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

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Purpose: **We demonstrated that vaccination with irradiated tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor (GM-CSF) stimulates potent, specific, and long-lasting antitumor immunity in multiple murine models and patients with metastatic melanoma. To test whether this vaccination strategy enhances antitumor immunity in patients with metastatic non–small-cell lung cancer (NSCLC), we conducted a phase I clinical trial.**

Patients and Methods: **Resected metastases were processed to single-cell suspension, infected with a replication-defective adenoviral vector encoding GM-CSF, irradiated, and cryopreserved. Individual vaccines consisted of 1×10^6, 4×10^6, or 1×10^7 cells, depending on overall yield, and were administered 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 513 ng/10^6 cells/24 h. Toxicities were restricted to grade 1 to 2 local skin reactions. Nine patients were withdrawn early because of rapid disease progression. Vaccination elicited dendritic cell, macrophage, granulocyte, and lymphocyte infiltrates in 18 of 25 assessable patients. Immunization stimulated the development of delayed-type hypersensitivity reactions to irradiated, dissociated, autologous, nontransfected tumor cells in 18 of 22 patients. Metastatic lesions resected after vaccination showed T lymphocyte and plasma cell infiltrates with tumor necrosis in three of six patients. Two patients surgically rendered as having no evidence of disease at enrollment remain free of disease at 43 and 42 months. Five patients showed stable disease durations of 33, 19, 12, 10, and 3 months. One mixed response was observed.**

Conclusion: **Vaccination with irradiated autologous NSCLC cells engineered to secrete GM-CSF enhances antitumor immunity in some patients with metastatic NSCLC.**

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THERE IS INCREASING evidence that non–small-cell lung cancer (NSCLC) can evoke specific humoral and cellular antitumor immune responses in some patients. Serologic-based cloning strategies have identified multiple tumor-associated antigens, including eIF4G, aldolase, annexin XI, Rip-1, and NY-LU-12.[1-4] Humoral responses to autologous lung cancer cells may be associated with prolonged survival.[5] T-cell–based cloning strategies similarly have revealed diverse targets in NSCLC, including Her2/neu, SART-1, SART-2, KIAA0156, ART-1, ART-4, cyclophilin B, mutated elongation factor 2, malic enzyme, and alpha-actinin-4.[6-15] The development of cytotoxic T-lymphocyte responses to NSCLC may also be correlated with prolonged survival.[14,15]

Notwithstanding these provocative findings, most patients do not generate anti-NSCLC immune reactions that are sufficiently potent to prevent lethal tumor progression. The recognition that tumor cells typically fail to stimulate optimal antigen presentation, however, has motivated the design of several novel strategies to augment antitumor immunity.[16] Among the approaches using ex vivo modification of tumor cells, 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 Lewis lung carcinoma.[17] Vaccination requires the participation of $CD4^+$ and $CD8^+$ T cells, CD1 days-restricted NKT cells, and antibodies and likely involves improved tumor antigen presentation by activated dendritic cells and macrophages.[17-20]

We recently reported a phase I clinical trial of vaccination with irradiated, autologous melanoma cells engineered by retroviral-mediated gene transfer to secrete GM-CSF in patients with metastatic melanoma.[21] Immunization sites showed intense infiltrates of dendritic cells, macrophages, eosinophils, and lymphocytes in all 21 assessable patients. Although metastatic

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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 patients examined.

To test whether this vaccination strategy augments antitumor immunity in patients with NSCLC, we used adenoviral-mediated gene transfer to engineer autologous GM-CSF–secreting tumor cell vaccines ex vivo. In contrast to conventional retroviral vectors, adenoviral vectors do not require target-cell replication for infection,[22] thus obviating the requirement for establishing primary tumor cell cultures. In this article, we present the results of a phase I clinical trial that establishes the feasibility, safety, and biologic activity of autologous GM-CSF–secreting NSCLC vaccines.

PATIENTS AND METHODS

Patients

This phase I Dana-Farber Partners Cancer Care clinical protocol received approval from local institutional review boards and biosafety committees, the National Institutes of Health Recombinant DNA Advisory Committee, and the United States Food and Drug Administration. Patients were eligible for enrollment if they had metastatic NSCLC, an Eastern Cooperative Oncology Group performance status of 0 or 1, an estimated life expectancy $\geq$ 6 months, age $\geq$ 18 years, 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 underwent staging scans and routine hematology and chemistry analysis.

Vaccine Preparation

Accessible metastases were resected and transported in sterile media to the Connell-O'Reilly Gene Transfer Laboratory (a dedicated biosafety level 2 [BL-2] facility for human gene transfer experiments) at the Dana-Farber Cancer Institute. Solid tumors were dissected to small fragments and processed to single-cell suspension with collagenase and mechanical digestion, and pleural fluid samples were concentrated by centrifugation. Tumor cells (2×10^6 cells) were irradiated (10,000 rads) and cryopreserved (90% fetal calf serum, 10% dimethyl sulfoxide) in 1×10^6 cell aliquots for use in delayed-type hypersensitivity testing. The remaining tumor cells were placed in media (α-minimal essential medium [MEM], 10% fetal calf serum, and gentamicin) and infected overnight at 37°C with a replication-defective adenoviral vector encoding human GM-CSF (Ad-GM) at a multiplicity of infection of 10. Pilot experiments using Ad-Lac Z (the same vector backbone with a beta-galactosidase cDNA insert) indicated that these conditions resulted in the infection of at least 50% of the tumor cells (not shown). In five patients, administered vaccines were also prepared from short-term cultures of resected tumors.

Ad-GM (manufactured by Cell Genesys, Foster City, CA) contains a GM-CSF expression cassette in the E1 region of adenovirus type 5 and a second deletion in the E3 region.[23] The GM-CSF expression cassette contains the cytomegalovirus immediate early promoter-enhancer,[24] a shortened human beta-globin second intron, the human GM-CSF gene,[25] and the beta-globin polyadenylation signal and 3′ untranslated region. The integrity of the virus was confirmed by restriction analysis. High-titer stocks of Ad-GM underwent extensive testing and certification before United States Food and Drug Administration approval for clinical use.

After overnight infection, the tumor cells were extensively washed and irradiated (10,000 rads). For 48 hours, 1×10^6 cells were placed into culture; the supernatants were collected, and GM-CSF levels were determined with an enzyme-linked immunosorbent assay (ELISA; EH-GMCSF [Endogen, Woburn, MA]) according to the manufacturer's instructions. Pilot experiments demonstrated that irradiation did not significantly influence GM-CSF production (not shown). Individual vaccine aliquots were cryopreserved on the basis of overall tumor cell yield as follows: $\leq 3 \times 10^7$ total, 1×10^6 aliquots (dose level 1); 3×10^7 to 1×10^8 total, 4×10^6 aliquots (dose level 2); and $\geq 1 \times 10^8$ total, 1×10^7 aliquots (dose level 3). Samples of nontransfected and infected tumor cells were tested for sterility, endotoxin, and mycoplasma. Before clinical administration, cryopreserved cells were thawed, washed extensively, and resuspended in 1 mL of sterile saline for the vaccines and 0.5 mL for the nontransduced cells used in delayed-type hypersensitivity analysis.

Treatment and Evaluation

Vaccines were administered intradermally (0.5 mL) and subcutaneously (0.5 mL) into normal skin on the limbs and abdomen on a rotating basis. Injections were given weekly for 3 weeks and then every other week until the vaccine supply was exhausted or the patient was removed from study. A minimum of six immunizations was required to consider a patient assessable for biologic activity. Patients were restaged at week 10 and then at 4-month intervals or when clinically indicated. Responding 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 CD1a, CD4, CD8, CD20, and immunoglobulin (Ig) kappa. Dendritic cells were identified by CD1a staining (in four patients) and/or by a characteristic morphology in hematoxylin and eosin sections that was previously reported[26] (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 responses were considered strong when the following were present: mononuclear cells admixed with eosinophils and basophils accumulated around blood vessels; swollen or necrotic endothelial cells, often with vessel luminal occlusion; and dermal edema and fibrin exudation. Tumor infiltrates were considered significant when they occupied at least 30% of multiple high-power microscopic fields.

An ELISA was developed to measure antiadenoviral antibodies. In brief, ELISA plates (COSTAR, Corning Costar, Acton, MA) were coated with intact Ad-Lac Z particles or viral lysates (prepared with aminocaproic acid, soybean trypsin inhibitor, leupeptin, pepstatin, 0.5% Nonidet P-40 [Calbiochem, San Diego, CA]) in a carbonate buffer. The wells were blocked overnight at 4°C with 2% nonfat dry milk (NFDM)/phosphate-buffered saline, washed, and incubated in duplicate with 100 μL of patient sera diluted 1:500 in 2% NFDM/phosphate-buffered saline overnight at 4°C. A goat antihuman IgG conjugated to horseradish peroxidase (HRP) (Zymed, San Francisco, CA) was added at room temperature, and the plate was developed with a one-step 3,3′,5,5′-tetramethylbenzidine (TMB/peroxide) reagent (DAKO, Glostrup, Denmark). All sample analyses were performed in duplicate. The values reported were the mean absorbance at 450 nm for virus-coating buffer only. The relative change in antibody titer was determined by serial dilution of the peak response to obtain a value equivalent to the pretreatment sample.

RESULTS

Patient Characteristics

Thirty-eight patients with metastatic NSCLC were enrolled onto this phase I trial between October 1997 and January 2000. One patient was removed from study after reanalysis of the tumor pathology established a diagnosis of thymoma. The characteristics of the remaining 37 patients are listed in Table 1. There were 21 males and 16 females, with a mean age of 56.5 years (range, 32 to 74 years). Most of the tumors were classified as adenocarcinomas (19 of 37 tumors), with smaller numbers of bronchioloalveolar, squamous cell, large-cell, and mixed histol-

Table 1. Patient Characteristics

Characteristic	No. of Patients	%
Age, years		
Mean	56.5	
Range	32 to 74	
Sex		
Male	21	57
Female	16	43
Race		
White	35	95
Other	2	5
Histology		
Adenocarcinoma	19	
Bronchioalveolar	4	
Squamous cell	3	
Large-cell	2	
NOS	4	
Mixed	5	
Sites of disease		
Lung	8	
Without pleural effusion	7	
With pleural effusion	1	
Lung (including pleural effusion) with one extra site of disease	13	
Bones	4	
Neck LN	4	
Liver	1	
Adrenal	1	
Abdomen	1	
Brain	2	
Lung with two extra sites of disease	4	
Bones and brain	1	
Bones and axilla	2	
Chest wall and muscles	1	
Lung with three or more extra sites of disease	12	
Prior therapy		
Surgery alone	4	
Chemotherapy alone	11	
One regimen	6	
Two regimens	2	
Three or more regimens	3	
Radiotherapy alone	4	
Chemotherapy and radiation	15	
One regimen	7	
Two regimens	4	
Three or more regimens	4	
None	3	

Abbreviations: NOS, not otherwise specified; LN, lymph node.

ogies. In the majority of patients (29 of 37 patients), metastases were present in at least one extrapulmonary site. Most patients had received prior chemotherapy (26 of 37 patients) and/or radiation therapy (19 of 37 patients).

Vaccine Production and Administration

Two patients were removed from study before tissue procurement because of disease progression. Tumor tissue was obtained in the remaining 35 patients, with the lung and lymph nodes the most common sites (26 patients), and pleural fluid, chest wall, and adrenal less frequent sources (Table 2). Solid tumors were processed to single-cell suspension by collagenase and mechanical digestion, and pleural fluid samples were concentrated by centrifugation. Tumor-cell suspensions were infected overnight with Ad-GM at a multiplicity of infection of 10, irradiated (10,000 rads), and cryopreserved. In five patients, additional vaccines were prepared from short-term tumor cultures.

Table 2. Sites of Tumor Procurement

Site	No. of Patients
Lung	13
Pleura	5
Adrenal	2
Chest wall	2
Lymph nodes	13

Vaccines were successfully manufactured for 34 of the 35 patients (97%; 90% confidence interval, 87% to 100%). These included 12 patients at dose level 1, 17 patients at dose level 2, and five patients at dose level 3. The average GM-CSF secretion after irradiation was 513 ng/10^6 cells/24 h, with a range from 6 to 3,017 ng/10^6 cells/24 h. The average cell viability was 66%, with a range from 2% to 100%. In general, the GM-CSF production correlated with the overall viability of the cell population (detailed cell processing data will be reported elsewhere).

Rapid disease progression resulted in the early withdrawal of nine patients. Twenty-five patients completed at least six vaccinations and were considered assessable for biologic activity (74% of the 34 patients for whom vaccines could be made; 90% confidence interval, 58% to 85%). Seven patients were treated at dose level 1, 14 patients were treated at dose level 2, and four patients were treated at dose level 3. Four patients with stable or indolent disease received additional courses of vaccination (eight to 38 total immunizations).

Toxicities

Vaccination consistently induced grade 1 to 2 erythema and induration at injection sites. Mild local pruritus was easily controlled with emollients. Occasional grade 1 to 2 fatigue and flu-like symptoms were reported. There were no significant hepatic, renal, pulmonary, cardiac, hematologic, gastrointestinal, or neurologic toxicities attributable to the vaccine. No autoimmune reactions were observed.

Vaccination Reactions

Injections of irradiated, autologous GM-CSF–secreting NSCLC cells elicited striking local reactions in 18 of 25 assessable patients. The intensity and frequency of these responses were related to vaccine dose, which reflected both total GM-CSF production and tumor cell number; strong reactions were observed in two of seven patients at dose level 1, 13 of 14 patients at dose level 2, and three of four patients at dose level 3. The intensity and duration of the responses generally increased in proportion to the number of vaccines administered. Clinically, the reactions were characterized by substantial erythema and induration (Fig 1A) that gradually declined over 48 to 72 hours. Patients who developed strong local reactions frequently manifested recall responses at sites of previous injection.

Pathologic examination of vaccination sites revealed brisk infiltrates of dendritic cells, macrophages, eosinophils, neutrophils, and lymphocytes that extended throughout the dermis and sometimes into the subcutaneous fat (Fig 1B and 1C). Endothelial cell

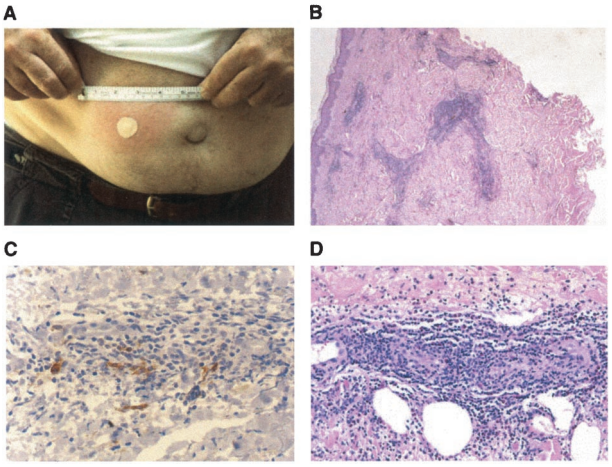

Fig 1. (A) Vaccine reaction; (B) vaccine infiltrate (×100); (C) vaccine-induced CD1a-positive dendritic cells (×400); (D) delayed-type hypersensitivity reaction (×400).

activation and damage were observed in the superficial venules of the upper dermis. Consistent with the clinical observations, the intensity of the infiltrates was related to vaccine dose.

Delayed-Type Hypersensitivity Reactions

Irradiated, dissociated, autologous nontransfected NSCLC cells were available for delayed-type hypersensitivity testing in 22 patients (insufficient cells precluded these studies in three of seven patients at dose level 1). Injections of nontransfected NSCLC cells failed to elicit significant clinical reactions or cellular infiltrates in all patients tested at the time of beginning treatment. However, these injections evoked strong responses in 18 of 22 patients when administered at the time of the fifth vaccination; these were observed in two of four patients at dose level 1, 13 of 14 patients at dose level 2, and three of four patients at dose level 3. Histopathologically, the responses were characterized by brisk infiltrates of T lymphocytes, eosinophils, and macrophages throughout the dermis (Fig 1D).

Antiadenoviral Humoral Responses

Although the adenoviral vector used to manufacture the vaccines is replication defective, the infected tumor cells may express some viral gene products, raising the possibility that vaccination might augment immune responses to adenoviral proteins. To test this possibility, we established an ELISA with intact and lysed adenoviral particles (Ad-LacZ). As expected from the epidemiology of adenoviral infections,[22] all nine patients examined at study entry showed detectable antibodies to both intact and lysed adenoviral particles. Vaccination stimulated increased antibody titers (three-fold) in five of the nine patients, and representative longitudinal analyses of two patients are depicted in Fig 2. No association between heightened antibody titers and either the number of vaccinations or levels of GM-CSF production was found. Similarly, there was no relationship between the development of augmented immunity to adenovirus and the generation of immunity to NSCLC cells. However, the small number of patients analyzed thus far limited our ability to detect relatively modest effects.

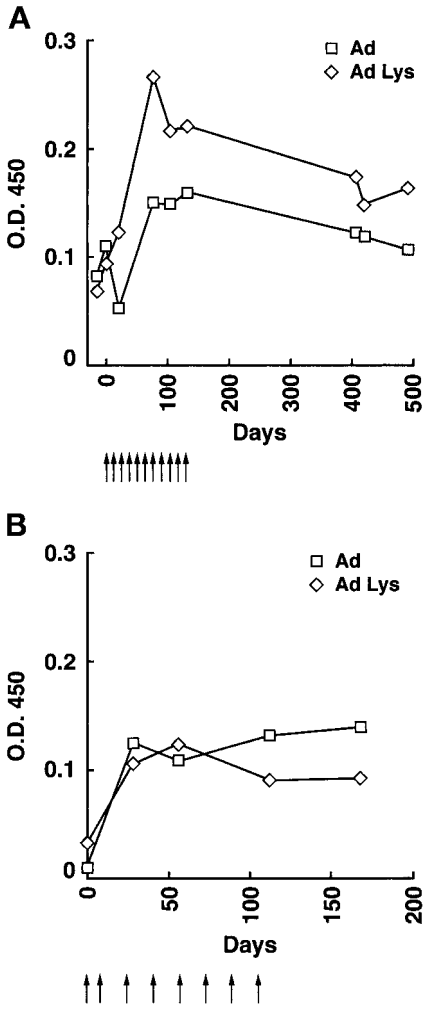

Fig 2. Antiadenoviral antibodies. Arrows denote immunizations; (A) patient L1, (B) patient L23. Changes shown represent three-fold increases.

Immune Responses in Metastases

To determine whether vaccination generated anti-NSCLC immune responses capable of inducing antitumor effects, we examined the host reactions to metastatic lesions resected before and after completing therapy. Metastatic lesions procured before the start of immunization revealed either the absence of host reactivity or only a modest inflammatory reaction in all patients. In contrast, significant T-lymphocyte and plasma cell infiltrates associated with tumor destruction were observed after vaccination in three of six patients examined. Immunohistochemistry revealed the presence of $CD4^+$ and $CD8^+$ T lymphocytes and $CD20^+$ B cells producing Ig in a responding metastasis (Fig 3A to 3F). All three infiltrated tumors were from patients treated at dose level 2. In contrast, two of the noninfiltrated tumors were from patients at dose level 1, whereas the remaining lesion was from a patient at dose level 2.

Clinical Outcomes

Five patients showed stable disease durations of 33, 19, 12, 10, and 3 months (all treated at dose level 2). One patient achieved a mixed response (lasting 4 months), with regression of the primary tumor and a lymph node metastasis but development

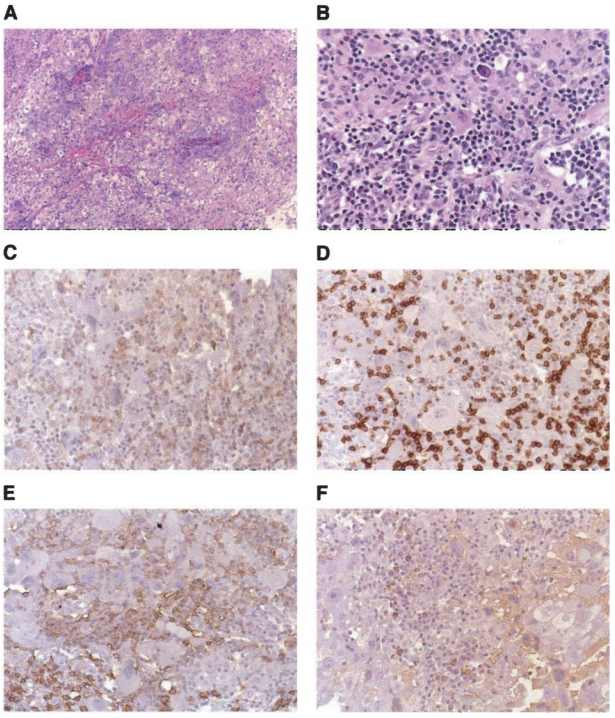

Fig 3. Infiltrated metastasis after vaccination in patient L23. (A) Metastasis without host response before treatment (×200); (B) metastasis with dense lymphocyte infiltrate after immunization (×400); (C) $CD4^+$ T cells (×400); (D) $CD8^+$ T cells (×400); (E) $CD20^+$ B cells (×400); and (F) immunoglobulin kappa (×400).

of a metastatic bone lesion (dose level 2). One patient with initial stable disease (19 months) is currently receiving chemotherapy for indolent progression at 36+ months.

Two patients surgically rendered as having no evidence of disease (NED) at the time of study entry remain free of disease. The first patient initially underwent a lobectomy for an adenocarcinoma of the right upper lobe with a synchronous undifferentiated large-cell carcinoma. Two years later, large-cell carcinoma recurred in the lung and adrenal gland. These metastases were resected and used to manufacture 22 immunizations at dose level 1. The patient was vaccinated, generated strong local and delayed-type hypersensitivity reactions, and is currently NED with more than 43 months of follow-up.

The second patient initially underwent a lingulectomy for bronchioloalveolar carcinoma. A segmentectomy was performed 2 years later for a tumor recurrence. Subsequent lung and mediastinal nodal metastases were resected 3 years later and used to manufacture 12 vaccines at dose level 2. The patient was immunized, developed striking local and delayed-type hypersensitivity reactions, and is currently NED with more than 42 months of follow-up.

DISCUSSION

This phase I clinical trial was undertaken in an effort to learn more about the host response to NSCLC. Serologic and T-cell–based cloning strategies have uncovered a large number of NSCLC-associated gene products that elicit immune recognition.[27] Antitumor antibody and T-cell reactions are associated with improved survival in some patients, although endogenous immunity to NSCLC usually is weak. The crafting of novel strategies to stimulate tumor antigen presentation has raised the possibility, however, that specific immunotherapies might enhance anti-NSCLC responses.

The data presented here indicate that vaccination with irradiated autologous NSCLC cells engineered to secrete GM-CSF augments antitumor immunity in some patients with metastatic NSCLC. Because primary NSCLC explants are difficult to establish in culture, a replication-defective adenoviral vector, which does not require target-cell replication for infection,[22] was used to transduce freshly processed samples. The ability to manufacture vaccines for 34 of 35 patients validates the high efficiency of this production scheme. Although a few preparations showed poor viability, likely reflecting the considerable necrosis evident in these surgical specimens, GM-CSF secretion rates typically were substantial. The average level of 513 ng/10^6 cells/24 h represents an increase of at least 2 logs over endogenous values. Moreover, the brief overnight infection protocol and the ex vivo use of a replication-defective vector of low pathogenicity enabled vaccine manufacture in a timely fashion. Although pre-existing antibodies to adenoviruses may limit certain in vivo applications of recombinant vectors,[28] no clear effect of antiadenoviral responses on tumor immunity was discerned in this study.

Despite previous cytotoxic therapies and extensive tumor burdens, 16 of 18 patients treated on dose levels 2 or 3 mounted strong vaccination responses. The presence of abundant macrophages and dendritic cells at immunization sites raises the possibility that NSCLC antigen presentation was augmented in response to GM-CSF.[19] As a consequence of treatment, 18 of 22 patients developed reactivity to injections of irradiated, dissociated, autologous nontransfected NSCLC cells. The prominent eosinophil component is characteristic of GM-CSF–based vaccinations and distinguishes these reactions from those evoked by other immunization schemes.[29,30]

The interpretation of the antigenic specificity of these local responses is complicated by the requirement to manipulate the autologous tumor cells ex vivo, with the attendant exposure to culture media. Although xenogeneic components may contribute to the delayed-type hypersensitivity reactions, NSCLC-associated gene products are likely to be important targets as well. In this context, we recently reported that three of the patients on this study developed, as a function of vaccination, increased humoral responses to ATP6S1, a putative subunit of the vacuolar H^+-ATPase complex that is highly expressed in NSCLC cells.[31] Because increasing evidence indicates that humoral and cellular responses may be coordinately induced,[27,32,33] the generation of high-titer IgG antibodies to ATP6S1 suggests that cognate $CD4^+$ T cells may also be produced, and these could participate in the skin responses. Further studies are underway to identify tumor-associated targets for other immunized patients.

Perhaps the strongest evidence for the generation of anti-NSCLC immunity in the clinical trial derives from the pathologic examination of distant metastases. Lesions procured for vaccine production disclosed minimal or absent host responses in all patients. In contrast, three of six tumors resected after immunization manifested significant lymphocyte infiltrates and tumor necrosis. The accumulation of $CD4^+$ and $CD8^+$ T cells and Ig-secreting $CD20^+$ B cells indicates a combined humoral

and cellular response. It is intriguing that these reactions were associated with tumor regression or prolonged periods of stable disease (33 and 12 months). Moreover, the infiltrating lymphocytes from these lesions should prove useful for efforts aimed at delineating the antigenic targets associated with tumor destruction.

The characteristics of the anti-NSCLC responses in this study bore striking similarity to the antimelanoma responses we previously reported in patients vaccinated with irradiated autologous melanoma cells, engineered by retroviral-mediated gene transfer, to secrete GM-CSF.[21] Indeed, several vaccinated melanoma patients also developed potent humoral responses to ATP6S1.[31] Taken together, these investigations underscore an intriguing conservation of biology across recombinant viral vectors and tumor types.

Overall, this phase I trial in NSCLC contributes to the accumulating evidence that GM-CSF–based cancer vaccines enhance immunity in diverse tumors.[21,34-36] The prolonged survival of some immunized NSCLC patients in the absence of significant toxicity should motivate the evaluation of this treatment strategy in early-stage disease. Moreover, the potential synergies of GM-CSF–secreting NSCLC vaccines with other immunologic schemes, such as anti-CTLA-4 antibody blockade,[37] or pharmacologic therapies also should be explored in patients with advanced disease.

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