

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

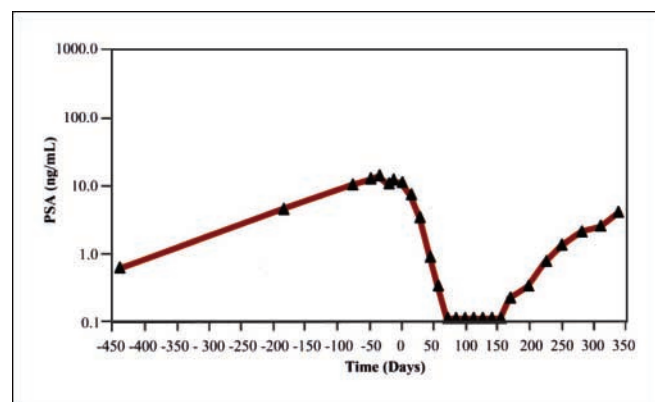


Fig. 1. Serum PSA over time in a patient in the radiologic group on the high dose of immunotherapy (patient G03-018-804SS).

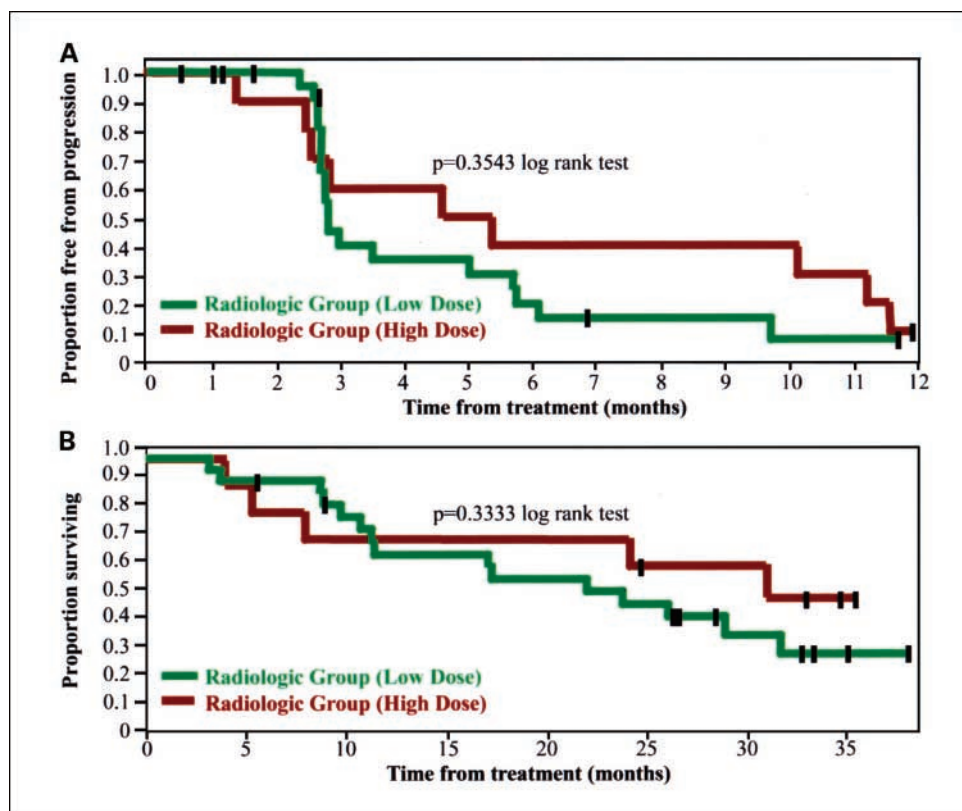


Fig. 2. Kaplan-Meier estimate of (A) time to bone scan progression and (B) overall survival time. Hash marks, patients who have not reached end point at the time of data analysis.

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

chemotherapy (taxane in 9/13); their median survival was more than 35.2 months (95% CI, 29, 44). The median survival of the nonchemotherapy-treated patients (21/34) was 17.2 months (95% CI, 9, 32). The differences in survival times were not statistically significant ($P = 0.1$). Median survival in the rising-PSA group was 37.5 months (95% CI, 29, 56). In the radiologic group, patients with reduced posttreatment PSA slopes had a longer survival time (26.2 months) compared with those with a stable or increasing PSA slope (11.5 months; $P = 0.12$). By contrast, in the rising-PSA group, median survival times were similar regardless of the direction of change in PSA slope.

Factors influencing outcome. Immunoreactivity was not a significant predictor of survival time or clinical response. Immunoblot data were available on 3/6 patients who had a >25% decrease in PSA, including the patient whose PSA dropped to 0.1 ng/mL. All three patients showed induction of immunoreactivity to one or both cell lines. There was no difference in treatment-associated changes in PSA slope, ICTP levels, time to PSA or bone scan progression, or overall survival based on induction of immunoreactivity to one or both cell lines. The improved median survival time observed in patients who received subsequent chemotherapy was evident in patients who were immunoreactive on immunoblot (29.8 months with chemotherapy versus 23.9 months without chemotherapy), but not in nonreactive patients (24.6 months with chemotherapy versus 24.8 months without chemotherapy). Although presence of a 250-kDa band on immunoblot was not a significant predictor of survival time, the median survival time in the 11 patients who showed this band was 31.2 months compared with 24.3 months in the 17 patients with immunoblot data that did not show the 250-kDa band.

The HLA class 1 type in 29 of 48 patients matched that of one or both immunotherapy cell lines (A2 and A24), including 20/31 (64.5%) in the radiologic group and 9/17 (52.9%) in the rising-PSA group. HLA type was not available in seven patients. HLA type did not significantly impact survival time or the

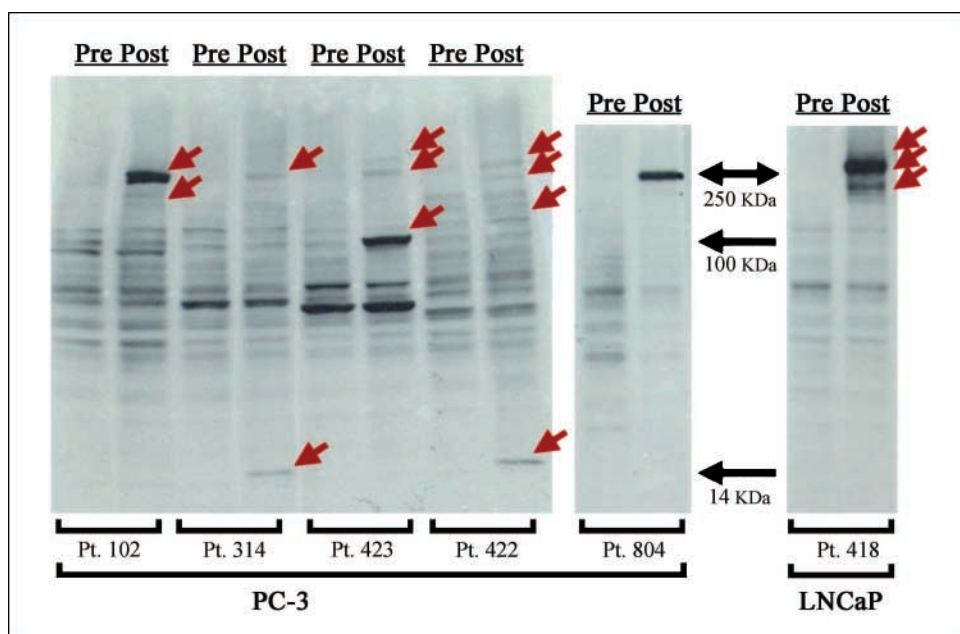
primary end points; however, the patient group whose HLA class 1 type was not a match to either cell line showed a greater response for every clinical end point (PSA, ICTP, time to progression, and survival) in comparison to the HLA-matched group. Immunoreactivity was noted in 7/9 (78%) mismatched and 11/18 (61%) HLA-matched patients.

Adverse events. Most adverse events (57%) were judged by the investigator to be not related to treatment. Of the 760 reported adverse events, 45% were grade 1, 49% were grade 2, 5% were grade 3, and <1% were grade 4 (five events in two patients). There was a higher incidence of a flu-like syndrome in the high dose (50%) than in the low-dose (8%) group, but the incidence of fatigue was higher in the low-dose (37%) than in the high-dose (20%) group; these adverse events resolved without sequelae. There were no other notable differences in the overall rate or NCI toxicity grade of adverse events between dose levels.

Injection site reaction was the most common treatment-related adverse event (Table 2). Fifty-three patients had a grade 2 injection site reaction consisting of pruritus, pain, and/or swelling, and one patient had a grade 3 reaction that included skin ulceration, all of which resolved without sequelae. Serious adverse events ($n = 21$) occurred in 16 patients, and none were related to treatment. One patient died due to disease progression within 30 days of the last treatment.

All posttreatment antinuclear antibody (ANA) titers (available for 53 patients) were $\leq 1:40$. One patient had a titer of 1:360 at screen and <1:40 at week 24. For 17 of 24 patients, the posttreatment ANA lab report stated "unidentified autoantibodies present," signifying the presence of IgG antibodies specific for non-nuclear antigens in the Hep2 cell line, which were not present at screening. For two patients, unidentified autoantibodies were noted at screen only. A review of the adverse events for these patients did not reveal symptoms of autoimmune disease. Five of the 17 patients with unidentified autoantibodies had a repeat ANA test at month 12, and no autoantibodies were observed.

Fig. 3. Immunoblot of pre- and posttreatment patient sera against lysates of the PC-3 and LNCaP cell lines. Red arrows, examples of posttreatment induction of new or enhanced tumor-reactive antibodies.



Discussion

This open-label multicenter study was undertaken to evaluate the safety, immunogenicity, and clinical activity of a GM-CSF-secreting, allogeneic cellular immunotherapy for prostate cancer. The immunotherapy was designed to stimulate an immune response through presentation of multiple tumor-associated antigens and targeted secretion of GM-CSF. This study showed that the immunotherapy was well tolerated and immunogenic in the majority of metastatic HRPC patients.

There seemed to be an advantage with the higher dose in patients with radiologically detectable metastases with regard to time to progression, PSA changes, and overall survival. The median survival time in the radiologic group was longer with the high dose compared with the low dose of immunotherapy (34.9 versus 24.0 months, respectively). The median time to bone scan progression was longer with the high dose (5.0 months versus 2.8 months). Similarly, the median time to PSA progression was longer with the high dose (3.7 months versus 2.3 months), and a higher frequency of patients in the high-dose group had a reduced PSA slope following treatment. One patient on the high dose had a complete response (a decline in PSA from 10 to 0.1 ng/mL that lasted 267 days and resolution of a bone lesion at week 12). The clinical relevance of posttreatment changes in PSA continues to be debated; however, the group of patients with reduced post-treatment PSA slopes had a longer survival time (26.2 months) compared with those with a stable or increasing PSA slope (11.5 months; $P = 0.12$), suggesting that these PSA changes are not random fluctuations. This study was not designed or powered for statistical comparison between dose groups or other subgroups of patients; nevertheless, these findings are

provocative and have helped to generate the hypothesis that the higher dose of immunotherapy may provide a clinical benefit.

Patients with metastatic HRPC are a heterogeneous population, and survival times are influenced by patient and tumor characteristics (20, 25–28). Because this study did not include a control arm, the observed survival times are difficult to interpret. Therefore, we calculated a predicted median survival time based on baseline patient characteristics following a validated pretreatment prognostic model, the Halabi Nomogram, to provide a context for the observed survival time (20). The Halabi Nomogram is based on the relationship between patient characteristics (PSA, alkaline phosphatase, hemoglobin, lactate dehydrogenase, Gleason sum, Eastern Cooperative Oncology Group (ECOG) performance status, and visceral disease) and overall survival observed during six chemotherapy trials of 1,101 patients with metastatic HRPC whose range of characteristics encompassed the immunotherapy study population. The median survival predicted by the Halabi nomogram for the 34 patients in the radiologic group in this trial was 19.5 months. The observed median Kaplan-Meier survival time for those 34 patients (26.2 months) exceeded that predicted by the Halabi Nomogram. These results should be interpreted with caution because the utility of this nomogram to evaluate survival data from immunotherapy trials has not been validated. Nevertheless, as a hypothesis-generating exercise, this result raises the possibility that this immunotherapy may improve survival time for patients with metastatic HRPC.

During the follow-up phase of this study, patients in the radiologic group who received subsequent chemotherapy had a longer survival time than those who did not receive chemotherapy (35.2 versus 17.2 months). Although this result could

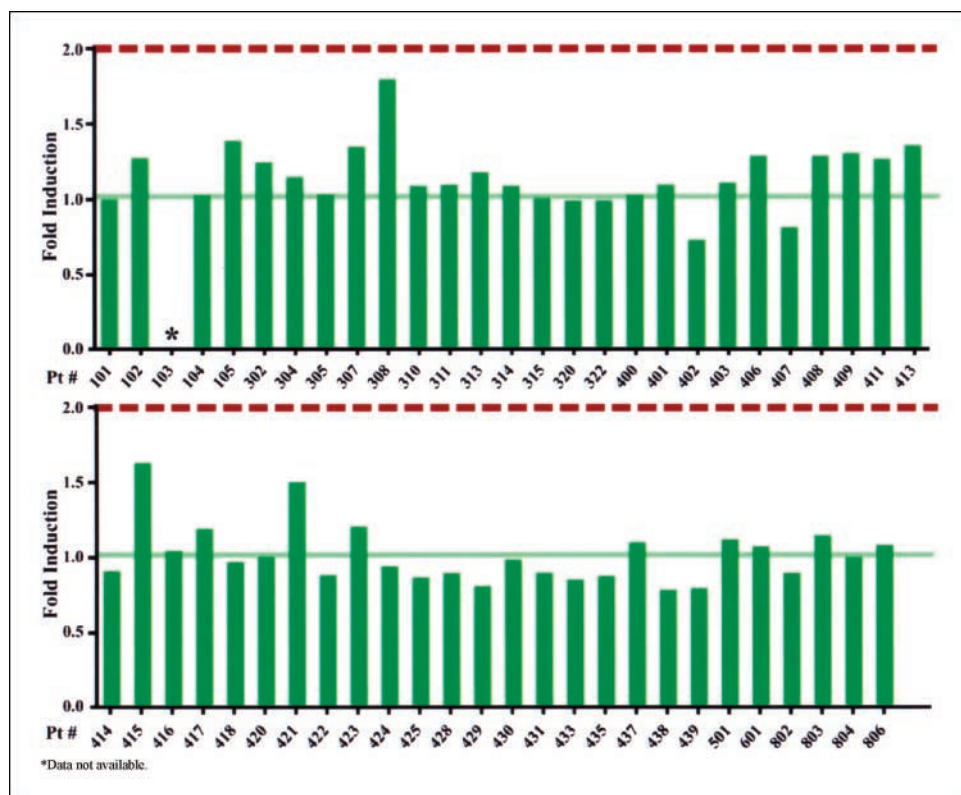


Fig. 4. PSA ELISA. Pre- to posttreatment fold induction of serum antibodies to PSA. Responses above 2-fold (dotted line) are considered induced.

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Table 2. Adverse events judged by the investigator to be possibly or probably related to treatment in >1 patient (total study group of 55)

Costart term	Grade 1, n (%)	Grade 2, n (%)	Grade 3, n (%)	Grade 4, n (%)
Body as a whole	19 (34)	9 (16)	2 (3)	0 (0)
Chills	8 (14)	0 (0)	0 (0)	0 (0)
Fatigue	9 (16)	5 (9)	1 (1)	0 (0)
Fever	7 (12)	3 (5)	0 (0)	0 (0)
Flu syndrome	8 (14)	1 (1)	1 (1)	0 (0)
Cardiovascular system	1 (1)	1 (1)	0 (0)	0 (0)
Vasodilation	1 (1)	1 (1)	0 (0)	0 (0)
Digestive system	6 (10)	2 (3)	0 (0)	0 (0)
Anorexia	4 (7)	1 (1)	0 (0)	0 (0)
Nausea	2 (3)	0 (0)	0 (0)	0 (0)
Injection site reaction	1 (1)	53 (96)	1 (1)	0 (0)
Musculoskeletal system	6 (11)	3 (5)	0 (0)	0 (0)
Arthralgia	2 (3)	2 (3)	0 (0)	0 (0)
Myalgia	4 (7)	0 (0)	0 (0)	0 (0)
Pain, pelvis	1 (1)	1 (1)	0 (0)	0 (0)
Nervous system	5 (9)	1 (1)	0 (0)	0 (0)
Dizziness	3 (5)	0 (0)	0 (0)	0 (0)
Myasthenia	0 (0)	1 (1)	0 (0)	0 (0)
Skin and appendages system	4 (7)	2 (3)	0 (0)	0 (0)
Pruritus	2 (3)	0 (0)	0 (0)	0 (0)
Rash	2 (3)	0 (0)	0 (0)	0 (0)

NOTE: Grade 1 treatment-related adverse events that occurred in one patient only included headache, constipation, flatulence, arthritis, hip pain, leg pain, shoulder pain, anxiety, somnolence, dyspnea, alopecia, conjunctivitis, and kidney pain. Grade 2 treatment-related adverse events that occurred in one patient only included lipoma, malaise, pain, diarrhea, vomiting, peripheral edema, myasthenia, skin disorder, dry skin, and hematuria. Grade 3 treatment-related adverse events that occurred in one patient only are shown above. There were no Grade 4 treatment-related adverse events.

simply reflect the selection of a better group of patients for subsequent chemotherapy, this chemotherapy-associated increased survival time seemed to be associated with the induction of immunoreactivity to one or both cell lines. This finding is consistent with other studies demonstrating an improved response to chemotherapy following a positive immune response to other types of immunotherapy (29–31). Further investigation of the coadministration of chemotherapy and this immunotherapy is under way in phase 3 trials.

Both PSA and ICTP levels are significantly higher in patients with metastatic prostate cancer as compared with prostate cancer patients without overt metastases. However, ICTP levels may be a more sensitive indicator of the extent of osseous metastasis than PSA levels and have been shown to decrease in patients with metastatic prostate cancer treated with androgen deprivation therapy (17, 32, 33). In the radiologic group, ICTP levels were stable or decreased in 78% of patients receiving the high dose and 65% receiving the low dose of immunotherapy. These changes in bone marker levels could not be attributed to hormone therapy or bisphosphonates, which were not allowed during the study. Antineoplastic immune responses in osseous metastases are suggested by these data.

The immunogenicity of this therapy was shown by immunoblots of patient sera against the immunotherapy cell lines, which showed development of new or enhanced posttreatment immunoreactive IgG bands for a majority of patients. These results indicated that patient immune responses were oligoclonal, with both shared and unique responses. The heterogeneity of antibody response between individual patients suggests that some immunodominant prostate cancer-associated antigens are shared, and some are unique to individual patients. This

supports the hypothesis that a polyvalent whole cell immunotherapy approach exposes patients to a broad array of potentially immunogenic antigens. The patient with a complete response was not an HLA class I match to either cell line, suggesting that an allogeneic approach can produce a patient-specific response despite lack of a HLA class I match. This clinical observation is consistent with reports that patient-specific T cell responses can be generated via cross-presentation of allogeneic antigens by host antigen-presenting cells as shown in a study of the GVAX platform-based, allogeneic, cellular immunotherapy for pancreatic cancer (15). HLA mismatching between the immunotherapy cells and the treated host might theoretically enhance the antitumor immune response by providing a danger signal associated with the strong allogeneic MHC response. This hypothesis is supported by preclinical data showing improved immunologic potency and survival in mice immunized with a murine GVAX immunotherapy product containing an allogeneic MHC antigen compared with mice immunized with a fully autologous GVAX product.⁹ An exploratory analysis done in this trial did not show a significant association between HLA class I match versus mismatch to either immunotherapy cell line and clinical outcome. However, nonsignificant improvements were noted in the HLA mismatched subgroup in every immunologic and clinical end point, including induction of tumor reactive antibodies, changes in PSA, ICTP, time to progression, and survival, suggesting that this hypothesis is worthy of further exploration in future trials.

⁹ Unpublished data.

The immune monitoring techniques that were readily available at the time this trial began (1999) did not allow detailed exploration of the immune response stimulated by this immunotherapy. However, generation of antibodies against PSA was evaluated in 52 patients, and none were identified. A previous study of this immunotherapy in hormone-naïve patients also showed that anti-PSA antibodies were not generated in any patients, including a patient with a partial PSA response to treatment (13). This suggests that PSA is not an immunodominant antigen in prostate cancer, at least when presented in the context of a whole cell immunotherapy. A 250-kDa band was noted on immunoblot for 11/19 immunoreactive patients, and the band was identified in one patient as filamin B (β). Filamin B is a cytoskeletal protein prevalent in endothelial cells that plays a central role in cell shape, division, adhesion, motility, signal transduction, and protein sorting (23, 24). Filamin B has been linked to cancer through functional studies and was identified as a candidate cancer gene in a study of mutation rates in breast and colorectal cancers (22).

An antibody response to a 250-kDa protein has been observed in other studies of this immunotherapy, including in a hormone-naïve patient with a partial PSA response to treatment (13), and Simons et al. reported a trend toward prolonged survival in 17 patients with this immunoreactive band compared with those without the 250-kDa band ($P = 0.08$, log-rank test; ref. 21). Immunoblot analysis using a mouse monoclonal antifilamin probe showed comigration of filamin and the 250-kDa band in these patients. Further assessment of immunogenicity including T-cell assays against defined prostate antigens and the relationship between B-cell and T-cell responses is warranted and under development.

This immunotherapy was well tolerated. The most frequent adverse event was injection site reaction. Immunization was often accompanied by the development of flu-like symptoms. Most (93%) of the adverse events were mild to moderate in severity and reversible; none of the patients discontinued the study due to adverse events related to treatment. Furthermore, there was no development of symptoms or signs suggestive of autoimmune disease and no increase in ANA titer, a measure of possible autoimmune reactivity. Although a maximum tolerable dose has not been established for this product, further dose escalation is limited from a feasibility standpoint by the number of injections required with each treatment.

The potential for efficacy with only minor toxicity suggests that the GM-CSF-secreting, cellular immunotherapy may be a therapeutic option for patients with advanced prostate cancer. The high dose level seemed to provide more favorable changes in PSA and ICTP levels, time to PSA progression, time to bone scan progression, and overall survival in patients with radiologically detectable metastases, although this observation warrants prospective confirmation. Randomized, controlled, phase 3 trials of this immunotherapy are under way to evaluate survival benefit and safety in a larger patient population.¹⁰

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¹⁰ Information on phase 3 trials can be found on www.clinicaltrials.gov (Keyword: GVAX).

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