Other vaccine		2

Table 2. Vaccine Production and Administration

Vaccine Characteristics		No. of Patients (N = 35)
Vaccine successfully prepared		34
Vaccine source		
Subcutaneous/soft tissue		17
Lymph node		8
Lung		5
Liver		1
Uterus		1
Omentum		1
Parotid gland		1
Adrenal gland		1
Cell viability, %		
Median	70	
Range	8-100	
GM-CSF secretion, ng/10^6 cells/24 hours		
Median	745	
Range	11-2,608	
Dose level		
1, 1×10^6 cells		9
2, 4×10^6 cells		11
3, 1×10^7 cells		14

Abbreviation: GM-CSF, granulocyte-macrophage colony-stimulating factor.

room temperature, and the plate was developed with a one-step 3,3′,5,5′-tetramethylbenzidine/peroxide reagent (DAKO, Glostrup, Denmark). All samples were performed in duplicate. The values reported were the mean absorbance at 450 nm for virus-coating buffer only.

RESULTS

Patient Characteristics

Thirty-five metastatic melanoma patients were enrolled onto this phase I trial between October 1997 and May 1999. The characteristics of the patients are listed in Table 1. There were 22 males and 13 females, with a median age of 55 years (range, 23 to 77 years). The average interval between the primary diagnosis of melanoma and study entry was 3 years (range, 3 months to 27 years). Sites of metastases on enrollment included subcutaneous/soft tissues (n = 21), lung (n = 14), lymph node (n = 11), liver (n = 2), omentum (n = 1), bone (n = 2), spleen (n = 2), uterus (n = 1), parotid gland (n = 1), and adrenal gland (n = 1). Seventeen patients showed involvement of multiple organs. Prior therapies for metastatic disease were surgery (n = 17), interferon (n = 10), chemotherapy (n = 8), radiation therapy (n = 8), interleukin-2 (n = 5), limb perfusion therapy (n = 2), and other vaccines (n = 2).

Vaccine Production and Administration

Tumor tissue was obtained from all 35 enrolled patients, most commonly from subcutaneous/soft tissues (n = 17) or lymph nodes (n = 8), but also from lung (n = 5), uterus (n = 1), omentum (n = 1), liver (n = 1), adrenal gland (n = 1), and parotid gland (n = 1, Table 2). Solid tumors were processed to single-cell suspension with mechanical agitation and collagenase digestion, transduced overnight with Ad-GM, irradiated, and cryopreserved.

Vaccines were successfully prepared for 34 (97%; 90% exact binomial confidence interval [CI], 87% to 100%) of the 35 patients (nine patients at dose level 1, 11 at dose level 2, and 14 at dose level 3). Insufficient numbers of viable cells precluded vaccine manufacture in one patient. The average GM-CSF secretion after irradiation was 745 ng/10^6 cells/24 hours, with a range from 11 to 2,608 ng/10^6 cells/24 hours and a median of 534 ng/10^6 cells/24 hours. The average cell viability was 70%, with a range from 8% to 100% and a median of 75%. The levels of GM-CSF produced generally were related to the overall viability of the cell population (detailed cell processing data will be presented elsewhere).

Rapid disease progression resulted in the early withdrawal of eight patients, two of whom never received any vaccinations; most early withdrawals were at dose level 3, likely reflecting higher tumor burdens. Twenty-six patients completed at least six immunizations and were considered assessable for biologic activity (76%; 90% exact binomial CI, 62% to 88%); eight of these patients were treated at dose level 1, nine were treated at dose level 2, and nine were treated at dose level 3. The average interval between tumor harvest and first vaccination was 3 weeks (range, 1 to 9 weeks). Seven patients with stable or indolent disease received additional courses of immunization. The median number of vaccinations was eight (range, one to 34 vaccinations).

Toxicities

Vaccination consistently elicited grade 1 to 2 erythema and induration at injection sites, often with associated pruritus. There

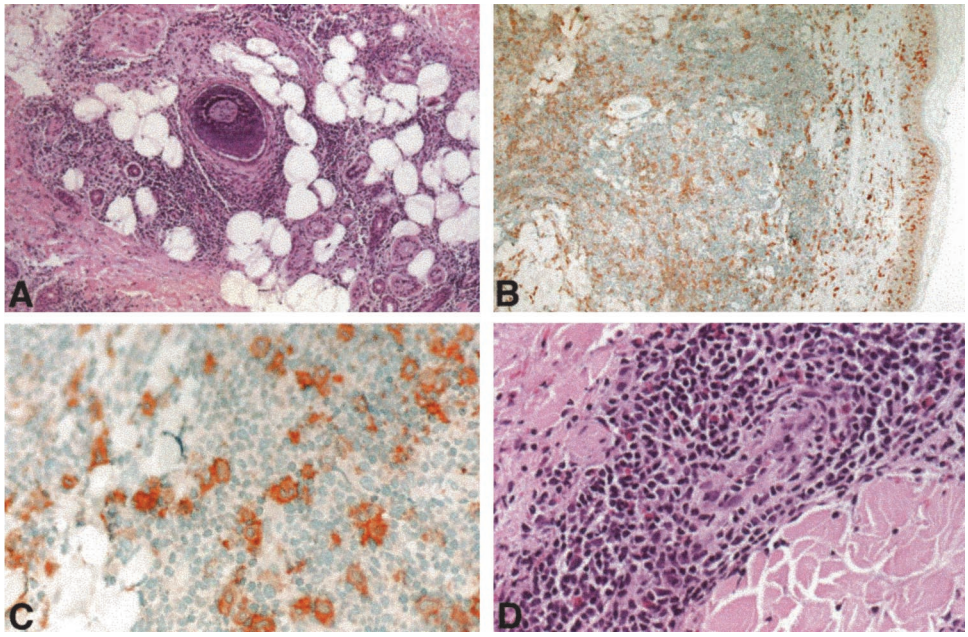

Fig 1. (A) Vaccination site reaction; (B, C) $CD1a^+$ dendritic cells; and (D) delayed-type hypersensitivity reaction with eosinophils.

were no significant hepatic, renal, pulmonary, cardiac, hematologic, gastrointestinal, or neurologic toxicities attributable to treatment. No autoimmune reactions or adenoviral infections were observed. One patient died from pneumococcal sepsis after the first immunization, although this was not related to vaccination.

Vaccination Reactions

Injections of irradiated, autologous GM-CSF–secreting melanoma cells elicited local reactions in all 26 assessable patients. The intensity and frequency of these responses were related to vaccine dose; strong reactions were observed in three of eight patients at dose level 1, eight of nine patients at dose level 2, and eight of nine patients at dose level 3. Erythema and induration at injection sites typically resolved over 48 to 72 hours. Local responses generally increased with subsequent immunizations, and patients with strong reactions sometimes developed recall responses at previous vaccination sites.

Immunization reactions were characterized by substantial dermal infiltrates of dendritic cells, macrophages, neutrophils, and lymphocytes (Fig 1A, 1B, and 1C). A prominent eosinophil component was present in seven patients (three patients at dose level 1, two at dose level 2, and two at dose level 3). Strong reactions included endothelial cell activation and damage in the superficial venules. The intensity of the infiltrates generally corresponded to the degree of erythema and induration.

Delayed-Type Hypersensitivity Reactions

Irradiated, dissociated, autologous nontransduced melanoma cells were available for delayed-type hypersensitivity testing in 25 patients (insufficient cells precluded these studies in one patient at dose level 1). Injections of nontransduced melanoma cells at the beginning of vaccination failed to provoke clinical or pathologic reactivity in any patients. However, three of seven patients at dose level 1, seven of nine patients at dose level 2, and seven of nine patients at dose level 3 developed significant reactions to these cells when administered at the time of the fifth vaccination or thereafter (68%; 90% exact binomial CI, 50% to 83%). Pathologic examination revealed T-lymphocyte and macrophage infiltrates throughout the dermis (Fig 1D). Eosinophils were also present in seven patients (three patients at dose level 1, two at dose level 2, and two at dose level 3).

Antiadenoviral Humoral Responses

Because the infected tumor cells may express some viral gene products, vaccination might stimulate immunity to adenoviral proteins. To explore this possibility, we developed an ELISA to measure antibodies against intact and lysed adenoviral particles (Ad-Lac Z). All eight patients who were examined showed detectable antibodies to both intact and lysed adenoviral particles at study entry, consistent with the high prevalence of adenoviral infection in the adult population.[37] Augmented antibody titers as a function of vaccination were found in five of the eight patients, and representative longitudinal studies are shown in Fig 2. Increases in antibodies were not related to the number of vaccinations or GM-CSF secretion levels. Moreover, the development of reactivity to autologous melanoma cells was not associated with changes in adenoviral antibody titers.

Immune Responses in Metastases

To evaluate whether the augmented antimelanoma immunity mediated tumor destruction, we compared the host responses in metastases resected before and after therapy. Before immunization, metastases showed either an absent or minimal cellular infiltrate in all patients. In contrast, 10 of 16 patients developed significant T-lymphocyte and plasma cell infiltrates associated with tumor destruction in metastases as a consequence of vaccina-

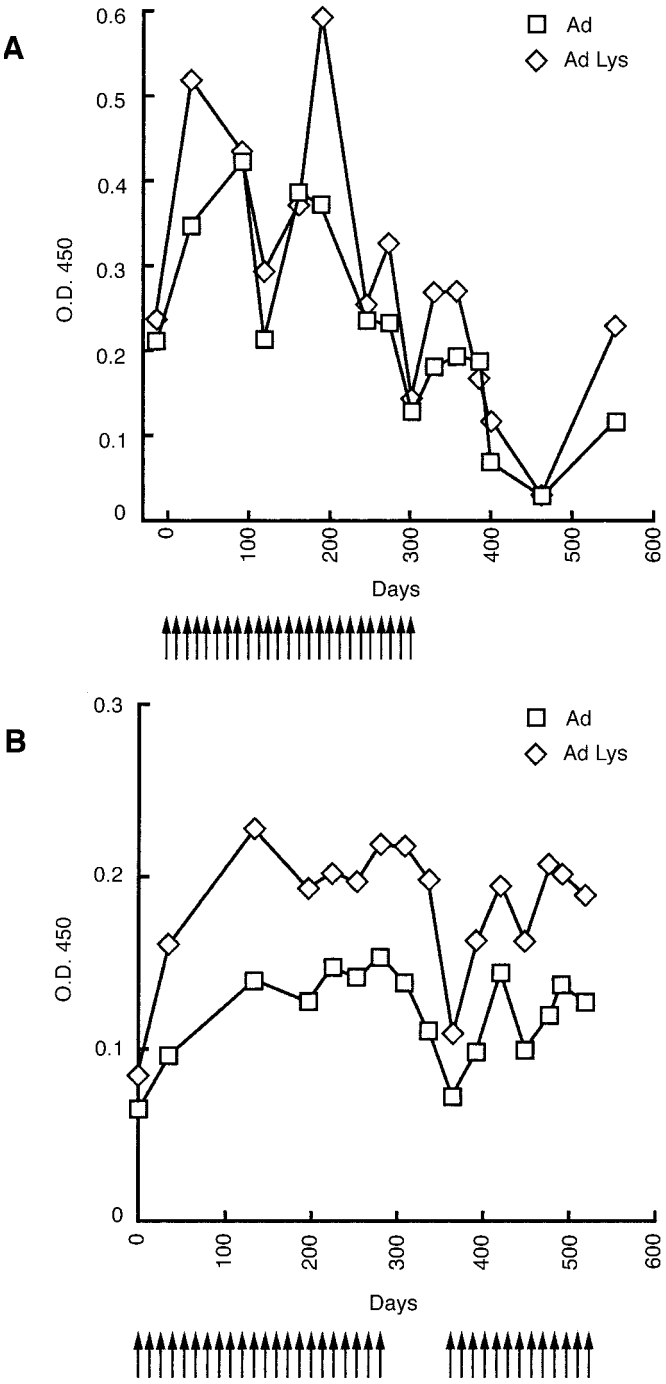

Fig 2. Vaccination stimulates increased antiadenoviral antibodies in some patients. Arrows denote immunizations. (A) Patient M28; and (B) patient M37. Abbreviations: O.D., optical density; Ad, intact adenoviral particles; Ad Lys, lysed adenoviral particles.

tion (four of five tumors at dose level 1, four of six tumors at dose level 2, and two of five tumors at dose level 3). A coordinated cellular and humoral response was indicated by the recruitment of $CD4^+$ and $CD8^+$ T lymphocytes and $CD20^+$ B cells secreting immunoglobulin (Fig 3). The T- and B-cell infiltrates were diffuse in six patients and focal in four patients; diffuse infiltrates were associated with more extensive tumor necrosis.

Clinical Outcomes

One complete, one partial, and one mixed response were observed. The regressing metastases were in subcutaneous tissues and the lung. Five patients demonstrated stable disease. The remaining 18 assessable patients exhibited progressive disease, although three achieved clinically meaningful responses to subsequent systemic therapy. Follow-up data are available on all 35 enrolled patients. Ten patients remain alive after a minimum of 36 months from study entry; four of these patients have no evidence of disease after further surgical treatment. The median survival time is 15 months, and the estimated 3-year overall survival rate is 29% (90% exact binomial CI, 16% to 41%; Fig 4). There was no association between prolonged survival and either cell dose level or GM-CSF secretion rate.

DISCUSSION

This phase I trial was undertaken in an effort to develop a biologically active, simplified method of manufacturing autologous GM-CSF–secreting melanoma vaccines. Our previous study using retroviral-mediated gene transfer revealed the ability of this immunization strategy to consistently augment cellular and humoral antimelanoma responses in patients with metastatic disease.[35] However, the complexity of establishing short-term tumor cultures and the requirement to certify the absence of replication competent retrovirus in the final product impeded further clinical development.

Because adenoviral vectors efficiently transduce resting target cells, we used this gene transfer technology to engineer freshly processed tumor samples. The ability to prepare vaccines for 34 of 35 patients demonstrates the high efficiency of this manufacturing scheme. Indeed, the average GM-CSF secretion rate of 745 ng/10^6 cells/24 hours exceeded the rate previously achieved with retroviral-mediated gene transfer.[35] The relatively low production levels in a few samples primarily reflected the extensive tumor necrosis present in the surgical specimens. Importantly, the brief overnight transduction protocol and the low pathogenicity of adenoviral vectors with ex vivo applications[37] enabled treatment to begin in a timely fashion.

Whereas the adenoviral vector yielded substantial improvements in vaccine manufacture, some differences in the antimelanoma immune response were observed compared with our previous clinical study. Vaccination with melanoma cells engineered by retroviral-mediated gene transfer consistently elicited local infiltrates composed of dendritic cells, macrophages, lymphocytes, and eosinophils.[35] Although immunization with melanoma cells engineered by adenoviral-mediated gene transfer stimulated strong dendritic cell, macrophage, and lymphocyte infiltrates in 19 of 26 patients, a prominent eosinophil component was generated in only seven patients. A comparable reduction in eosinophil recruitment to delayed-type hypersensitivity reactions was also observed. Preliminary studies in three patients indicate that peripheral-blood mononuclear cells secreted minimal or no interleukin-5 in response to autologous melanoma cells (data not shown), compared with the robust production consistently induced in the retroviral trial[35]; however, additional experiments

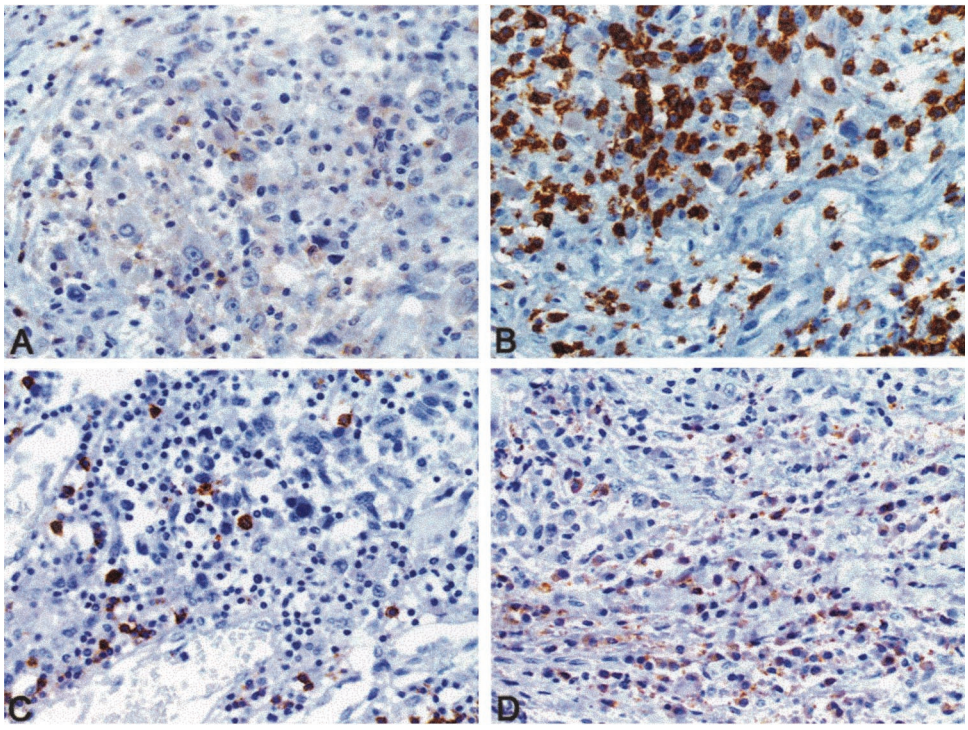

Fig 3. Infiltrated metastasis with extensive tumor destruction after vaccination. (A) $CD4^+$ T cells; (B) $CD8^+$ T cells; (C) $CD20^+$ B cells; and (D) immunoglobulin kappa.

are necessary to examine these differences in more detail. The interpretation of the antigenic specificities of the local responses is, moreover, complicated by the requirement to manipulate the autologous tumor cells ex vivo, with the attendant exposure to culture media. Thus, in addition to tumor-associated antigens, xenogeneic components may contribute to the reactions.

The mechanisms underlying the differences in antitumor immunity elicited by the adenoviral and retroviral vectors are currently unclear. One possibility might involve the use of freshly dissociated tumor suspensions versus cultured tumor cells. To explore this idea, we established short-term tumor cultures from several patients and transduced the cells with Ad-GM; vaccination with these products nevertheless failed to provoke eosinophil responses. Another possibility might involve specific characteristics of the adenoviral vector. However, a second phase I study in which we prepared irradiated, autologous GM-CSF–secreting non–small-cell lung carcinoma vaccines using the identical manufacturing scheme revealed the consistent induction of eosinophil-rich reactions that were indistinguishable from those in the retroviral melanoma trial.[42] A third possibility that remains to be investigated involves whether melanoma and non–small-cell lung carcinoma cells manifest important differences in transgene expression after in vivo administration.

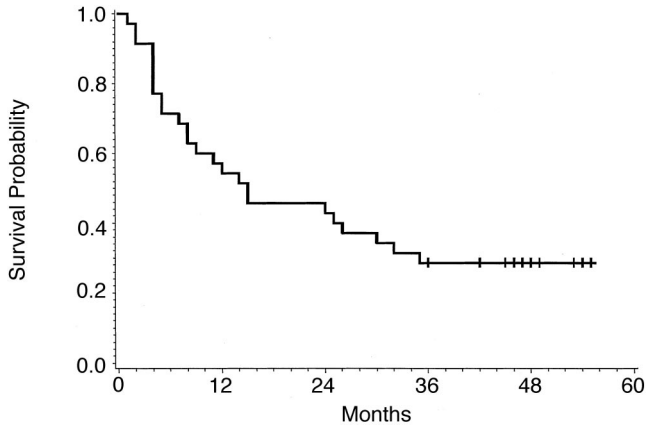

Fig 4. Survival probability of all 35 enrolled patients.

Vaccination with irradiated melanoma cells engineered to secrete GM-CSF by adenoviral-mediated gene transfer did stimulate immunity that was sufficiently potent to mediate antitumor effects. Ten of 16 patients developed significant lymphocyte infiltrates and tumor necrosis in distant metastases as a function of vaccination. The accumulation of $CD4^+$ and $CD8^+$ T cells and immunoglobulin-secreting $CD20^+$ B cells indicated the evolution of a coordinated humoral and cellular response. By undertaking a detailed analysis of one of the patients who developed vaccine-induced lymphocyte infiltrates, we identified the melanoma inhibitor of apoptosis protein as one of the targets mediating tumor destruction.[43]

In conclusion, this phase I study demonstrates that vaccination with irradiated, autologous melanoma cells engineered to secrete GM-CSF by adenoviral-mediated gene transfer augments antitumor immunity in patients with metastatic melanoma. The ongoing survival of 10 of 35 patients, with a minimum follow-up of 36 months, is intriguing and raises the possibility that vaccination might be effectively combined with other treatment approaches. Indeed, several patients manifested prolonged responses to subsequent chemotherapy or surgery. Similarly, the infusion

of a humanized monoclonal antibody to cytotoxic T lymphocyte-associated antigen 4 stimulated extensive tumor necrosis with lymphocyte and granulocyte infiltrates in three of three previously immunized patients.[44] Finally, alternative vaccine production protocols should also be explored, such as the admixing of generic GM-CSF delivery sources with irradiated autologous melanoma cells.[45,46] Methods like these may allow for improved standardization of vaccine manufacture.

ACKNOWLEDGMENT

The acknowledgment is included in the full text version of this article only, available on-line at www.jco.org. It is not included in the PDF version.

REFERENCES

1. Chen Y-T, Gure A, Tsang S, et al: Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc Natl Acad Sci USA 95:6919-6923, 1998

2. Sahin U, Tureci O, Schmitt H, et al: Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci USA 92:11810-11813, 1995

3. Old L, Chen Y-T: New paths in human cancer serology. J Exp Med 187:1163-1167, 1998

4. Anichini A, Mortarini R, Maccalli C, et al: Cytotoxic T cells directed to tumor antigens not expressed on normal melanocytes dominate HLA-A2.1-restricted immune repertoire to melanoma. J Immunol 156:208-217, 1996

5. Boon T, van der Bruggen P: Human tumor antigens recognized by T lymphocytes. J Exp Med 183:725-729, 1996

6. Romero P, Dunbar P, Valmori D, et al: Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high numbers of antigen-experienced tumor-specific cytolytic T lymphocytes. J Exp Med 188:1641-1650, 1998

7. Zeng G, Wang X, Robbins P, et al: $CD4^+$ T cell recognition of MHC class II-restricted epitopes from NY-ESO-1 presented by a prevalent HLA DP4 allele: Association with NY-ESO-1 antibody production. Proc Natl Acad Sci USA 98:3964-3969, 2001

8. Jäger E, Nagata Y, Gnjatic S, et al: Monitoring CD8 T cell responses to NY-ESO-1: Correlation of humoral and cellular immune responses. Proc Natl Acad Sci USA 97:4760-4765, 2000

9. Clark W, Elder D, Guerry D, et al: Model predicting survival in stage I melanoma based on tumor progression. J Natl Cancer Inst 81:1893-1904, 1989

10. Clemente C, Mihm M, Bufalino R, et al: Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. Cancer 77:1303-1310, 1996

11. Mihm M, Clemente C, Cascinelli N: Tumor infiltrating lymphocytes in lymph node melanoma metastases: A histopathologic prognostic indicator and an expression of local immune response. Lab Invest 74:43-47, 1996

12. Mach N, Dranoff G: Cytokine-secreting tumor cell vaccines. Curr Opin Immunol 12:571-575, 2000

13. Jäger E, Jäger D, Knuth A: Clinical cancer vaccine trials. Curr Opin Immunol 14:178-182, 2002

14. Berd D, Murphy G, Maguire HC, et al: Immunization with haptenized, autologous tumor cells induces inflammation of human melanoma metastases. Cancer Res 51:2731-2734, 1991

15. Oratz R, Cockerill C, Speyer J, et al: Induction of tumor-infiltrating lymphocytes in human malignant melanoma metastases by immunization to melanoma antigen vaccine. J Biol Response Mod 8:355-358, 1989

16. Mitchell MS, Harel W, Kempf RA, et al: Active-specific immunotherapy for melanoma. J Clin Oncol 8:856-869, 1990

17. Hersey P: Active immunotherapy with viral lysates of micrometastases following surgical removal of high risk melanoma. World J Surg 16:251-260, 1992

18. Morton DL, Foshag LJ, Hoon DSB, et al: Prolongation of survival in metastatic melanoma after active specific immunotherapy with a new polyvalent melanoma vaccine. Ann Surg 216:463-482, 1992

19. Slingluff C, Vollmer R, Siegler H: Stage II malignant melanoma: Presentation of a prognostic model and an assessment of specific active immunotherapy in 1,273 patients. J Surg Oncol 39:139-147, 1988

20. Livingston P, Wong G, Adluri S, et al: Improved survival in stage III melanoma patients with GM2 antibodies: A randomized trial of adjuvant vaccination with GM2 ganglioside. J Clin Oncol 12:1036-1044, 1994

21. Marchand M, van Baren N, Weynants P, et al: Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. Int J Cancer 80:219-230, 1999

22. Jäger E, Bernhard H, Romero P, et al: Generation of cytotoxic T-cell responses with synthetic melanoma-associated peptides in vivo: Implications for tumor vaccines with melanoma associated antigens. Int J Cancer 66:162-169, 1996

23. Nestle F, Alijagic S, Gilliet M, et al: Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nature Med 4:328-332, 1998

24. Rosenberg S, Yang J, Schwartzentruber D, et al: Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nature Med 4:321-327, 1998

25. Jäger E, Gnjatic S, Nagata Y, et al: Induction of primary NY-ESO-1 immunity: CD8+ T lymphocyte and antibody responses in peptide-vaccinated patients with NY-ESO-1+ cancers. Proc Natl Acad Sci USA 97: 12198-12203, 2000

26. Wang F, Bade E, Kuniyoshi C, et al: Phase I trial of a MART-1 peptide vaccine with incomplete Freund's adjuvant for resected high-risk melanoma. Clin Cancer Res 5:2756-2765, 1999

27. Lee P, Wang F, Kuniyoshi J, et al: Effects of interleukin-12 on the immune response to a multipeptide vaccine for resected metastatic melanoma. J Clin Oncol 19:3836-3847, 2001

28. Thurner B, Haendle I, Roder C, et al: Vaccination with Mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med 190:1669-1678, 1999

29. Banchereau J, Palucka AK, Dhodapkar M, et al: Immune and clinical responses in patients with metastatic melanoma to CD34(+) progenitor-derived dendritic cell vaccine. Cancer Res 61:6451-6458, 2001

30. Slingluff CL Jr, Yamshchikov G, Neese P, et al: Phase I trial of a melanoma vaccine with gp100(280-288) peptide and tetanus helper peptide in adjuvant: Immunologic and clinical outcomes. Clin Cancer Res 7:3012-3024, 2001

31. Dranoff G, Jaffee E, Lazenby A, et al: Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. Proc Natl Acad Sci USA 90:3539-3543, 1993

32. Huang AY, Golumbek P, Ahmadzadeh M, et al: Role of bone marrow-derived cells in presenting MHC class I-restricted tumor antigens. Science 264:961-965, 1994

33. Mach N, Gillessen S, Wilson SB, et al: Differences in dendritic cells stimulated in vivo by tumors engineered to secrete granulocyte-macrophage colony-stimulating factor or flt3-ligand. Cancer Res 60:3239-3246, 2000

34. Reilly R, Machiels J-P, Emens L, et al: The collaboration of both humoral and cellular HER-2/neu-targeted immune responses is required for

the complete eradication of HER-2/*neu*-expressing tumors. Cancer Res 61:880-883, 2001

35. Soiffer R, Lynch T, Mihm M, et al: Vaccination with irradiated, autologous melanoma cells engineered to secrete human granulocyte-macrophage colony stimulating factor generates potent anti-tumor immunity in patients with metastatic melanoma. Proc Natl Acad Sci USA 95:13141-13146, 1998

36. Mulligan RC: The basic science of gene therapy. Science 260:926-932, 1993

37. Shenk T: Adenoviridae: The viruses and their replication, in Fields BN, Knipe DM, Howley PM (eds): Fields Virology (ed 3). Philadelphia, PA, Lippincott-Raven Publishers, 1996, pp 2111-2148

38. Hardy S, Kitamura M, Harris-Stansil T, et al: Construction of adenovirus vectors through cre-lox recombination. J Virol 71:1842-1849, 1997

39. Cullen BR: Trans-activation of human immunodeficiency virus occurs via a bimodal mechanism. Cell 46:973-982, 1986

40. Dranoff G, Soiffer R, Lynch T, et al: A phase I study of vaccination with autologous, irradiated melanoma cells engineered to secrete human granulocyte-macrophage colony stimulating factor. Hum Gene Ther 8:111-123, 1997

41. Murphy G, Bhan A, Sato S, et al: A new immunologic marker for human Langerhan cells. N Engl J Med 304:791-792, 1981

42. Salgia R, Lynch T, Skarin A, et al: Vaccination with irradiated autologous tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor augments antitumor immunity in some patients with metastatic non-small-cell lung carcinoma. J Clin Oncol 21:624-630, 2003

43. Schmollinger JC, Vonderheide RH, Hoar KM, et al: Melanoma inhibitor of apoptosis protein (ML-IAP) is a target for immune-mediated tumor destruction. Proc Natl Acad Sci U S A 100:3398-3403, 2003

44. Hodi FS, Mihm MC, Soiffer RJ, et al: Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. Proc Natl Acad Sci USA 100:4712-4717, 2003

45. Yu J, Burwick J, Dranoff G, et al: Gene therapy for metastatic brain tumors by vaccination with granulocyte-macrophage colony-stimulating factor-transduced tumor cells. Hum Gene Ther 8:1065-1072, 1997

46. Borrello I, Sotomayor E, Cooke S, et al: A universal granulocyte-macrophage colony-stimulating factor-producing bystander cell line for use in the formulation of autologous tumor cell-based vaccines. Hum Gene Ther 10:1983-1991, 1999