

## Phase I/II Trial of an Allogeneic Cellular Immunotherapy in Hormone-Naïve Prostate Cancer

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**Abstract Purpose:** To determine the toxicity, immunologic, and clinical activity of immunotherapy with irradiated, allogeneic, prostate cancer cells expressing granulocyte macrophage colony-stimulating factor (GM-CSF) in patients with recurrent prostate cancer.

**Patients and Methods:** A single-institution phase I/II trial was done in hormone therapy – naïve patients with prostate-specific antigen (PSA) relapse following radical prostatectomy and absence of radiologic metastases. Treatments were administered weekly via intradermal injections of  $1.2 \times 10^8$  GM-CSF gene – transduced, irradiated, cancer cells ( $6 \times 10^7$  LNCaP cells and  $6 \times 10^7$  PC-3 cells) for 8 weeks.

**Results:** Twenty-one patients were enrolled and treated. Toxicities included local injection-site reactions, pruritus, and flu-like symptoms. One patient had a partial PSA response of 7-month duration. At 20 weeks post first treatment, 16 of 21 (76%) patients showed a statistically significant decrease in PSA velocity (slope) compared with prevaccination ( $P < 0.001$ ). Injection site biopsies showed intradermal infiltrates consisting of CD1a<sup>+</sup> dendritic cells and CD68<sup>+</sup> macrophages, similar to previous clinical trials using autologous GM-CSF-transduced cancer cells. Post-treatment, patients developed new oligoclonal antibodies reactive against at least five identified antigens present in LNCaP or PC-3 cells. A high-titer antibody response against a 250-kDa antigen expressed on normal prostate epithelial cells was induced in a patient with partial PSA remission; titers of this antibody decreased when treatment ended, and subsequent PSA relapse occurred.

**Conclusions:** This non-patient-specific prostate cancer immunotherapy has a favorable safety profile and is immunologically active. Continued clinical investigation at higher doses and with longer boosting schedules is warranted.

New therapies for prostate cancer are urgently needed for the 28,000 men in the United States predicted to die from metastatic disease in 2005. Cancer immunotherapy represents a novel treatment approach currently in development (1). As many prostate cancer-associated antigens are also expressed by normal prostate epithelial cells, an important objective of cancer immunotherapy strategies is to break immune tolerance to antigens expressed by prostate cancer metastases.

The granulocyte macrophage colony-stimulating factor (GM-CSF) – secreting cancer cell immunotherapy platform (GVAX), generated from whole tumor cells by *ex vivo* GM-CSF gene transfer, has been shown to elicit potent, long-lasting, tolerance-breaking, tumoricidal immune responses in a variety of poorly immunogenic animal tumor models (2–8). We and others have shown the efficacy of such polyvalent immunotherapies preclinically in both spontaneously arising and transgenic rodent models of prostate cancer (9, 10). Treatments were shown to induce antitumor immune responses by recruitment and activation of antigen-presenting cells, such as dendritic cells, to injection sites leading to activation of tumor antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Recent preclinical studies have suggested that CD4<sup>+</sup> T cells activated by GM-CSF-secreting tumor cell immunotherapies simultaneously elicit both Th1 and Th2 CD4<sup>+</sup> T-cell responses, leading to cancer cell killing by several mechanisms that can kill MHC class 1-null tumor cells present in many prostate cancers (11).

In phase I clinical trials using GM-CSF-secreting tumor cell immunotherapies, induction of systemic antitumor immune responses and clinical activity was observed in renal-cell, melanoma, and pancreatic cancer (12–16). More recently, clinical responses have been reported in advanced non-small-cell lung cancer (17, 18) and with such autologous

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immunotherapies used in combination with CTLA-4 antibody in melanoma and ovarian cancer (19). In our pilot phase I study of eight patients treated with GM-CSF-secreting autologous tumor cell injections following radical prostatectomy, we observed a dose-dependent increase in systemic T-cell and B-cell immune responses against autologous prostate cancer-associated antigens (20).

A major limitation to further development of such autologous immunotherapies was the small size of the resected prostate tumors, which led to failure of vaccine manufacturing in over half of the patients. In addition, for patients with micrometastatic prostate cancer [increasing prostate-specific antigen (PSA)] after prostatectomy, which cannot feasibly be harvested, allogeneic tumor cell lines provide the only practical source of tumor cells for product development. For these reasons, two established prostate cancer cell lines were selected for clinical development, which were originally derived from lymph node (LNCaP) and bone (PC-3) metastases and expressed, by transcriptome analyses and immunoblots, hundreds of genes identified in human prostate cancer metastases, including previously described prostate cancer-associated antigens. The results of the first phase I/II study of treatment with these GM-CSF gene-modified allogeneic tumor cells in prostate cancer are presented.

## Patients and Methods

**Selection of patients.** Study candidates were men with adenocarcinoma of the prostate with isolated PSA recurrence following radical prostatectomy. This was defined as increasing PSA on two independent determinations over at least 4 weeks, with an absolute value of  $>1$  ng/mL. PSA recurrence more than 4 years after primary surgery, previous radiation therapy, hormonal therapy or chemotherapy, and evidence of radiologic metastases were exclusion criteria. Additional eligibility criteria included Eastern Cooperative Oncology Group performance status of 0 or 1, no previous history of autoimmune diseases, and intact T-cell response to delayed-type hypersensitivity challenge with common microbial recall antigens (Multitest CMI panel, Pasteur-Merieux-Connaught, Swiftwater, PA).

**Study design.** Investigational product was administered weekly for 8 weeks in the outpatient clinic at a total dose of  $1.2 \times 10^8$  cells ( $6 \times 10^7$  per cell line). The dose for all treatments was the same. LNCaP and PC-3 injections were given on separate limbs and injection sites were rotated with each treatment cycle. Patients were monitored for cutaneous and systemic toxicities by National Cancer Institute Common Toxicity Criteria. Evaluation for the appearance of autoimmune diseases, including serology studies, was conducted. Treated men were assessed for PSA response and progression using serum PSA (TOSOH assay) determinations monthly for 12 months and then every 3 months. Radiographic imaging studies, including bone and computer-assisted tomography scans, were employed if PSA progression was documented. The primary end points of the trial were safety and efficacy based on assessment of PSA response. Secondary end points included time to PSA and clinical progression and assessment of local and systemic immune responses.

The study was reviewed and approved by the Johns Hopkins Institutional Review Board, Food and Drug Administration, and the NIH Office of Recombinant DNA Activities (protocol 9708-205). All subjects gave written informed consent. The conduct of the study was sponsored by the Prostate Cancer Foundation Clinical Trials Consortium with manufacture and supply of the investigational product provided by Cell Genesys, Inc. (South San Francisco, CA). The clinical investigators had no conflict of interest with Cell Genesys under the review of Johns Hopkins University.

**Table 1. Patient characteristics**

Characteristic	No.
Enrolled in the study	21
Completed 8 weekly treatments	21
Median age, y (range)	64 (51-74)
Time from prostatectomy, mo	
Median	43
Range	8-127
Tumor stage at diagnosis	
I	0
II	5
III	12
IV	2
NA	1
Gleason score, median	7
Gleason score, frequency	
6	4
7	7
8	8
9	2
Baseline PSA, ng/mL	
Median	9.5
Mean	16.8
Range	4.2-74.1
Eastern Cooperative Oncology Group performance status	
0	21
1	0

**Investigational product manufacturing.** The LNCaP (21) and PC-3 (22) cell lines were characterized for PSA, carcinoembryonic antigen (CEA), androgen receptor, prostate-specific membrane antigen, urokinase-type plasminogen activator, and glutathione S-transferase expression (23). LNCaP was shown to express androgen receptor, PSA, urokinase-type plasminogen activator, and prostate-specific membrane antigen, whereas PC-3 was shown to express mutant p53, glutathione S-transferase, CEA, and urokinase-type plasminogen activator.<sup>5</sup> Both were transduced to express human GM-CSF as previously described (9, 13, 15). The genetically modified cells were cloned and banked. Clinical lots were manufactured and lethally irradiated to prevent cell replication. Final product was characterized for GM-CSF secretion by ELISA (R&D Systems, Minneapolis, MN), evaluated for vector integration by quantitative Southern blot analysis (9), cryopreserved at a concentration of  $3 \times 10^7$  cells/mL, and tested according to Food and Drug Administration specifications before use (13, 15). To administer the product, the frozen cells were thawed quickly in a 37° water bath, immediately drawn into syringes without any post-thaw manipulation, and administered intradermally. For each administration, four injections of 1 mL each were given (two injections per cell line) to deliver a total cell dose of  $1.2 \times 10^8$  cells ( $6 \times 10^7$  per cell line). GM-CSF secretion from the clinical lots used in this trial was 150 ng/10<sup>6</sup> cells/24 h (LNCaP) and 450 ng/10<sup>6</sup> cells/24 h (PC-3).

**Immune response evaluation.** Assessment of immune response included analyses of injection-site biopsies and induction of LNCaP and PC-3 reactive serum antibodies. For each patient, a skin biopsy was obtained before at baseline and then 3 days following the first, fourth, and eighth treatment. Biopsies were split into formalin-fixed and snap-frozen samples. Infiltrating tumor cells were detected by immunohistochemical staining for cytokeratins, PSA, and prostate-specific acid

<sup>5</sup> Unpublished results.

phosphatase. Infiltrating inflammatory cells were detected by staining for CD68 (macrophages), CD1a and S-100 (Langerhans cells; ref. 24), CD56 (natural killer cells), leukocyte common antigen, T cells (CD3, CD4, CD8, and CD45RO), CD20 (B cells), Ki-67 (proliferation marker), and with Diff Quick (eosinophils and neutrophils). Frozen sections were stained for eosinophil major basic protein.

Sera were analyzed for induced antibody responses against prostate cancer-associated antigens by immunoblot analysis against prostate cancer cell lines (LNCaP and PC-3) as well as primary cultures of human prostate epithelial, stromal, and smooth muscle cells, and lung fibroblasts (Clonetics Corporation, Walkersville, MD) as previously described (25). Briefly, cell lysates were separated by denaturing gel electrophoresis (Novex, San Diego, CA), blotted onto nitrocellulose membrane, and incubated with patient serum (1:1,000 dilution). Bound antibodies were detected using horseradish peroxidase-conjugated goat anti-human antibodies (Zymed, South San Francisco, CA) by chemiluminescence (Amersham, Piscataway, NJ).

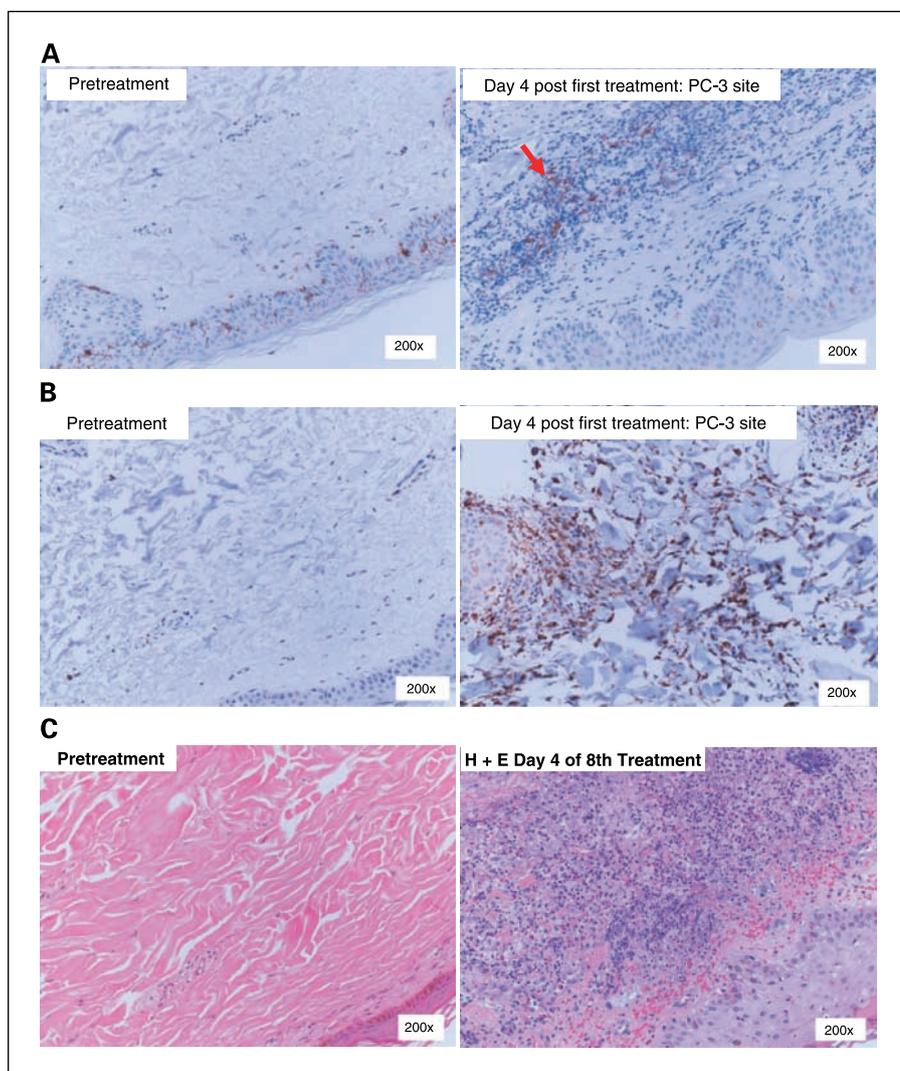
**Immunoreactive antigen identification.** To identify the antigens recognized by the serum antibodies of treated patients, lysates of LNCaP and PC-3 were separated by two-dimensional gel electrophoresis according to the method of O'Farrell (26). Duplicate gels were run for each lysate. One gel was stained with Coomassie blue whereas the other was transferred onto a polyvinylidene difluoride membrane that was blotted with pretreatment and posttreatment patient serum to identify reactive antigens. New spots induced posttreatment were

excised from the Coomassie blue-stained gel, subjected to trypsin digest, and analyzed by matrix-assisted laser desorption/ionization mass spectrometry at the Protein Chemistry Core Facility, Howard Hughes Medical Institute, Columbia University. The identified peptides were analyzed using the SWISS-PROT database.

**Statistical methods.** PSA response was the primary study end point and was defined according to the PSA Working Group criteria (27). Analysis of PSA kinetics was not a prespecified study end point but was done post hoc. Pretreatment and posttreatment PSA slopes (slope of the natural log of PSA) were defined as the least square lines calculated from at least three PSA measurements recorded within a minimum of 4 months before first treatment (pretreatment slope; ref. 28). This was compared with posttreatment PSA slope calculated from at least five PSA measurements over 5 months following the first treatment (posttreatment slope). The slope of natural log PSA both pretreatment ( $m_1$ ) and posttreatment ( $m_2$ ) and the difference ( $m_2 - m_1$ ) were calculated for each patient. A two-sided  $t$  test was used to compare the results across all 21 patients. Statistical software employed was STATA.

## Results

**Safety.** Twenty-one patients were enrolled. Baseline patient characteristics are shown in Table 1. Forty-eight percent had a



**Fig. 1.** Injection site biopsies pre- and post-treatment. *A*, infiltration of CD1a<sup>+</sup> cells (arrow) with the appearance of dendritic cells at PC-3 injection site day 4 post first treatment. *B*, infiltration of CD68<sup>+</sup> cells consistent with intradermal macrophages at day 4 post first treatment. *C*, intense neutrophil- and eosinophil-rich intradermal infiltrate at PC-3 injection site at day 4 post eighth treatment. Similar results were seen at LNCaP injection sites.

Gleason score of >7 in their primary tumor. Every patient received the full 8-week treatment course for a total of 168 treatments administered.

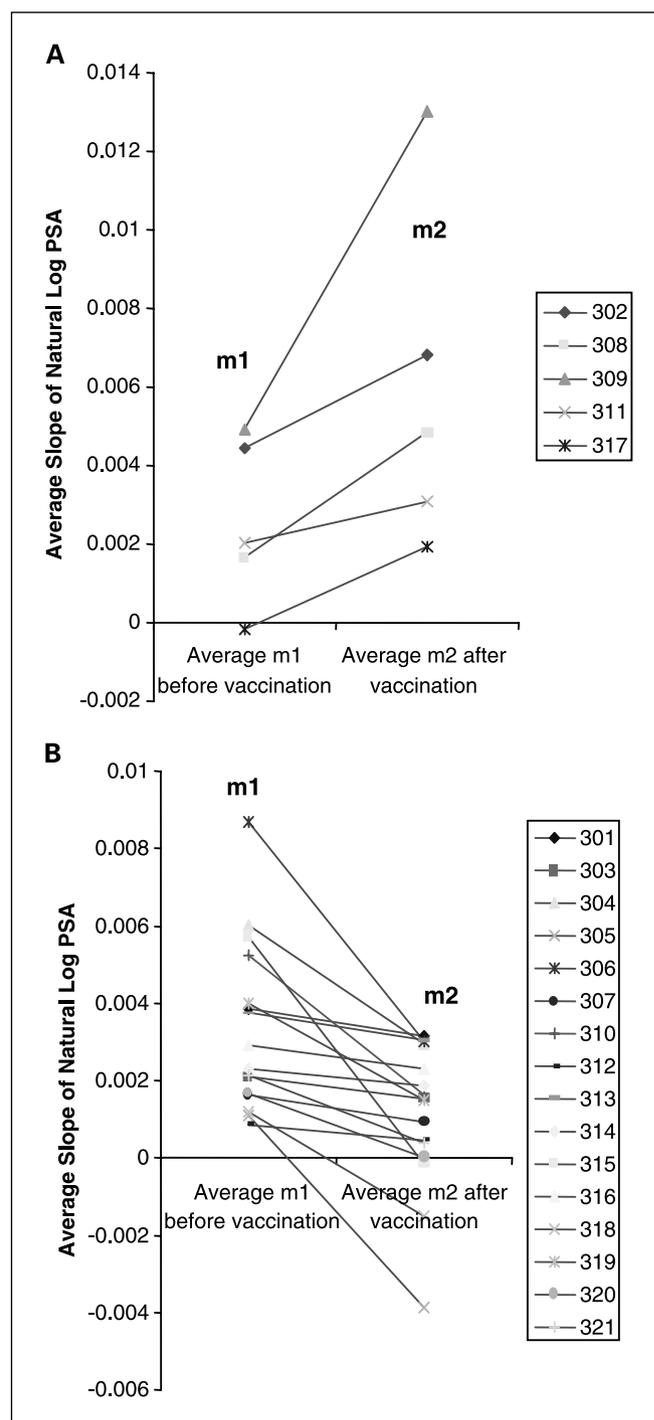
Self-limited injection-site reactions including local discomfort, erythema, swelling, and pruritis were seen in all patients. Injection site reactions were recorded as severe in six patients due largely to local pruritis and erythema, but did not meet criteria for National Cancer Institute grade 3 toxicity. No skin ulceration was noted. Injection reactions were recorded as grades 1 to 2 in the remaining patients. In general, the diameter of PC-3 reactions was greater than LNCaP. Reactions typically peaked within 48 hours and resolved within 7 days. Rash was reported in 10 patients and pruritis in 16. Mild fevers, chills, malaise, peripheral edema, and vasodilation were reported by 10% to 20% of patients. There were no dose-limiting toxicities and no other grade 3 or 4 adverse events reported. No significant alterations in serum chemistries or hematology counts were noted except for mild eosinophilia in five patients. Importantly, there were no significant alterations from baseline in autoantibody titers for antinuclear antibodies, rheumatoid factor, microsomal antibodies, or thyroid stimulating hormone in any patient.

**Recruitment of antigen-presenting cells to injection sites.** Intradermal administration was chosen because of the abundance of Langerhans cells, the skin dendritic cells, at this site. Injection site biopsies were obtained 3 days after the first, fourth, and eighth treatment. Extensive analyses of these injection-site biopsies were undertaken to characterize immune effector cell infiltrates. These were compared with our previous published experience with day 3 injection-site biopsies from autologous renal-cell, melanoma, and prostate carcinoma GM-CSF gene-transduced cancer cell immunotherapy trials (12, 15, 20).

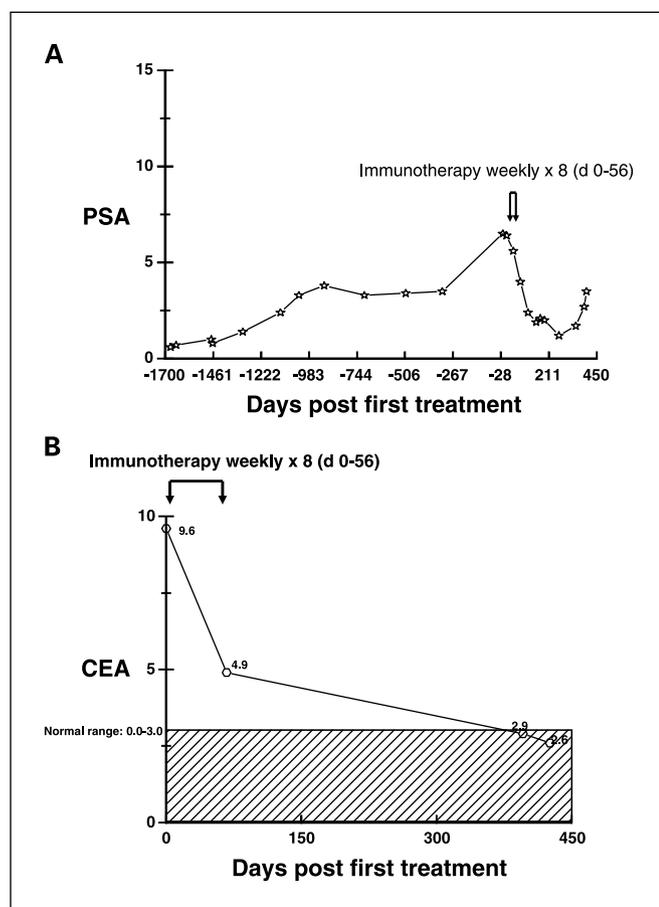
Infiltrating tumor cells, characterized by positive staining for cytokeratins, were present intradermally in injection-site biopsies (data not shown). CD1a<sup>+</sup> dendritic cells could be observed following the first treatment (Fig. 1A). Langerhans cells were evident at the junction of the epidermis and dermis and also in the dermis near areas of infiltrating tumor cells. As predicted from previous trials with autologous products (12, 15, 18, 19), intense antigen-presenting cell infiltrates with CD68<sup>+</sup> macrophages were also detected in these same interdermal areas for both LNCaP and PC-3 injection sites (Fig. 1B). Neutrophils and eosinophils were abundantly present near tumor cells in the dermis (Fig. 1C). Eosinophils showed evidence of activation, with eotaxin-positive staining of dermal vessels and massive levels of intradermal degranulation seen by eosinophil major basic protein staining (data not shown). Small blood vessels near tumor cells were surrounded by mononuclear inflammatory cells, including CD4<sup>+</sup> and CD8<sup>+</sup> T cells, by the eighth treatment cycle (data not shown). Overall, the histologic findings for allogeneic GM-CSF-modified LNCaP and PC-3 injection sites seemed to be similar to autologous GM-CSF-modified tumor cell injection sites we have previously reported and in our preclinical studies (25). Taken together, these data suggest that both transduced LNCaP and PC-3 cells secrete bioactive GM-CSF *in vivo*.

**Clinical activity.** All 21 patients were monitored for changes in PSA pre- and post-treatment. One patient had a partial PSA response (>50% reduction of PSA) of 7-month duration. In addition, an analysis of treatment-associated change in PSA

slope was done. At 3 months post completion of treatment (five PSA determinations over 5 months), natural log of PSA slope was increased in 5 of 21 patients (Fig. 2A). Increasing natural log of PSA slope is consistent with no treatment effect on the natural history. In contrast, 16 of 21 (76%) patients showed a decrease in natural log of PSA slope after treatment, consistent with treatment effect (Fig. 2B). This difference



**Fig. 2.** Change in average slope of natural log PSA before ( $m_1$ ) and after ( $m_2$ ) 8 weekly treatments. **A**, 5 of 21 patients with increased PSA slope posttreatment. **B**, 16 of 21 patients with decreased PSA slope posttreatment. Difference between groups was significant ( $P < 0.001$ ).



**Fig. 3.** Tumor marker response in patient 305. Partial PSA response (>50% reduction) following 8 weekly treatments (A) with corresponding complete CEA response (B). Maximal tumor marker responses were delayed and occurred following completion of treatment.

between 16 PSA slope responders and 5 nonresponders achieved statistical significance ( $P < 0.001$ , two-sided  $t$  test). The kinetics of a maximal PSA response required weeks after treatment initiation. The one patient with a partial PSA response had a Gleason grade 8 cancer and manifested a decline in PSA by week 8 with the PSA nadir observed over 5 months after the completion of study treatment (Fig. 3A). In addition to PSA, elevated serum levels of CEA in this patient, who was not a smoker, fell within the reference range following treatment (Fig. 3B), suggesting antitumor activity against tumor cells expressing both CEA and PSA.

**Induction of tumor-reactive antibodies.** Dendritic cell loading of tumor-associated antigens present on immunizing tumor cells can generate new antibody responses (12, 13, 15, 25). We were the first to report antibody induction following treatment with GM-CSF-transduced autologous prostate cancer cells (25). Testing for antibody induction in this trial of allogeneic cells was therefore undertaken. Immunoblot analyses, two-dimensional gels, and subsequent mass spectroscopy using sera from patients, pre- and post-treatment, showed the appearance of new antibodies recognizing LNCaP- and PC-3-associated tumor antigens. Antibodies reactive against both PC-3 and LNCaP were induced. Most responses were oligoclonal in nature, with one to five new or induced bands per patient. Whereas many responses seemed to be patient specific, several patients mounted PC-3- and LNCaP-reactive antibodies against proteins of similar molecular weight, including five patients with reactivity against a 14-kDa protein and three patients with reactivity against a 250-kDa protein on PC-3. Table 2 summarizes the results for five induced high-titer antibodies reactive against the PC-3 cell line for which the protein antigens they recognize have been identified. We specifically tested for the induction of new antibodies against PSA or CEA that might have confounded the analysis of these serum markers of disease activity. In no case, including the patient with the partial PSA response, were antibodies against either PSA or CEA identified (Fig. 4A).

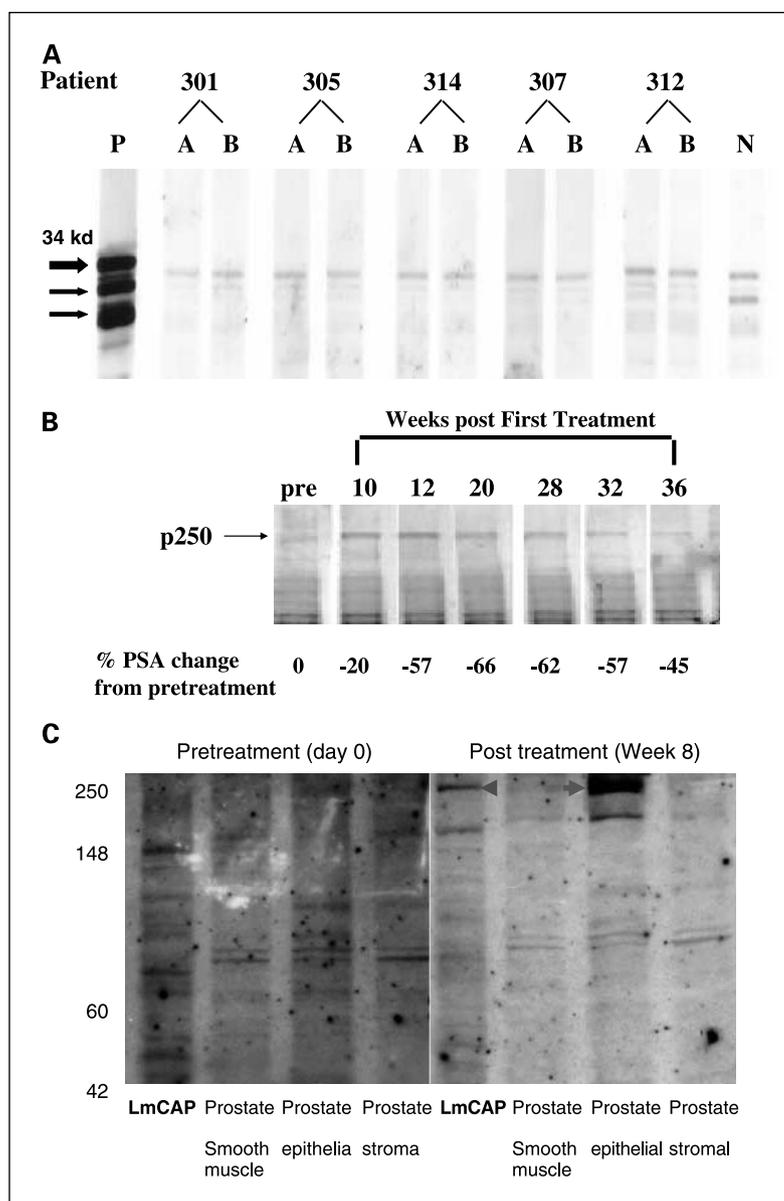
Of particular interest, induction of one of these new antibodies and its titer was correlated with the clinical course. The patient with the 7-month partial PSA response (Fig. 3A) developed a new immunoglobulin G antibody reactive against LNCaP with titers  $>1:10,000$  by week 10 (Fig. 4B). On further analysis, this antibody recognized an epitope on LNCaP and normal untransformed prostate epithelium, but not prostate stroma or smooth muscle (Fig. 4C). The titer for this antibody decreased to barely detectable at 1:1,000 dilution 2 months before the time serum PSA began to increase from its nadir (Fig. 4B). These data suggest a possible association between cessation of vaccination and a subsequent decline in antibody titers and tumor progression by PSA.

## Discussion

The results of this phase I/II trial of a GM-CSF-secreting allogeneic prostate cancer cell immunotherapy showed that the treatment was well tolerated and can be administered in an outpatient setting. Treatment-related toxicities were mostly cutaneous and self-limited and no evidence of autoimmune activity was observed. The pruritis and erythema observed at the

**Table 2.** Post treatment immunoglobulin G antibody induction against PC-3: proteomic characterization of candidate antigens

Patient sera	Molecular weight (theoretical/apparent)	Candidate antigen	Sequence coverage (%)
305	15.8/14	Ubiquitin cross reactive protein (ISG15)	62
306	17.3/14	Nucleoside diphosphate kinase B (NM23-H2)	94
306	24.8/32	High mobility group-1 protein	44.8
310	54.3/60	Probable protein disulfide isomerase ER-60 precursor (ERP60)	62
310	70.9/71	Heat shock cognate 71 kDa protein	38.4



**Fig. 4.** Treatment-induced antibody responses against PC-3 and LNCaP. **A**, immunoblots using LNCaP lysates blotted against patient serum did not detect induction of anti-PSA antibodies posttreatment. [A, pretreatment; B, post eighth injection; P, PSA-positive control (LNCaP blotted with anti-PSA immunoglobulin G antibody; arrows, bands associated with 34-kDa full-length PSA secretory protein and its major degradation products); N, normal serum without anti-PSA antibody]. **B**, immunoblots at 1:1,000 dilution of serum against PC-3 lysates showing kinetics of immunoglobulin G antibody induction against 250-kDa protein present on PC-3 in patient 056 with PSA response. Antibody response was detectable by 10 weeks and waned by week 36 when PSA began to increase. **C**, immunoreactive 250-kDa protein (arrows) recognized by patient 305 is expressed on normal prostate epithelium and LNCaP, but not prostate smooth muscle or stroma. Serum dilution, 1:1,000.

injection sites were correlated with significant eosinophil infiltration. GM-CSF is a well-known activator of eosinophils, and the eosinophilic reaction to GM-CSF-secreting tumor cell immunotherapies has been observed in earlier trials (12, 13, 25).

The original rationale for choosing a single phase I/II dose of  $1.2 \times 10^8$  cells was based on three observations. First, dosing was based on extrapolating to human body size from preclinical studies showing efficacy against preestablished tumors in this range of tumor cell dose and GM-CSF secretion rate (9, 10). Second, similar tumor cell dose and GM-CSF secretion levels were well tolerated in a phase I study using autologous GM-CSF-modified tumor cells in prostate cancer patients (29). Third, this tumor cell dose (secreting GM-CSF > 150 ng/million cells/24 h) conferred a partial remission in a patient with metastatic renal cell carcinoma (15).

In this study, injection-site biopsies stained positive for biomarkers of infiltrating antigen-presenting cells including CD68<sup>+</sup> macrophages and CD1a<sup>+</sup> dendritic cells. These pat-

terns were similar to those reported for the autologous prostate cancer immunotherapy trial on day 3 following first treatment (15). A partial PSA response in 1 of 21 patients and a reduction in PSA velocity posttreatment in 16 of 21 patients provide preliminary evidence of clinical antitumor activity. The efficacy of this GM-CSF-secreting cellular immunotherapy was not compared in this study to recombinant GM-CSF protein alone, which has shown effects on PSA in prostate cancer trials (30). However, animal studies have convincingly shown a more potent induction of injection-site inflammatory cell infiltration and antitumor immunity with tumor cells genetically modified to secrete GM-CSF than with recombinant GM-CSF alone.<sup>6</sup> The dose and schedule employed in this trial, while showing signatures of GM-CSF-transduced tumor cell injection-site immune responses, were on the low end of a potential

<sup>6</sup> K. Jooss, unpublished data.

dose-response relationship. Higher cell doses and a more prolonged schedule of boost injections are warranted.

This allogeneic whole-cell immunotherapy strategy was developed using LNCaP and PC-3 as a polyvalent source of candidate antigens from prostate lymph node and bone metastases for patients in whom autologous products could not be easily created with current biotechnology. Specifically, our approach was conceived for men who still have low tumor burden at relapse as measured by isolated PSA recurrence after prostatectomy.

Of interest in use of PSA as a surrogate of antitumor activity is our observation that neutralizing antibodies against PSA were not generated after treatment with LNCaP, a cell line that expresses PSA, suggesting that PSA is not an immunodominant antigen. Thus, PSA changes in this study are an evaluable biomarker of disease activity and treatment effects.

The patients in this trial had no available autologous tumor for harvest. As a result, delayed-type hypersensitivity testing and measurement of *in vitro* T-cell responses against autologous tumor cells could not be done. However, induction of LNCaP- and PC-3-reactive antibodies does suggest that the B-cell arm of the immune system could be clinically involved in antitumor activity. This is supported by the observation of an association between induction of a new antibody, recognizing a 250-kDa antigen on human prostate cancer, and PSA response in one patient. With the cessation of weekly treatments, antibody titers decreased and subsequent PSA progression was noted. This observation makes a strong argument for testing periodic booster injections to sustain new antibody levels and potentially delay PSA progression. In addition, new antibodies were generated against ubiquitin cross-reactive protein (ISG-15), the metastasis suppressor gene nucleoside diphosphate kinase B (NM23-H2), high mobility group-1 protein, probable protein disulfide isomerase ER-60 (ER-60), and heat shock cognate 71-kDa protein. Of note, ubiquitin cross-reactive protein (ISG15) has been reported as a key protein that modulates innate immune responses and sensitivity of melanoma cells to IFN- $\alpha$  (31). NM23-H2, a metastasis suppressor gene, has been implicated in breast and colon cancer and is expressed in prostate

cancer adenocarcinomas (32, 33). The clinical significance of breaking tolerance against these antigens in prostate cancer, and other antigens not yet fully characterized, requires further study.

The platform of GM-CSF-transduced tumor cell immunotherapy (GVAX) represents only one of several new approaches for active specific immunotherapy of prostate cancer. Other examples include treatment with defined peptide antigens like PSA (34), carbohydrate antigens (35), vaccinia vectors expressing PSA (36), and infusions of GM-CSF-activated autologous dendritic cells loaded *ex vivo* with defined prostate-specific peptides, such as prostate-specific membrane antigen and prostatic acid phosphatase (37, 38), or even amplified prostate tumor RNA (39). New tumor-associated antigens and antibodies discovered in this and other immunotherapy trials could themselves become candidate therapeutic molecules using recombinant DNA technologies. Whereas the immune monitoring techniques employed in this trial were relatively unsophisticated, more sophisticated techniques to monitor tumor antigen-reactive antibodies have been developed in recent years. Additional antigen discovery efforts have been applied in subsequent clinical trials to explore the relationship between B-cell and T-cell responses induced by this immunotherapy.

This report suggests that systemic immune responses to candidate prostate cancer-associated antigens can be induced and identified in a tumor type that has been conventionally viewed as refractory to immunotherapy using a non-patient-specific allogeneic tumor cell-based strategy. Clinical trials of higher doses and more prolonged boosting schedules to optimize efficacy have been initiated on the basis of these findings. In addition, strategies to boost immune response to this immunotherapy, including combination therapy with antibodies to CTLA-4, are being pursued (19, 40).

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