BRIEF COMMUNICATION

Granulocyte–Macrophage Colony-Stimulating Factor Gene-Modified Autologous Tumor Vaccines in Non–Small-Cell Lung Cancer

John Nemunaitis, Daniel Sterman, David Jablons, John W. Smith II, Bernard Fox, Phil Maples, Scott Hamilton, Flavia Borellini, Andy Lin, Sayeh Morali, Kristen Hege

To evaluate the feasibility, safety, and efficacy of vaccination with autologous tumor cells genetically modified with an adenoviral vector (Ad-GM) to secrete human granulocyte–macrophage colony-stimulating factor (GM-CSF), we conducted a phase I/II multicenter trial in patients with early and advanced stage non–small-cell lung cancer (NSCLC). Vaccines were generated from autologous tumor harvests. Intradermal injections were given every 2 weeks for a total of three to six vaccinations. Tumors were harvested from 83 patients, 20 with early-stage NSCLC and 63 with advanced- stage NSCLC; vaccines were successfully manufactured for 67 patients, and 43 patients were vaccinated. The most common toxicity was a local injection-site reaction (93%). Three of 33 advanced-stage patients, two with bronchioloalveolar carcinoma, had durable complete tumor responses (lasting 6, 18, and ≥22 months). Longer survival was observed in patients receiving vaccines secreting GM-CSF at more than 40 ng/24 h per 10^6 cells (median survival = 17 months, 95% confidence interval [CI] = 6 to 23 months) than in patients receiving vaccines secreting less GM-CSF (median survival = 7 months, 95% CI = 4 to 10 months) (P = .028), suggesting a vaccine dose–related survival advantage. [J Natl Cancer Inst 2004;96:326–31]

Given the considerable toxicity and modest benefit of chemotherapy for non–small-cell lung cancer (NSCLC) *(1),* immune-based therapies have been explored as potential treatment options. Treatment with interferon α *(2),* lymphokine-activated killer cells, and interleukin 2 *(3)* has been explored with limited success. Therapeutic cancer vaccines derived from whole tumor cells *(4–8)* or lysates *(9)* mixed with adjuvant have been tested in patients with resected early-stage NSCLC and have demonstrated immunologic activity and the suggestion of a survival advantage. Genetically modified tumor cells used as vaccines have demonstrated activity in murine tumor models (including Lewis lung) with a variety of immune-modulatory cytokine genes *(10–15).* In head-to-head comparisons, vaccines secreting granulocyte–macrophage colony-stimulating factor (GM-CSF) have shown greater activity than vaccines using other cytokines *(14).*

Several phase I/II human trials using GM-CSF–secreting autologous or allogeneic tumor cell vaccines have been performed *(16–21).* In this study, we evaluated such autologous vaccines in a multicenter phase I/II trial of patients with early-stage and advanced-stage NSCLC. Patients were enrolled in two cohorts (A and B) at five clinical sites after the study design received institutional review board approval; the patients provided written informed consent. Patients in cohort A had stage IB or II (according to the American Joint Committee on Cancer staging system) NSCLC with planned primary surgical resection and no pre- or postoperative chemotherapy or radiotherapy. Patients in cohort B had stage III or IV NSCLC with an accessible tumor to harvest for vaccine processing and no chemotherapy or radiotherapy within 4 weeks of tumor harvest or vaccine treatment. Eligibility criteria for both cohorts included age of at least 18 years, Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 at tumor harvest, histologic confirmation of NSCLC, successful vaccine processing, and acceptable organ function. Patients were excluded for the following reasons: previous treatment with cancer vaccines or gene therapy, active or untreated brain metastases, systemic corticosteroid use, active autoimmune disease, or infection with human immunodeficiency virus.

For vaccine preparation, tumor tissue was obtained surgically or by thoracentesis in the case of malignant effusions. A tissue or cytological sample was submitted for pathological evaluation to confirm the diagnosis of NSCLC. The remaining tumor or pleural effusion was shipped at 4 °C to a central processing facility (US Oncology, Dallas, TX). Solid tumors were processed to a single-cell suspension by mechanical and enzymatic digestion for 45–60 minutes in medium containing collagenase (Life Technologies, Grand Island, NY) and fetal bovine serum (JRH Biosciences, Lenexa, KS) using a Stomacher laboratory blender (Brinkmann, Westbury, NY). Pleural effusions were subjected to Ficol (Amersham Pharmacia, Uppsala, Sweden) density gradient separation. Tumor cells were set aside for use in delayed-type hypersensitivity (DTH) skin testing (1×10^6 tumor cells per test) and, when cell yields were sufficient, for immunologic studies. The remaining cells were exposed overnight at 37 °C to vector supernatant (Ad-GM) at a multiplicity of infection of 10 plaque-forming units per cell in medium containing 10% fetal bovine serum. The Ad-GM replication-defective vector (manufactured at Cell Genesys, South San Francisco, CA) was constructed by replacing the E1 gene of adenovirus type 5 with the gene for human GM-CSF and deleting an additional segment in the E3 region *(22).* After overnight infection (or culture for DTH cells), vaccine and DTH cells were washed in serum-free medium, irradiated at 10 000 cGy (^{137}Cs Nordion Gammacell 3000 irradiator; Kanata, Ontario, Canada) to prevent tumor cell proliferation, and cryopreserved in liquid nitrogen. The total process was completed within 36

Affiliations of authors: US Oncology, Dallas, TX (JN, PM); University of Pennsylvania, Philadelphia (DS); University of California, San Francisco (DJ); Earl A. Chiles Research Institute/Providence Medical Center, Portland, OR (JWS, BF); Cell Genesys Inc., South San Francisco, CA (SH, FB, AL, SM, KH).

Correspondence to: John Nemunaitis, MD, 3535 Worth St., Collins Bldg., 5th Fl., Dallas, TX 75246 (e-mail: john.nemunaitis@usoncology.com).

See "Notes" following "References."

DOI: 10.1093/jnci/djh028

Journal of the National Cancer Institute, Vol. 96, No. 4,

hours. Irradiated prostate adenocarcinoma PC-3 cells were used as a control in DTH skin tests (Cell Genesys). Successful vaccine processing required a minimum yield of three vaccines at 5×10^6 tumor cells per vaccine. All procedures were performed in compliance with regulatory guidelines for gene therapy.

Vaccines were administered intradermally every 2 weeks for a total of three to six vaccinations. The vaccine dose was individualized on the basis of yield, and each dose contained 5×10^6 to 100×10^6 tumor cells. Patients were stratified into three dose ranges for analysis: 5×10^6 to 10×10^6 cells per vaccination, 10×10^6 to 30×10^6 cells per vaccination, and 30×10^6 to 100×10^6 cells per vaccination. Vaccine and DTH cells were thawed and directly injected into the extremities. Autologous tumor DTH cells were administered on the same day on the first and fourth vaccinations and at month 9. PC-3 DTH cells were administered with the fourth vaccination only.

Immune response of the vaccine and DTH skin reactions was determined by use of the diameter of induration. Punch biopsy specimens were assessed immunohistochemically for CD3, CD4, CD8, and CD1a (a dendritic cell marker) with corresponding monoclonal antibodies (Impath Laboratories, Los Angeles, CA). Serum antibodies against autologous lung tumor (when available); allogeneic lung tumor cell lines 157, 441, 520, 596, 1435, 1437, and 2347; the prostate tumor cell line PC-3; and adenovirus before and after vaccination were assayed by immunoblotting (Cell Genesys).

For statistical analysis, the primary end points were safety, manufacturing feasibility, and immunologic activity. Secondary end points were tumor response, disease progression, and survival. Adverse events were recorded by use of National Cancer Institute Common Toxicity Criteria. Manufacturing feasibility assessment included analysis of vaccine yields, viability, GM-CSF secretion, and sterility. Tumor staging was performed at baseline and week 12, and response was evaluated with standard Southwest Oncology Group criteria *(23)*. Tumor responses were confirmed by repeat imaging studies more than 4 weeks after the initial response. Progression-free and overall survival were calculated by the Kaplan–Meier method from the date of tumor harvest. Univariable and multivariable association analyses between manufacturing, clinical, and immunologic variables were performed with Spearman's correlation coefficient, Wilcoxon signed rank test, Fisher's exact test, and Cox proportional hazards regression, depending on the nature of the variables analyzed (continuous or categorical). The assumptions for using the Cox proportional hazards regression test were met. All statistical tests were two-sided.

Eighty-three patients underwent tumor harvest (20 in cohort A, 63 in cohort B) and 43 initiated vaccine treatment (10 in cohort A, 33 in cohort B). Patient baseline characteristics are shown in Table 1. All 10 patients in cohort A completed vaccine treatment. The median number of vaccines administered in cohort B was five.

The median size of processed solid tumor was 14 g (range = 0.5–96 g), and the median volume of pleural fluid processed was 675 mL (range = 127–2600 mL). Among vaccine-treated patients, the median tumor cell dose was 23×10^6 cells (range = 5×10^6 to 100×10^6 cells), with a GM-CSF secretion level (post-thaw) of 104 ng/(24 h) per 10^6 cells (range = 50–1871 ng/(24 h) per 10^6 cells) and viability of 62% (range =

Table 1. Baseline patient characteristics

	Tumors harvested	Initiated vaccine*
Total No. (%)	83	43
Cohort, No. (%)		
A	20 (24)	10 (23)
B	63 (76)	33 (77)
Age, y		
Median	64	61
Range	32–89	32–81
Sex, No. (%)		
Male	41 (49)	21 (49)
Female	42 (51)	22 (51)
Tumor stage, No. (%)		
IB	N/A†	8 (19)
IIA	N/A	2 (5)
IIIA	N/A	3 (7)
IIIB	N/A	2 (5)
IV	N/A	28 (65)
Performance status, No. (%)		
0	N/A	13 (30)
1	N/A	30 (70)
Histology, No. (%)		
Adenocarcinoma	39 (47)	17 (40)
Squamous cell	19 (23)	14 (33)
Large cell	6 (7)	4 (9)
Bronchioloalveolar	4 (5)	3 (7)
Undifferentiated/other	15 (18)	5 (12)
Source of tumor harvest, No. (%)		
Lung	47 (57)	26 (60)
Pleural effusion	15 (18)	4 (9)
Lymph node	13 (16)	7 (16)
Subcutaneous	3 (4)	1 (2)
Brain	2 (2)	2 (5)
Adrenal	2 (2)	2 (5)
Liver	1 (1)	1 (2)
No. of prior regimens, cohort B only, No. (%)		
0	N/A	7 (21)
1	N/A	1 (3)
2	N/A	11 (33)
≥3	N/A	14 (42)
Dose, No.		
Cohort A		
5×10^6 to 10×10^6 tumor cells	N/A	3
10×10^6 to 30×10^6 tumor cells	N/A	1
30×10^6 to 100×10^6 tumor cells	N/A	6
Cohort B		
5×10^6 to 10×10^6 tumor cells	N/A	13
10×10^6 to 30×10^6 tumor cells	N/A	10
30×10^6 to 100×10^6 tumor cells	N/A	10

*Reasons for not proceeding to vaccine treatment included vaccine processing failure (16 patients), death (11 patients), withdrawal (11 patients), and ineligibility (two patients).

†N/A = not available.

11%–94%). The median number of days from tumor harvest to vaccine release was 31 and that from harvest to initiation of vaccine treatment was 49. Vaccines were successfully manufactured in 80% of patients in cohort A and 81% of patients in cohort B. The majority of manufacturing failures (15 of 16 failures) resulted from an insufficient number of tumor cells; two cases of bacterial contamination were noted. The success rate was higher for solid tumors (82%) than for pleural effusions (53%) ($P = .02$). GM-CSF secretion varied by 300-fold between vaccine lots. This variability was not associated with any baseline tumor characteristic evaluated, with the exception of higher levels of GM-CSF secreted from pleural effusions than from solid tumors ($P < .001$). Tumor cells had a broad range of viability after thawing that was not consistently associated with any immunologic or clinical end point.

The most common vaccine-related adverse events were local vaccine injection site reactions (93%), followed by fatigue (16%), and nausea (12%) and then by pain, arthralgia, and upper respiratory infection (each at 5%). All injection site reactions except one were grade 1 or 2 in severity and consisted of local, self-limited erythema, induration, and pruritis (Fig. 1). Two grade 4 (pericardial effusion) and six grade 3 (dyspnea, fatigue, injection site reaction, hypokalemia, malignant ascites, and pulmonary embolism) possibly related events were reported. There was no association between vaccine dose and the total number of adverse events or grade 3 or 4 adverse events.

Immune response to vaccination was measured by vaccine and DTH skin reactions and the induction of tumor-reactive antibodies. After the first injection, 81% of patients developed vaccine site induration, which increased to 90% after repeated vaccination. Vaccine reaction size was positively associated with vaccine GM-CSF secretion ($P = .01$) and increased with repeated vaccinations. Analysis of vaccine site biopsy specimens showed dense infiltration with $CD4^+$ and $CD8^+$ T cells, $CD1a^+$ dendritic cells, and eosinophils (Fig. 1).

Cellular immune response to vaccination was monitored by DTH skin reactions to injections of irradiated, autologous tumor cells or control PC-3 prostate cancer cells. Autologous tumor DTH testing was positive (>5-mm induration) in four (9%) of 43 patients at baseline (all four were negative on repeat testing). After four vaccinations, 10 (34%) of 29 patients tested positive (Fig. 1). No positive DTH reactions (of a total of 10) were seen at doses of fewer than 10×10^6 cells compared with 10 (53%) of 19 at higher doses ($P = .04$), suggesting a possible dose–response effect. DTH reactions against PC-3 were present in 15 (50%) of 30 patients.

Five of 33 patients induced serum antibodies reactive against autologous tumors after vaccination. This analysis was technically limited by insufficient tumor material from most patients and, therefore, was extended to a panel of seven allogeneic lung cancer cell lines. In 32 (78%) of 41 patients, antibody reactivity was induced against at least one lung cancer line, whereas only 13 (32%) of 41 patients induced reactivity against PC-3. Because residual adenoviral proteins are a component of the final vaccine and might serve as an immunologic adjuvant, we measured the impact of vaccination on adenoviral immunity. Most patients (98%) had anti-adenoviral antibodies before vaccination, and 95% had an increased anti-adenoviral antibody titer after vaccination. No statistically significant differences were noted between the early- and advanced-stage cohorts in any of the immune response end points.

Three patients in cohort B achieved durable, complete tumor regressions lasting 6 months, 18 months, and ongoing at 22 months. In addition, there was one minor response (30% decrease in a lung nodule) and two mixed responses; seven patients had stable disease (median duration = 7.7 months; range = 4.7 to >28 months). Prior chemotherapy for advanced disease had failed for two of the three complete responders, and two had bronchioloalveolar histology (Fig. 2), a relatively uncommon subtype of NSCLC. Complete responses occurred at doses of 6.7×10^6 to 10×10^6 tumor cells per vaccine and at vaccine GM-CSF secretion rates of 44–236 ng/(24 h) per 10^6 cells. Vaccine viability ranged from 19% to 90% among the six patients with any evidence of tumor regression. Immunologic end points were inconsistent; none of the complete responders developed DTH reactions to autologous tumor, but DTH reactions were detected in two of three patients with minor responses. One complete responder showed an *in vitro* T-cell response to autologous tumor–pulsed dendritic cells after vaccination (data not shown). Antibody responses against autologous tumor were not measured in the complete respond-

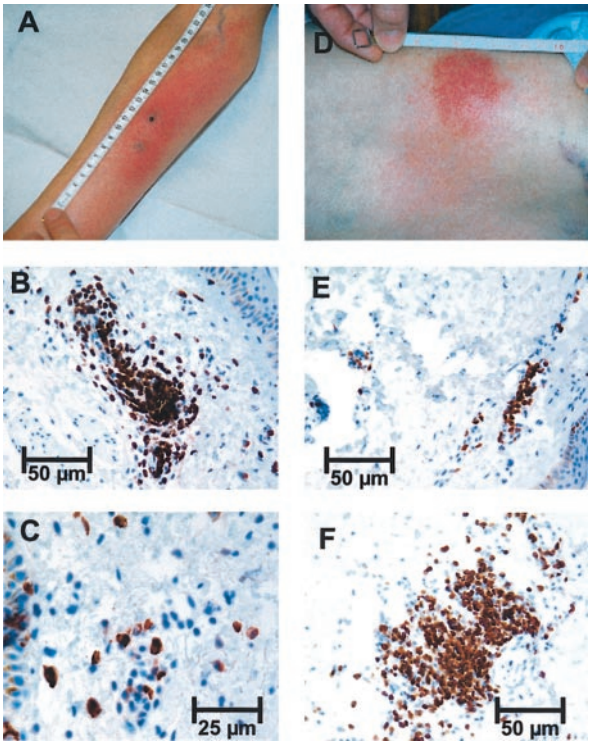

Fig. 1. Vaccine and autologous tumor delayed-type hypersensitivity (DTH) skin reactions. Grade II vaccine injection site reaction is shown in **A**, $CD3^+$ T-cell infiltrate in **B**, and $CD1a^+$ dendritic-cell infiltrate in **C**. **D**) Autologous tumor DTH injection site reaction after vaccination. **E**) Minimal $CD3^+$ T-cell infiltrate at the DTH injection site before vaccination. **F**) Extensive $CD3^+$ infiltrate at the DTH site after vaccination.

ers because of a lack of tumor cells. Six recurrences have been observed among the 10 cohort A patients, with a median follow up of 20 months. There were no statistically significant associations between immunologic end points and tumor response.

Survival analysis was performed on cohort B. The median progression-free survival was 4 months (95% confidence interval [CI] = 3 to 6 months), and the median overall survival was 9 months (95% CI = 6 to 12 months) among all 63 patients who underwent tumor harvest. The median progression-free survival was 4 months (95% CI = 2 to 6 months), and the median overall survival was 12 months (95% CI = 6 to 19 months) among the 33 treated patients. Survival at 1 year was 39% (95% CI = 34% to 44%) among all patients who underwent tumor harvest and was 44% (95% CI = 37% to 52%) among treated patients. Vaccine-associated GM-CSF secretion was statistically significantly associated with survival (Fig. 3). Median survival among patients receiving vaccines secreting GM-CSF at a rate of at least 40 ng/24 h per 10^6 cells was 17 months (95% CI = 6 to 23 months) compared with 7 months (95% CI = 4 to 10 months) for those receiving vaccines secreting less GM-CSF (P = .028). Corresponding 1-year survivals were 56% and 0%, respectively. This GM-CSF cutoff was predetermined from the threshold required for reliable induction of antitumor immunity in murine models *(24)*. In a multivariable analysis of prognostic factors for overall survival among treated cohort B patients that included manufacturing (dose, GM-CSF secretion, viability, and solid tumor versus pleural effusion), clinical (performance status and prior chemotherapy), and immunologic (vaccine site reaction, tumor DTH reaction, and antibody induction) parameters, only vaccine-associated GM-CSF secretion was statistically significantly associated with improved survival. Results of this initial preliminary investigation will be assessed prospectively in future studies.

NSCLC is not considered an immune-sensitive malignancy. However, durable tumor regressions in this trial were seen in three of 33 treated patients with metastatic NSCLC. We are not aware of previous reports in which immune therapy was the sole therapy associated with complete and durable regression of refractory metastatic NSCLC lesions, particularly those lasting more than 1 year, as observed in two of three complete responders in our study.

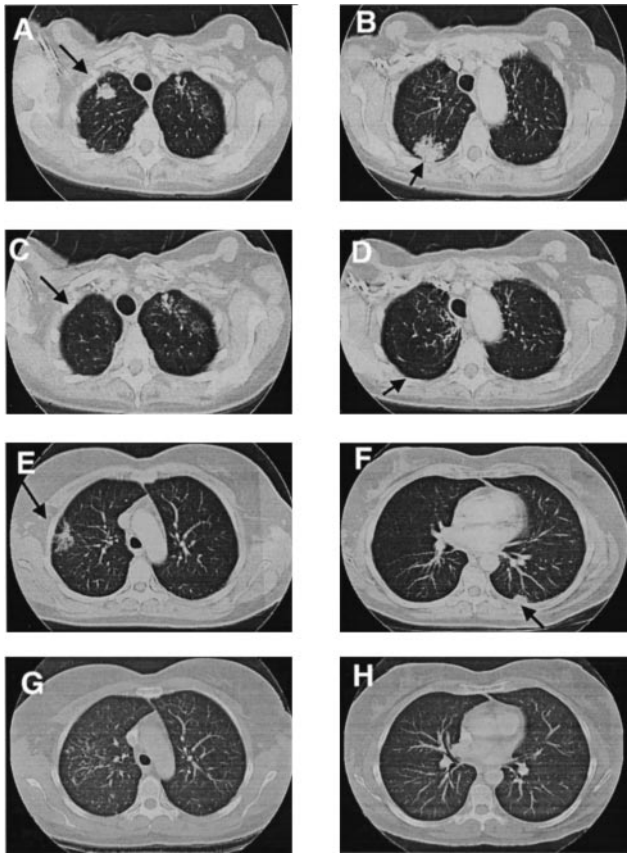

Fig. 2. Complete tumor responses in subjects with bronchioloalveolar carcinoma, as shown by radiologic assessment. **A–D**) Patient 1. **A** and **B**) Baseline tumor staging showing two lung tumors, one of which (**B**) was resected for vaccine processing. **C** and **D**) Complete tumor regression after vaccination. **E–H**) Patient 2. **E** and **F**) Baseline tumor staging showing two lung lesions, one of which (**E**) was resected. **G** and **H**) Complete tumor regression after vaccination.

Interestingly, two of three treated patients with the bronchioloalveolar subtype of NSCLC achieved a complete response (Fig. 2). This subtype is clinically and pathologically distinct and is more common in younger patients, nonsmokers, and women *(25)*. A viral etiology for bronchioloalveolar carcinoma has been proposed, potentially resulting in expression of immunogenic viral an-

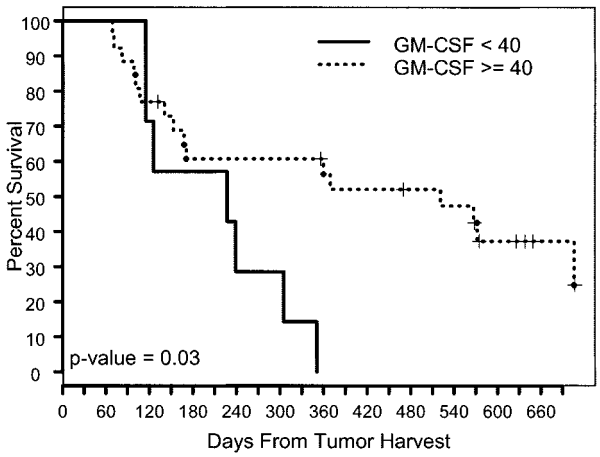

Fig. 3. Kaplan–Meier estimates of overall survival in cohort B. Treated advanced-stage patients analyzed by vaccine-associated granulocyte–macrophage colony-stimulating factor (GM-CSF) secretion. **Dashed lines** = at least 40 ng/24 h per 10^6 cells (26 patients); **solid lines** = less than 40 ng/24 h per 10^6 cells (seven patients). Median survival = 17 months (95% confidence interval [CI] = 6 to 23 months) for GM-CSF at greater than or equal to 40 ng/24 h per 10^6 cells versus 7 months (95% CI = 4 to 10 months) for GM-CSF at less than 40 ng/24 h per 10^6 cells (P = .028). Corresponding 1-year survival = 56% (95% CI = 46% to 69%) versus 0% (95% CI = indeterminate), respectively.

tigens *(26,27)*. This subtype is commonly held to be less responsive to chemotherapy than other subtypes of NSCLC and to have a more indolent clinical course, although few trials focused on bronchioloalveolar carcinoma have been conducted *(28)*. Whether the activity of this vaccine is truly more pronounced in this particular subtype of NSCLC is the subject of future research. Multivariable analysis suggested that survival of patients who received vaccines secreting levels of GM-CSF associated with optimal induction of antitumor immunity in preclinical models *(24)* was longer than that of patients receiving vaccines secreting lower levels of GM-CSF. These data suggest that this vaccine has a broader therapeutic benefit than that noted in bronchioloalveolar carcinoma.

Measures of immunologic response were not consistently associated with either tumor regression or survival and, therefore, did not function as useful surrogates of clinical activity in this study. Consistent associations between immunologic and clinical end points have been rare in the history of cancer vaccine development, with a few exceptions *(29–32)*. This study had the additional challenge that the relevant immunodominant antigens in NSCLC have not been identified, and the availability of autologous tumor cells for immunologic analyses was limited.

A primary study end point was assessment of manufacturing feasibility. The overall success rate for vaccine processing was 81%. Although this was lower than the 97% success rate reported in a similar trial using the same vaccine platform *(20)*, the minimum required tumor cell dose in this trial was fivefold higher. GM-CSF secretion varied by 300-fold from lot to lot, probably because of intrinsic heterogeneity among tumors in the expression of receptors critical for adenoviral infection, namely, coxsackie adenoviral receptor and $\alpha_v\beta$ integrins *(33,34)*. This variability in transduction efficiency may be overcome by a "bystander" vaccine approach in which autologous tumor cells and cells secreting GM-CSF are mixed *(35)*. This approach is currently being evaluated in clinical trials in NSCLC and hematologic cancers. Although vaccine viability varied, cancer vaccine strategies have included the use of tumor cell lysates *(36,37)*, membrane extracts *(9)*, and tumor-derived heat-shock protein preparations *(38)*, suggesting that viable tumor cells may not be required to induce antitumor immunity. Finally, the overall feasibility of this approach was limited by the long delay between tumor harvest and vaccine treatment, resulting in an overall dropout rate of 48%. Expedited vaccine release should be possible in future studies through the use of a validated closed system for vaccine manufacturing. This approach should increase the proportion of patients who undergo vaccine treatment and improve the overall feasibility of this approach.

GM-CSF gene-modified tumor vaccines have been tested in multiple human cancers, and immunologic activity has been observed in all studies *(16–21)*. Objective tumor responses were noted in melanoma and renal cell carcinoma *(17,18,21)*. More specifically, a previous phase I study *(20)* of such vaccines in metastatic NSCLC, with the same adenoviral vector and vaccine platform used in this study, demonstrated immunologic activity, a mixed tumor response, and prolonged recurrence-free survival (>42 months) in two subjects rendered surgically disease-free before vaccination. Although the DTH response rate reported in that trial was higher than reported here (82% versus 34%), criteria for assessing a positive DTH reaction differed. In contrast, vaccine site reactions were more frequent in our trial (93% versus 72%), and objective tumor responses were more common. Thus, these trials provide evidence for both immunologic and clinical activity of this vaccine approach in NSCLC.

REFERENCES

(1) Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. Non-small Cell Lung Cancer Collaborative Group. BMJ 1995;311:899–909.

(2) Grunberg S, Kempf R, Itri L, Venturi C, Boswell W, Mitchell M. Phase II study of recombinant alpha interferon in the treatment of advanced non-small cell lung carcinoma. Cancer Treat Rep 1985;69:1031–2.

(3) Kimura H, Yamaguchi Y. A phase III randomized study of interleukin-2 lymphokine-activated killer cell immunotherapy combined with chemotherapy or radiotherapy after curative or noncurative resection of primary lung carcinoma. Cancer 1997;80:42–9.

(4) Schulof R, Mai D, Nelson M, Paxton H, Cox J, Turner M, et al. Active specific immunotherapy with an autologous tumor cell vaccine in patients with resected non-small cell lung cancer. Mol Biother 1988;1:30–6.

(5) Perlin E, Oldham RK, Weese JL, Heim W, Reid J, Mills M, et al. Carcinoma of the lung: immunotherapy with intradermal BCG and allogeneic tumor cells. Int J Radiat Oncol Biol Phys 1980;6:1033–9.

(6) Stack BH, McSwan N, Stirling JM, Hole DJ, Spilg WG, McHattie I, et al. Autologous x-irradiated tumour cells and percutaneous BCG in operable lung cancer. Thorax 1982; 37:588–93.

(7) Souter RG, Gill PG, Gunning AJ, Morris PJ. Failure of specific active immunotherapy in lung cancer. Br J Cancer 1981;44:496–501.

(8) Takita H, Takada M, Minowada J, Han T, Edgerton F. Adjuvant immunotherapy of stage III lung carcinoma. New York (NY): Raven Press; 1978.

(9) Hollinshead A, Stewart T, Takita H, Dalbow M, Concannon J. Adjuvant specific active lung cancer immunotherapy trials. Tumor-associated antigens [published erratum appears in Cancer 1988;61:1090]. Cancer 1987; 60:1249–62.

(10) Golumbek PT, Lazenby AJ, Levitsky HI, Jaffee LM, Karasuyama H, Baker M, et al. Treatment of established renal cancer by tumor cells engineered to secrete interleukin-4. Science 1991;254:713–6.

(11) Gansbacher B, Zier K, Daniels B, Cronin K, Bannerji R, Gilboa E. Interleukin 2 gene transfer into tumor cells abrogates tumorigenicity and induces protective immunity. J Exp Med 1990;172:1217–24.

(12) Gansbacher B, Bannerji R, Daniels B, Zier K, Cronin K, Gilboa E. Retroviral vector-mediated gamma interferon gene transfer into tumor cells generates potent and long lasting antitumor immunity. Cancer Res 1990;50:7820–5.

(13) Aoki T, Tashiro K, Miyatake S, Kinashi T, Nakano T, Oda Y, et al. Expression of murine interleukin 7 in a murine glioma cell line results in reduced tumorigenicty in vivo. Proc Natl Acad Sci U S A 1992;89:3850–4.

(14) Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K, 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 U S A 1993; 90:3539–43.

(15) Lee CT, Wu S, Ciernik IF, Chen HL, Nadaf-Rahrov S, Gabrilovich D, et al. Genetic immunotherapy of established tumors with adenovirus-murine granulocyte-macrophage colony-stimulating factor. Hum Gene Ther 1997;8:187–93.

(16) Simons JW, Mikhak B, Chang JF, DeMarzo AM, Carducci MA, Lim M, et al. Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer. Cancer Res 1999;59:5160–8.

(17) Soiffer R, Lynch T, Mihm M, Jung K, Rhuda C, Schmollinger J, et al. Vaccination with irradiated autologous melanoma cells engineered to secrete human granulocyte-macrophage colony-stimulating factor generates potent antitumor immunity in patients with metastatic melanoma. Proc Natl Acad Sci U S A 1998;95:13141–6.
(18) Simons J, Jaffee E, Weber C, Levitsky H, Nelson W, Carducci M, et al. Bioactivity of autologous irradiated renal cell carcinoma vaccines generated by ex vivo granulocyte-macrophage colony-stimulating factor gene transfer. Cancer Res 1997;57:1537–46.
(19) Jaffee E, Hruban R, Laheru D, Schepers K, Sauter P, Goemann M, et al. Novel allogeneic GM-CSF-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol 2001;19: 145–56.
(20) Salgia R, Lynch T, Skarin A, Lucca J, Lynch C, Jung K, 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 2003;21:624–30.
(21) Soiffer R, Hodi FS, Haluska F, Jung K, Gillessen S, Singer S, et al. 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. J Clin Oncol 2003;21:3343–50.
(22) Hardy S, Kitamura M, Harris-Stansil T, Dai Y, Phipps ML. Construction of adenovirus vectors through Cre-lox recombination. J Virol 1997;71:1842–9.
(23) Green S, Weiss GR. Southwest Oncology Group standard response criteria, endpoint definitions, and toxicity criteria. Invest New Drugs 1992;10:239–53.
(24) Jaffee E, Thomas M, Huang A, Hauda K, Levitsky H, Pardoll D. Enhanced immune priming with spatial distribution of paracrine cytokine vaccines. J Immunother Emphasis Tumor Immunol 1996;19:176–83.
(25) Barkely J, Green M. Bronchioloalveolar carcinoma. J Clin Oncol 1996;14:2377–86.
(26) Stinson J, Leibovitz A, Brindley G, Hayward R, Turner R, McCombs W. Filamentous particles in human alveolar cell carcinomas: electron microscopy studies of six cases (preliminary report). J Natl Cancer Inst 1972;49: 1483–93.
(27) Min KW, Song J. Virus-like intranuclear particles in bronchiolar-alveolar cell carcinoma. J Iowa Med Soc 1982;72:319–21.
(28) Vance R, Crowley J, Livingston R, Gandara D. A phase II Southwest Oncology Group trial (S9714) utilizing paclitaxel by 96-hour infusion in stage IIIb and IV bronchioloalveolar carcinoma of the lung (BAC) [abstract]. Proc ASCO 2001;20:336a.
(29) Hsueh EC, Famatiga E, Gupta RK, Qi K, Morton DL. Enhancement of complement-dependent cytotoxicity by polyvalent melanoma cell vaccine (CancerVax): correlation with survival. Ann Surg Oncol 1998;5:595–602.
(30) Barth A, Hoon DS, Foshaag LJ, Nizze A, Mamatiga E, Okun E, et al. Polyvalent melanoma cell vaccine induces delayed-type hypersensitivity and in vitro cellular immune response. Cancer Res 1994;54: 3342–5.
(31) Livingston P, Wong G, Adluri S, Tao Y, Padavan M, Parente R, et al. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. J Clin Oncol 1994;12:1036–44.
(32) Takahashi T, Johnson TD, Nishinaka Y, Morton DL, Irie RF. IgM anti-ganglioside antibodies induced by melanoma cell vaccine correlate with survival of melanoma patients. J Invest Dermatol 1999;112:205–9.
(33) Tomko RP, Xu R, Philipson L. HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc Natl Acad Sci U S A 1997;94:3352–6.
(34) Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 1997;275:1320–3.
(35) Borrello I, Sotomayor E, Cooke S, Levitsky H. 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 1999;10: 1983–91.
(36) Wallack MK, Sivanandham M, Balch CM, Urist MM, Bland KI, Murray D, et al. A phase III randomized, double-blind, multiinstitutional trial of vaccinia melanoma oncosylate-active specific immunotherapy for patients with stage II melanoma. Cancer 1995; 75:34–42.
(37) Mitchell MS. Perspective on allogeneic melanoma lysates in active specific immunotherapy. Semin Oncol 1998;25:623–35.
(38) Tamura Y, Peng P, Liu K, Daou M, Srivastava PK. Immunotherapy of tumors with autologous tumor-derived heat shock protein preparations. Science 1997;278:117–20.

NOTES

Supported by grants from Cell Genesys, Inc.

We acknowledge the contributions of the following individuals: Casey Cunningham, James Arseneau, Sasha Vukelja, Barry Berman, Don Richards, Mitchell MaGee, Richard Wood, Tom Meyers, Robert Hebeler, Robert Mennel, Joseph Kuhn, Adrienne Williams, Thierry Jahan, Bob Kuhn, Adriana Recio, Larry Kaiser, Teri Doran, Bernie Fox, Richard Whyte, and Karen Nelson.

Manuscript received July 16, 2003; revised November 25, 2003; accepted December 10, 2003.