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Granulocyte Macrophage Colony-Stimulating Factor–Secreting Allogeneic Cellular Immunotherapy for Hormone-Refractory Prostate Cancer

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Abstract

Purpose: This trial evaluated the safety, clinical activity, and immunogenicity of an allogeneic cellular immunotherapy in 55 chemotherapy-naïve patients with hormone-refractory prostate cancer (HRPC). The immunotherapy, based on the GVAX platform, is a combination of two prostate carcinoma cell lines modified with the granulocyte macrophage colony-stimulating factor (GM-CSF) gene.

Experimental Design: HRPC patients with radiologic metastases (n = 34) or rising prostate-specific antigen (PSA) only (n = 21) received a prime dose of 500 million cells and 12 boost doses of either 100 million cells (low dose) or 300 million cells (high dose) biweekly for 6 months. End points were changes in PSA, time to progression, and survival.

Results: Median survival was 26.2 months (95% confidence interval, 17, 36) in the radiologic group: 34.9 months (8, 57) after treatment with the high dose (n = 10) of immunotherapy and 24.0 months (11, 35) with the low dose (n = 24). The median time to bone scan progression in the radiologic group was 5.0 months (2.6, 11.6) with the high dose and 2.8 months (2.8, 5.7) with the low dose. In the rising-PSA group (n = 21) receiving the low dose, the median time to bone scan progression was 5.9 months (5.6, not reached), and median survival was 37.5 months (29, 56). No dose-limiting or autoimmune toxicities were seen; the most common adverse events were injection site reaction and fatigue.

Conclusions: These results suggest that this GM-CSF–secreting, allogeneic cellular immunotherapy is well tolerated and may have clinical activity in patients with metastatic HRPC. Phase 3 trials to confirm these results are under way.

Approximately 27,050 men die annually from metastatic hormone-refractory prostate cancer (HRPC; ref. 1). Although chemotherapy with docetaxel has been shown to prolong survival in HRPC (2, 3), alternatives to chemotherapy remain of considerable interest to many patients and physicians. Recent advances in the understanding of cancer immunology have led to the development of new cancer treatments specifically designed to stimulate the patient's immune system. Although prostate cancer has traditionally been thought of as poorly immunogenic, numerous studies have shown that tumor tolerance can be reversed (4–6). Prostate cancer is a good target for immunotherapy due to the typically slow growth rate of most prostate tumor cells, which in turn permits an appropriately stimulated immune system time to mount antitumor responses (4, 5).

Immunotherapy typically involves presenting one or more tumor antigens to the patient's immune system *in vivo* or to harvested immune cells *in vitro* (4, 6). An immune system stimulant may be included in the treatment to enhance the immune response to the antigens. Whole tumor cells have been

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Statement of significance: This translational study showed that the GM-CSF–secreting, allogeneic cellular immunotherapy was well tolerated, clinically active, and broke immunologic tolerance to prostate cancer in chemotherapy-naïve patients with metastatic HRPC.

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proposed as an antigen source in immunotherapy because relevant prostate cancer tumor-rejection antigens have not been convincingly identified, and a polyvalent source of antigens can better address "antigen escape" resulting from the modulation and down-regulation of antigens during tumor growth (7). The rationale for employing a granulocyte macrophage colony-stimulating factor (GM-CSF)–transduced whole cell immunotherapy is to use whole tumor cells as the source of multiple tumor-associated antigens and to use GM-CSF to induce growth, maturation, and recruitment of dendritic cells, which process and present antigens, to the immunotherapy injection sites (8). Preclinical studies in several poorly immunogenic rodent HRPC models have shown prolonged survival in animals treated with GM-CSF–transduced whole cell immunotherapy (4, 9–11).

The first clinical trial of GM-CSF–secreting, cellular immunotherapy for prostate cancer was conducted with autologous cells derived from resected tumor material in patients with hormone-naïve prostate cancer following prostatectomy (12). Treated patients exhibited tumor-associated humoral immune responses, delayed-type hypersensitivity reactions to autologous prostate cancer cells, and new T-cell and B-cell responses against prostate cancer-associated antigens. However, the small number of cells that can be obtained from surgically removed tumors limits the practicality of this approach (12). Therefore, two cell lines, derived from a lymph node metastasis (LNCaP) and a bone metastasis (PC-3), were selected for an allogeneic cellular immunotherapy with the expectation that their combined antigenic profile would broadly represent the spectrum of metastatic prostate cancer (13). These cell lines were modified with a human GM-CSF gene to secrete high levels of bioactive GM-CSF. An initial phase 1/2 trial in hormone-naïve patients with prostate cancer showed a favorable safety profile, statistically significant changes in the slope of prostate-specific antigen (PSA) velocity, and a PSA decline of >50% in one patient, suggesting an antitumor effect (13).

An open-label, phase 1/2, multicenter trial was therefore conducted to evaluate the safety, clinical activity, and immunogenicity of the GM-CSF–secreting, allogeneic cellular immunotherapy in chemotherapy-naïve patients with metastatic HRPC. The protocol was amended to allow administration of a higher dose level after an interim analysis showed that the initial dosage tested was well tolerated.

Materials and Methods

This study was conducted according to the precepts established by the Helsinki Declaration and the NIH Guidelines for Research Involving Recombinant DNA. The protocol was approved by each site's Human Investigations Committee. Each patient provided signed informed consent. The study was initiated on May 19, 1999, and completed on January 16, 2001.

Materials. This immunotherapy is based on the GVAX platform (Cell Genesys, Inc.) and consists of two prostate cancer cell lines, PC-3 and LNCaP, modified to express the *human GM-CSF* gene. The cell lines are propagated, frozen, and irradiated to arrest further cell division (13). The product is stored and shipped on dry ice and thawed before administration. All manufacturing is conducted according to good manufacturing practice and NIH containment guidelines for recombinant DNA.

Patients. Men with histologically confirmed adenocarcinoma of the prostate and disease progression despite androgen deprivation were eligible. All patients had metastatic disease, had two or more successive increases in serum PSA (≥2 ng/mL) taken at least 2 weeks apart, and were asymptomatic (without bone pain due to HRPC). Patients in the radiologic group had overt metastatic disease (positive bone scan, bidimensionally measurable disease, or both). Patients in the rising-PSA group had biochemical metastases with increasing PSA levels but negative bone scan, computed tomography (CT) scan (abdomen and pelvis) and chest X-ray. Patients were excluded for primary HRPC, brain metastases, uncontrolled medical problems, or previous chemotherapy, bisphosphonate therapy, biological therapy, immunotherapy, or gene therapy for cancer.

Treatment. All patients received a priming dose of 500 million cells (250 million cells of each cell line). This was deemed a maximum feasible dose due to the number of injections required. Patients in the rising-PSA group and the first 24 patients in the radiologic group received the low dose boost of 100 million cells (50 million of each cell line). Because no dose-limiting toxicities were seen at this dose level, a high boost dose of 300 million cells (150 million of each cell line) was given to 10 additional patients in the radiologic group. Although the 500 million cell priming dose was well tolerated, a boost dose higher than 300 million cells was avoided due to the number of injections required. Dose levels were selected based on an earlier trial of a similar GVAX platform–based immunotherapy in pancreatic cancer, which showed that 100–500 million cell dose levels were immunologically and clinically active (14, 15). The increase in boost dose level allowed further exploration of tolerability and a potential dose response in patients with radiologically detectable metastases, presumably with a heavier disease burden than patients in the rising-PSA group. Each cell type was injected intradermally in opposite limbs every 2 weeks for 6 months.

Evaluation. The prospectively defined primary study end points were PSA decline of at least 50%, time to PSA and bone scan progression (16), change in PSA over time (slope), local or systemic immune response, and safety. PSA was tested at a central laboratory (Abbott AxSYM) at 2-week intervals during treatment and monthly during the 6-month follow-up period. Bone scans, CT scans (abdomen and pelvis), and chest X-rays were done at screening and months 3, 6, 9, and 12 in the radiologic group or at screening and when clinically indicated for the rising-PSA group. Serum levels of carboxyterminal telopeptide of type I collagen (ref. 17; ICTP) were measured in the radiologic group. B-cell immune responses were measured in the radiologic group pre- and posttreatment by immunoblot analyses (two-dimensional electrophoresis) using lysates of the LNCaP and PC-3 cell lines against patient sera as in the earlier studies (12, 13). The two-dimensional electrophoresis was done according to the method of O'Farrell (18) by Kendrick Labs, Inc. A posttreatment 250-kDa band from a PC-3 immunoblot was of interest, and the protein spot was excised from a Coomassie blue–stained 10% acrylamide slab gel. Mass spectrometry (MS) fingerprinting of the protein spot was done by subsequent digestion with endoproteinase Lys-C and analysis by matrix-assisted laser desorption ionization MS (Protein Chemistry Core Facility, Howard Hughes Medical Institute/Columbia University). Serum samples were tested for antibodies against PSA by ELISA using donkey antihuman immunoglobulin G (IgG) IgM horseradish peroxidase (HRP) and compared with a negative control (normal serum) and two positive controls (rabbit anti-PSA with donkey anti-rabbit IgG HRP; human IgG). A greater than 2-fold induction in titer posttreatment was considered positive. Patients were assessed for human leukocyte antigen (HLA) type on enrollment, but HLA type was not an exclusion criteria. Safety assessments included physical examinations, laboratory evaluations, and recording of adverse events, which were graded by the National Cancer Institute (NCI) Common Toxicity Criteria, version 2. All patients were followed for survival. Data collection was monitored according to Good Clinical Practice Guidelines, and data were double entered into a database before analysis.

Statistical analysis. A sample size of 30 patients with radiologic metastases and 20 patients with biochemical metastases (rising PSA)

Table 1. Patient characteristics at baseline

	All patients	Radiologic group: low dose	Radiologic group: high dose	Rising-PSA group: low dose
Patients enrolled	55	24	10	21
Age (y), median (range)	71 (58-88)	73 (58-85)	70 (58-76)	70 (62-88)
PSA* (ng/mL), median (range)	35.9 (1.3-1,207)	49.5 (3.8-1,207)	79.6 (3.7-846.7)	16.5 (1.3-92.5)
Alkaline phosphatase (units/L), median (range)	77.0 (48-659)	97.5 (63-659)	78.5 (48-263)	67.0 (49-99)
Hemoglobin (g/dL), median (range)	13.2 (9.3-16.1)	12.6 (9.3-15.1)	13.7 (10.3-16.0)	13.4 (11.7-16.1)
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Ethnic group				
Caucasian	52 (95)	23 (96)	9 (90)	20 (95)
African-American	2 (4)	1 (4)	1 (10)	0 (0)
Asian	1 (2)	0 (0)	0 (0)	1 (5)
Extent of disease				
Bone disease only	20 (36)	15 (63)	5 (50)	0 (0)
Soft tissue only	8 (15)	6 (25)	2 (20)	0 (0)
Bone and soft tissue	6 (11)	3 (13)	3 (30)	0 (0)
PSA-only disease	21 (38)	0 (0)	0 (0)	21 (100)
ECOG performance status				
0	45 (81.8)	18 (75.0)	10 (100.0)	17 (81.0)
1	10 (18.2)	6 (25.0)	0 (0.0)	4 (19.0)
Gleason score ≥7	34 (62)	15 (63)	7 (70)	12 (57)

*PSA: prostate-specific antigen.

was calculated to allow detection of adverse events that occur at an underlying rate of >5% and a single PSA response if the underlying rate was 10%. Analyses were conducted on these two populations separately. Variables measured on a continuous scale were characterized by summary statistics (mean and SD). Variables that were dichotomous in nature or categorical in outcome were summarized using counts and proportions with exact binomial confidence limits. The time to progression was measured from the first day of treatment to the day progression was documented. Log-transformed PSA values were plotted against time, and a linear regression model was used to calculate the pretreatment slope based on at least three successive PSA values taken at least 2 weeks apart and the posttreatment slope based on all PSA values collected during the treatment and follow-up period. Survival time and time to progression (PSA and bone scan) were estimated according to the Kaplan-Meier method (19). Patients who had not reached an end point by the date of analysis were censored. In a post hoc analysis, a predicted median survival time was calculated based on baseline patient characteristics [including PSA, alkaline phosphatase, hemoglobin, lactate dehydrogenase (LDH), Gleason score, performance status, and visceral disease] following a validated pretreatment prognostic model developed by Halabi et al. (20) and compared with the observed survival time. Because LDH was not collected during this trial, the median LDH collected from a similar population of HRPC patients in a subsequent immunotherapy trial was used (21). Exploration of factors influencing survival time and the primary clinical end points (PSA decrease, time to progression, change in PSA velocity) was assessed by categorizing patients by HLA type and separately by posttreatment immunoreactivity to the tumor cell lines (immunoblot) regardless of dose group.

Results

Patients. All 55 patients had metastatic HRPC. The radiologic group consisted of 34 men: 24 received the low dose, and 10 received the high dose. The rising-PSA group consisted of 21 men: all received the low dose. Patient characteristics are summarized in Table 1. Of the 55 patients enrolled, 29 (53%) completed the 6-month treatment period. The primary reasons for the discontinuation of treatment were progressive disease (17), initiation of alternative treatment (4), unrelated adverse events (4), and other (nonspecified) reasons (1). Twelve patients completed the 1-year study, and 17 discontinued during the 6-month follow-up phase due to initiation of alternative treatment (9), progressive disease (5), and other reasons (3).

Clinical response. Six of the 55 patients (11%) had a decrease of more than 25% in PSA, including a decrease of more than 50% in one patient in the radiologic group (high dose). This patient had a baseline PSA value of 10 ng/mL, which began to drop 2 weeks after the first dose, reached 0.1 ng/mL at 10 weeks, and subsequently began to increase at 24 weeks. The duration of response was 267 days (Fig. 1). The

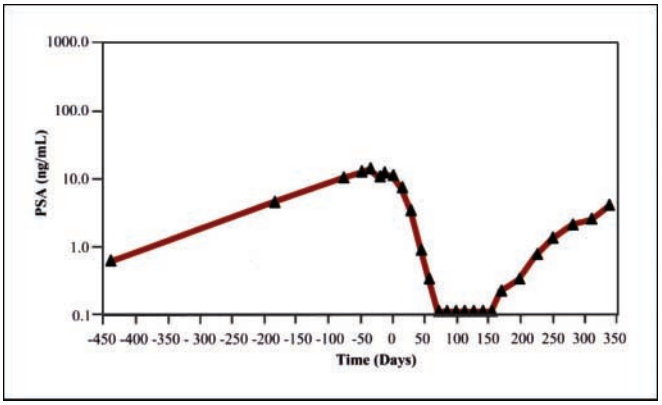

Fig. 1. Serum PSA over time in a patient in the radiologic group on the high dose of immunotherapy (patient G03-018-804SS).

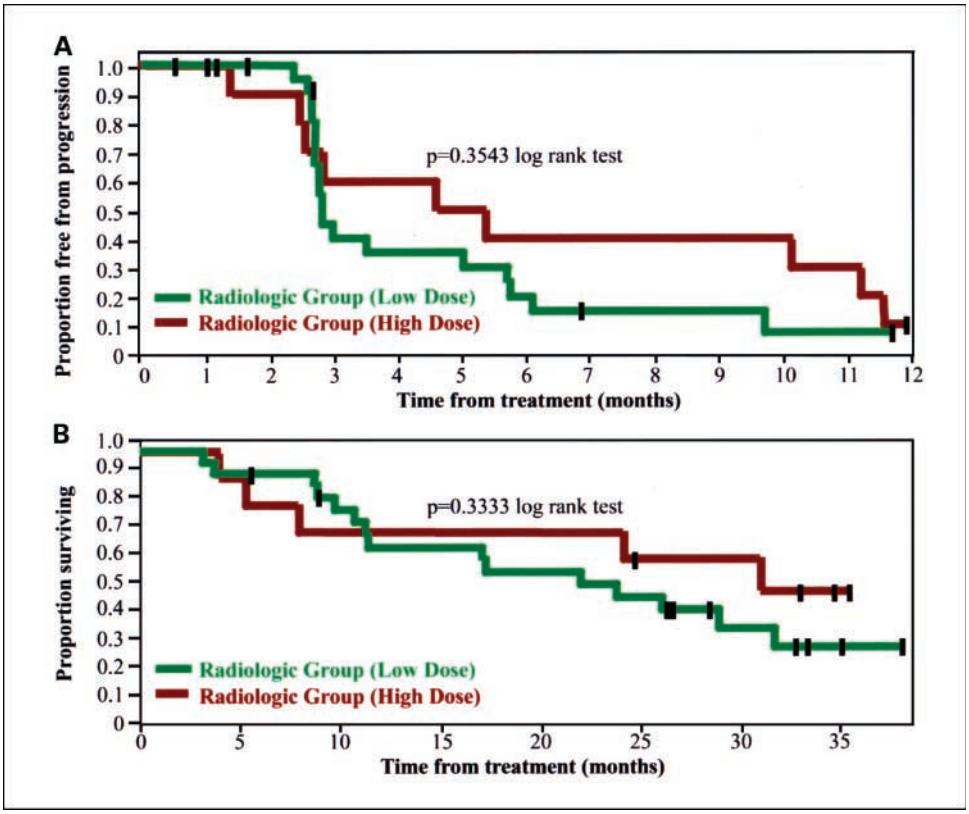

Fig. 2. Kaplan-Meier estimate of (*A*) time to bone scan progression and (*B*) overall survival time. Hash marks, patients who have not reached end point at the time of data analysis.

patient had resolution of a bone lesion on bone scan at week 12 and developed no new lesions during the trial. This patient was not an HLA class I match to either cell line comprising the immunotherapy and had no evidence of antibodies against PSA. A posttreatment reduction in PSA slope was observed in 25 of 34 (73.5%) patients in the radiologic group, including 16 of 24 (66.6%) receiving the low dose and 8 of 10 (80%) receiving the high dose, and 11 of 21 (52.4%) patients in the rising-PSA group.

Time to progression. The median time to PSA progression was 2.6 months in the radiologic group, including 2.3 months [95% confidence interval (95% CI), 1.8, 3.2] with the low dose and 3.7 months (95% CI, 3.2, 5.5) with the high dose. In the rising-PSA group, the median time to PSA progression was 3.9 months (95% CI, 3.2, 7.8). The median time to bone scan progression was 3.0 months in the radiologic group, including 2.8 months (95% CI, 2.8, 5.7) with the low dose and 5.0 months (95% CI, 2.6, 11.6) with the high dose of immunotherapy (Fig. 2A). The median time to a positive bone scan in the rising-PSA group was 5.9 months (95% CI, 5.6, not reached).

ICTP. Serum levels of ICTP, a biological marker of metastatic bone turnover, were analyzed in the radiologic group. At 12 weeks, levels of ICTP were decreasing or stable (<25% change) in 20/29 (69%) patients in the radiologic group: 13/20 (65%) on the low dose and 7/9 (78%) on the high dose of immunotherapy (5 patients did not have data).

B-cell immune responses. Immunoblot analysis of patient serum against lysates of the two immunotherapy cell lines, PC-3 and LNCaP, was done in the radiologic group to assess the induction of antibody responses reactive against the prostate cancer cells. New or enhanced immunoreactive bands appeared posttreatment in 19/28 (67.8%) patients in the radiologic group, including 13/19 (68.4%) on the low dose and 6/9 (66.7%) on the high dose (6 patients did not have data). A larger percentage of patients showed immunoreactivity to the PC-3 cell lysate (18/28; 64.3%) compared with the LNCaP lysate (12/28; 42.8%). The immune response to prostate antigens was oligoclonal; some bands were shared between multiple patients, and others were unique to individual patients (Fig. 3). A median of 2 new or enhanced bands (range, 1-6) were induced against PC-3 and a median of 1 (range 1-3) against LNCaP. Induction of serum antibodies against PSA was evaluated by ELISA in 52 patients, and no evidence of induced anti-PSA antibodies was observed (Fig. 4). A more than 250-kDa band was present on immunoblot for 11/19 immunoreactive patients, including 8 on the low dose and 3 on the high dose (all in the radiologic group). In the patient whose PSA dropped to 0.1 ng/mL, the band was excised and identified by mass spectrometry as filamin B (β), a cytoskeletal protein that has been linked to cancer and is involved in cell shape, division, adhesion, motility, signal transduction, and protein sorting (22–24).

Survival. In the radiologic group, the overall median survival time after initiation of treatment was 26.2 months (95% CI, 17, 36), including 24.0 months (95% CI, 11, 35) with the low dose and 34.9 months (95% CI, 8, 57) with the high dose (Fig. 2B). Based on a pretreatment prognostic model developed by Halabi et al. (20), an expected median survival time of 19.5 months (95% CI, 17, 22) was estimated for the 34 patients in the radiologic group. At the end of the study, 13/34 patients in the radiologic group received subsequent

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