Clinical and Biological Effects of Neoadjuvant Sargramostim and Thalidomide in Patients with Locally Advanced Prostate Carcinoma

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Abstract

Purpose: Granulocyte macrophage colony-stimulating factor (GM-CSF) and thalidomide are active agents in prostate cancer. This study assessed the biological effects and safety of GM-CSF and thalidomide in patients with localized prostate cancer before radical prostatectomy.

Experimental Design: Locally advanced prostate cancer patients undergoing radical prostatectomy were recruited for this study. Treatment consisted of two 28-day cycles of GM-CSF (250 μg, s.c., thrice weekly) and thalidomide (200 mg, orally, daily) on days 1 to 28 of each cycle. Radical prostatectomy occurred within 7 to 10 days after completion of therapy. Pretreatment and posttreatment specimens were used to assess the expression of CD3, CD68, Ki-67, S100, PTEN, and CD31. Peripheral blood was examined for dendritic cells, regulatory T cells, and cytokines.

Results: Twenty-eight patients were enrolled. No pathologic responses (P0) were observed and no unexpected toxicities or surgical complications occurred. Eighty-one percent of patients had a prostate-specific antigen decline (mean ± SD decrease was 21.1 ± 15.4%; median, 18.0%). With a median follow-up of 32 months, five patients have experienced progression. Radical prostatectomy tumor tissue specimens showed significant CD3 and S100 overexpression when compared with pretreatment biopsies. No significant changes in tumor macrophage infiltration were observed. Increased number of serum dendritic cell, as well as high serum levels of interleukin-8, basic fibroblast growth factor, and vascular endothelial growth factor, was also observed.

Conclusions: Neoadjuvant GM-CSF and thalidomide was safe and feasible and did not affect the perioperative morbidity of radical prostatectomy. Although no pathologic complete responses were observed, significant posttreatment tumor T-cell and dendritic cell infiltration was noted. No significant changes in serum cytokines, dendritic cells, or regulatory T cells were induced.

Despite recent advances in surgical and radiation therapy techniques, the outcome for patients with locally advanced prostate cancer defined by unfavorable pretreatment characteristics [prostate-specific antigen (PSA) >10 ng/mL, biopsy Gleason sum of ≥7, or clinical stage T2b–T2c or T3] remains poor (1). For most of these patients, micrometastatic disease or residual disease at the primary site ultimately leads to disease recurrence (2–4).

A major area of prostate cancer research now focuses on the evaluation of systemic approaches with the potential of improving outcome when given before or after local definitive treatments, such as radical prostatectomy or external beam radiation therapy.

Although initial trials evaluating neoadjuvant androgen deprivation seemed promising, subsequent randomized studies evaluating short- and long-term neoadjuvant androgen deprivation failed to show an improvement in time to biochemical progression and overall survival (5–8). The shown activity of docetaxel-based chemotherapy in patients with metastatic disease led to exploratory studies in the neoadjuvant setting. Although these studies provided evidence that neoadjuvant chemotherapy was safe and feasible, the lack of pathologic response or improved biochemical disease-free survival was disappointing (9–12).

Both passive and active immunotherapies have recently regained popularity as potential nontoxic therapeutic strategies in the management of patients with asymptomatic, castrate-resistant prostate cancer. Overexpression of molecules that can serve as targets for immune recognition ultimately leading to antitumor activity has been the background for the development of immunotherapy in prostate cancer.

Several immunomodulatory agents capable of inducing passive immune activation by way of enhancing antigen recognition by T cells have been evaluated. Granulocyte macrophage colony-stimulating factor (GM-CSF), a pleiotropic
cytokine and a member of a large family of growth factors that acts at multiple levels of hematopoietic cell differentiation and development, has shown some promise in prostate cancer therapeutics (13–19). Systemic administration of GM-CSF promotes the uptake of prostate cancer antigens by dendritic cells, leading to subsequent cross-priming of T cells that ultimately generate an appropriate immune response (16–18).

Various studies evaluating the activity of GM-CSF as a single agent in prostate cancer have been reported (13–15). Although the experience remains small and single institutional in nature, these trials suggest clinical activity in both the castrate and noncastrate setting. Biologically, analysis of peripheral blood in patients receiving GM-CSF shows that the number of circulating monocytes and dendritic cells is increased following each cycle of GM-CSF, suggesting an immunologic basis for the treatment effect observed in these trials (19).

Thalidomide, an immunomodulatory agent with T-cell stimulatory and antiangiogenic activity through blockade of basic fibroblast growth factor, vascular endothelial growth factor (VEGF), and interleukin (IL)-6, has shown clinical activity in patients with castrate-resistant prostate cancer (20–22). Recently, the results of a phase II study evaluating the clinical effects of GM-CSF and thalidomide in castrate-resistant metastatic prostate cancer patients were reported (23). In this small trial, 2 patients achieved a Response Evaluation Criteria in Solid Tumors–defined objective response and 23% of patients (5 of 22) achieved a PSA decline >50%. Albeit provocative, this report together with other published studies are limited due to the lack of information about any potential immune response generated by any of these agents, thus failing to address their potential mechanism(s) of antitumor activity.

In an effort to assess the potential application of GM-CSF and thalidomide in the neoadjuvant setting as well as to elucidate the potential mechanism by which GM-CSF and thalidomide exert their clinical activity in prostate cancer, we conducted a neoadjuvant study in patients with locally advanced prostate cancer undergoing radical prostatectomy.

Materials and Methods

Twenty-eight patients were enrolled in the study between May 2003 and June 2006. Eligibility criteria included histologically documented intermediate- or high-risk localized prostate cancer defined by a serum PSA level of ≥10 ng/mL (any grade or stage), clinical stage T2b, T2c, or T3 (any PSA level or grade), biopsy Gleason sum of 7 (4 + 3 only) or 8 or greater (any stage or PSA level), or any stage/PSA/Gleason patients with a 35% or greater chance of biochemical failure at 5 y based on Kattan’s nomogram (24). Clinical stage was assigned based on the digital rectal examination findings according to the 1997 American Joint Committee on Cancer criteria (25). All patients had no evidence of metastatic disease and were candidates for radical prostatectomy based on their clinical stage, general medical condition, performance function was required as defined by a serum creatinine of 2.0 mg/dL, and aspartate aminotransferase/alanine aminotransferase <35 mmol/L, and bilirubin <1.5 mg/dL, granulocytes ≥1,800 mm$^3$, and platelets ≥100,000 mm$^3$.

The Cleveland Clinic Institutional Review Board reviewed and approved the trial in accordance with an assurance filed with and approved by the Department of Health and Human Services. All patients provided written informed consent before registration and compliance with the Food and Drug Administration–mandated System for Thalidomide Education and Prescribing Safety Program. The exclusion criteria included prior hormonal therapy (except finasteride for the treatment of obstructive voiding symptoms), prior chemotherapy or pelvic radiotherapy, and any malignancy other than basal cell carcinoma of the skin within 5 y of study entry. Patients with underlying grade ≥1 peripheral neuropathy regardless of the etiology were also excluded. The pretreatment evaluation included history and physical examination, complete blood count, serum chemistry panel, and testosterone and PSA measurement. Patients were required to have a whole-body bone scan and computed tomography of the abdomen and pelvis.

Treatment consisted of GM-CSF at 250 μg administered s.c. thrice weekly for 8 wk. Thalidomide was administered orally starting at 100 mg for 5 d and then escalated to reach 200 mg/d with each 4-wk period considered one cycle of therapy. While on study, no prophylactic anticoagulation was administered.

PSA values were collected at baseline and at weeks 4 and 8 after treatment with GM-CSF and thalidomide. Patients were required to have an absolute neutrophil count of ≥1,500/mm$^3$ and a platelet count of ≥100,000/mm$^3$ before proceeding with the next cycle of therapy. Dose modifications were stipulated for thalidomide-related and GM-CSF–related toxicity. Radical prostatectomy and bilateral pelvic lymphadenectomy were done within 7 to 10 d from the last dose of thalidomide. Surgery was done according to a standardized technique under epidural anesthesia (26, 27). Resection of both neurovascular bundles was done at the discretion of the surgeon. For most patients, a cell salvage device was used for intraoperative autotransfusion. Routine postoperative care was provided as previously described (27).

Pretreatment and posttreatment tissue specimens (diagnostic biopsies and radical prostatectomy surgical pathology) were assessed by central review of a single pathologist (C.M.G.). The histologic analysis included evidence of residual cancer, necrosis, atrophy, extraprostatic extension, seminal vesicle invasion, lymph nodes, and margin status. Extraprostatic extension was defined as evidence of prostate cancer in the periprostatic adipose tissue and positive margins as tumor touching ink. A pathologic complete response was defined as complete eradication of tumor. Pathologic down-staging was defined as evidence of decreased pathologic stage or Gleason score when compared with pretreatment clinical stage. Postoperatively, patients were followed up with a PSA determination at 6 wk after surgery and every 3 mo thereafter. Biochemical relapse was defined as a PSA level >0.2 ng/dL measured in two different occasions confirmed at least 1 wk apart. No additional therapy was initiated until biochemical or clinical relapse was evident.

Construction of tissue microarray and collection of pretreatment specimens. A prostate tissue microarray (TMA) containing samples from the radical prostatectomy specimens was constructed, including prostate cancer and corresponding nonneoplastic prostate tissue as control per patient. Three different paraffin blocks from each case were used to construct the TMA to ensure sampling from different prostate cancer areas. Briefly, areas of interest (prostate cancer and nonneoplastic tissue) were traced with a marker pen on H&E-stained slides. The least differentiated regions of each individual formalin-fixed, paraffin-embedded prostate tumor were chosen. The corresponding areas were then marked on the corresponding paraffin blocks (donor blocks) and precisely arrayed into a new recipient paraffin block (35 mm × 20 mm) with a manual TMA arrayer (Beecher Instruments). Three 1.0-mm-thick tissue cores from prostate cancer and two from nonneoplastic tissue were taken for each specimen. After the block construction was completed, 5-μm sections were cut with a microtome using an adhesive-coated tape sectioning system (Instrumedics) and mounted on Superfrost Plus glass slides and stored for future use. A H&E stain was done on the initial TMA slide to verify the histologic diagnosis for each core. The number of samples varied slightly between the individual marker analyses because of variability in the number of interpretable specimens on TMA sections.
Pretreatment diagnostic prostate biopsies (PBx) done at other institutions were reviewed, and representative unstained sections or corresponding paraffin blocks were requested, after patient’s consent was obtained.

**Immunohistochemistry.** For immunohistochemical analysis, 5-μm tissue sections from formalin-fixed, paraffin-embedded blocks of TMA and PBx were stained with antibodies recognizing the following markers: CD3P (1:600 dilution; Cell Marque), CD68 (1:10 dilution; Dako), CD31 (1:20 dilution; Dako), S100 (1:200 dilution; Dako), PTEN (1:100 dilution; Zymed), and Ki-67 (MIB-1, 1:5 dilution; Novocastra). Standard indirect immunoperoxidase procedure (ABC Elite, Vector Laboratories) was used for detection of the secondary antibodies. Microwave and pressure cooker antigen retrieval methods were used for all antibodies with the exception of S100. The primary antibody was omitted for negative controls. All the slides were reviewed and scored by a single pathologist (C.M.-G.) and the microscope used for this analysis was an Olympus BX40.

Granular cytoplasmic staining for PTEN was detected in the secretory cells of benign prostatic glands (internal control). Based on the PTEN pattern of staining, the prostate cancer cases were initially divided into three groups: positive (entire tumor staining), mixed (both positive and negative tumor cells present), and negative (no staining). Quantification of Ki-67 (MIB-1) labeling index was estimated as a percentage of positively stained tumor nuclei of at least 100 cells counted in multiple viewing fields of a given tumor. Immunoreactivity for CD3, CD68, and S100 was scored as percentage of positive stromal cells intimately related to the epithelial component of the tumor. CD31 was evaluated at ×40 magnification and scored as mean number of vessels per high-power field. Up to 10 high-power fields were scored when available. Aside from CD31, all the other markers were evaluated at ×10 and ×20 magnifications.

**Blood correlates.** Blood samples were obtained before and at the end of cycle 1 and 2 posttreatment initiation. Dendritic cells were isolated from peripheral blood mononuclear cells using MACS with the Blood Dendritic Cell Isolation Kit II (Miltenyi Biotec). The number of dendritic cell isolated was expressed per mL of blood collected. Total RNA was extracted from the isolated dendritic cell using the RNasy Mini kit (Qiagen). The blood collected for dendritic cell studies was also used to assess regulatory T cells. The flow through fraction from the dendritic cell isolation, which contains all of the T cells from the peripheral blood mononuclear cells, was processed using MACS and the CD4/CD25 Regulatory T Cell Isolation kit (Miltenyi Biotec). Total RNA was extracted and quantitative reverse transcription-PCR was done in triplicates using prestandardized Taqman primer and probes or FoxP3 and g-lyceraldehyde-3-phosphate dehydrogenase to normalize. Aliquots of a standard cDNA prepared from peripheral blood mononuclear cells collected from a healthy donor were used as standards in each plate to normalize for inter-plate variability. Results for the expression of FoxP3 were normalized using glyceraldehyde-3-phosphate dehydrogenase signals and linearly transformed. An index was calculated by multiplying the number of CD4/CD25+ cells per mL of blood by the relative FoxP3 mRNA expression.

Th1-associated cytokines were assessed by flow cytometry with the Human Th1/Th2 Cytokine Cytometric Bead Array kit (BD PhaMingen) using patient’s existing serum. Capture beads were mixed with culture supernatants and phycerothrin detection reagent and incubated for 3 h at room temperature. The beads were then washed with wash buffer and analyzed. The assay sensitivities for IL-2, IL-4, IL-5, IL-10, tumor necrosis factor-α, and IFN-γ were 6.6, 6.5, 2.8, 4.7, 4.3, and 15.6 pg/mL, respectively. Serum VEGF, tumor necrosis factor-α, and basic fibroblast growth factor were also evaluated in similar fashion.

**Statistical methods.** The primary end points of the study were to determine the clinical and tumor tissue effects of GM-CSF and thalidomide administered before radical prostatectomy, including pathologic response (histologic P0, margin status, extraprostatic extension, seminal vesicle, and lymph node invasion), surgical outcome, and immune changes observed within the prostatic tumor tissue. Secondary end points included safety, feasibility, changes in serum PSA levels, and time to biochemical failure.

A two-stage accrual design with a maximum goal of 29 patients was used to test the hypothesis that the underlying pathologic response rate was essentially 0% versus ≥15%. Sixteen patients were planned for the first accrual stage and early stopping rules were built in for both positive and negative results. At the end of the first accrual stage, the trial was to be stopped if more than one pathologic complete response was observed or if none was observed. Accrual was to continue if one response was observed. The type I and II errors for the study were 0.03 and 0.10, respectively. Toxicity was graded using the Common Toxicity Criteria, version 2.0. Spearman rank correlations and the Jonckheere-Terpstra test were used to assess the associations between pathologic stage and factors, such as clinical stage and PSA. The Wilcoxon signed rank test and Kendall’s τ were used to assess changes in immunologic, genetic, and angiogenic markers. All tests of statistical significance were two sided and no adjustment was made for multiple comparisons.

### Results

**Patient characteristics.** Between May 2003 and June 2006, 27 patients with either intermediate- or high-risk prostate cancer were enrolled on this trial. One patient withdrew consent and therefore is excluded from all analyses. Another patient (excluded from the tissue analysis) was found to have unresectable disease at the time of surgery. Radical prostatectomy was aborted, and the patient was treated with external beam radiation therapy. Table 1 summarizes patient and disease characteristics for the 26 analyzed patients with available pretreatment and posttreatment tissue specimens. The pretreatment serum PSA level ranged from 2.94 to 64.87 ng/mL. All patients had documented normal testosterone values at some time before undergoing radical prostatectomy. Median testosterone value for the entire cohort was 398 ng/dL (range, 174-628 ng/dL). At the time of initial digital rectal examination, almost half of the patients (52%) had T1c tumors meeting...
study eligibility by having either a high Gleason score (42%) or a high PSA value (≥10; 19%). Three patients (12%) had T3 tumors or greater. Eighteen patients (67%) had a pretreatment biopsy Gleason score of 8 or 9. No Gleason score 10 biopsies were observed. When using the preoperative Kattan nomogram, 13 (48%) patients had a >35% risk of biochemical failure at 5 years.

Pathologic responses. Although neoadjuvant therapy with GM-CSF and thalidomide resulted in a significant decrease in PSA levels, there were no pathologic complete responses. Of the 26 radical prostatectomy specimens available for pathologic analysis, 11 patients (42%) had organ-confined disease (pT2b or pT2b), and 15 patients (58%) had either extraprostatic extension (5 of 17) or seminal vesicle invasion (9 of 17). When the Gleason score from the pretreatment biopsy was compared with the Gleason score from the posttreatment prostatectomy specimen, the prostatectomy-based Gleason score was three and two points lower in 1 patient each (4% and 4%), one point lower in 8 patients (30%), the same in 12 patients (44%), one point higher in 3 patients (11%), and two points higher in 2 patients (7%).

When we evaluated the correlation between clinical and pathologic stage with different PSA levels (diagnostic PSA, posttreatment PSA level, and absolute and percentage change in PSA level induced by therapy), the only factor associated with surgical outcome (pathologic stage) was clinical stage (Spearman \( r = 0.39; P = 0.04 \)); however, it is perhaps worth noting that, with the exception of the two patients with clinical stage T3 disease, the pathologic stage was always higher than the clinical stage.

PSA responses. The vast majority of patients (81%) achieved a PSA decline while on therapy. The changes in PSA for the entire cohort are summarized graphically in Fig. 1. Five patients (18%) had an increase in their PSA value that, at its maximum during treatment, ranged from 3% to 53%. Only one patient achieved a PSA response (PSA decline ≥50% compared with baseline value). Of the remaining 21 patients, 10 (37%) and 11 (41%) experienced a decline in their PSA ranging from 20% to 48% and 2% to 16%, respectively. For all 27 patients, the overall mean ± SD change in PSA decline was 14.0 ± 22.1% (95% confidence interval, 5.3–22.7; \( P = 0.002, \) Wilcoxon signed rank test). Postoperatively, nadir PSA values of ≤0.2 ng/mL were observed in all but four patients who were initiated on androgen deprivation immediately after surgery. A statistically significant reduction in the pretreatment versus posttreatment mean PSA level was observed for the entire group (16.26 ± 14.30 ng/mL and 13.59 ± 13.51 ng/mL; \( P = 0.002, \) Wilcoxon signed rank test).

Biochemical failure outcomes. Of the 27 patients undergoing radical prostatectomy, 1 patient was found to have gross palpable disease invading the bladder, and therefore, his radical prostatectomy was aborted. This patient’s bilateral lymph node dissection, however, failed to show metastatic disease in nine of nine lymph nodes resected. The patient was initiated on androgen deprivation and subsequently received primary definitive external beam radiotherapy. Of the remaining 26 patients, 22 (84%) had undetectable PSA values 6 weeks after surgery. With a median follow-up of 32 months (range, 12–51 months), 5 of 26 patients (19%) have developed biochemical failure. Four patients failed to achieve an undetectable PSA value after radical prostatectomy and one patient developed disease progression by PSA and Response Evaluation Criteria in Solid Tumors criteria 17 months after surgery.

Systemic toxicities. Treatment with GM-CSF and thalidomide was generally well tolerated and caused mild to moderate toxicity. Twenty-four patients (89%) received all 8 planned weeks of treatment, and 3 patients discontinued therapy secondary to drug intolerance (see toxicity and safety data below). No dose adjustments were required for patients completing 8 weeks of therapy. Of the three patients who discontinued therapy, one patient experienced a significant grade 2 injection site reaction 2 weeks into his first cycle and was removed from the study. Similarly, a second patient developed grade 3 urticaria after 5 days of initiating therapy. This patient had a previous history of angioedema, and shortly after initiating steroids, antihistamines, and H2 blockers, his symptoms subsided.

A third patient with borderline platelet count at the time of study enrollment developed grade 1 thrombocytopenia after completing one cycle of GM-CSF and thalidomide. After a complete hematologic workup, he was diagnosed with idiopathic thrombocytopenia purpura and then was removed from study. Four weeks after treatment discontinuation, his platelets normalized. The patient subsequently underwent uneventful radical prostatectomy. Overall toxicity consisted of mostly grade 1/2 constipation (67%), grade 1/2 injection site reaction (59%), grade 1 peripheral neuropathy (26%), grade 1 fatigue (48%), and somnolence/dizziness (33%). Other toxicities less commonly observed included taste changes (11%) and peripheral lower extremity edema (11%). No significant changes in WBC counts were observed. One patient developed a grade 3 deep vein thrombosis after completing 8 weeks of therapy. Patient was initiated on anticoagulation and had an inferior vena cava filter placed. Subsequently, this patient had an uneventful surgery.

Surgical morbidity. The median operative time (skin incision to skin closure) was similar to that observed in previous neoadjuvant trials conducted at our institution (10). The estimated blood loss ranged from 250 to 2,900 mL (mean, 1,233 mL). A cell salvage device was used for intraoperative autotransfusion in the vast majority of patients, and no patient
received a transfusion of nonautologous blood. Similar to previous neoadjuvant docetaxel reports, marked evidence of periprostatic fibrosis after the administration of GM-CSF and thalidomide was observed, which increased the difficulty of resection compared with untreated patients (10, 28). The transurethral catheter was removed within 2 to 3 weeks after surgery (range, 14-24 days). All patients undergoing surgery in our study became continent, and only four patients have required protection for mild stress incontinence after 6 months from radical prostatectomy. In this study, the mean time to continence was 11.9 weeks (range, 4-32 weeks), which is similar to that previously reported in other neoadjuvant trials. As of today, only two patients have required urethral dilations for bladder neck contractures. No intraoperative or immediate postoperative complications developed and no operative mortalities occurred.

**Biological effects.** Tumor tissue changes in prostate cancer tumors treated with GM-CSF and thalidomide were assessed by immunohistochemical analysis from constructed TMA from radical prostatectomy and PBx tumor tissues. Marker selection was determined based on the proposed mechanism of action for GM-CSF and thalidomide. Evaluated markers included Ki-67, PTEN, CD3, CD68, S100, and CD31. Immunohistochemical data comparing PBx and radical prostatectomy tumor tissue are summarized in Tables 2 and 3. Twenty-four patients have matched pretreatment and posttreatment tumor tissue material available for analysis. Two patients did not have their PBx available for further analysis. Nineteen of 24 patients (79%) had available matched pretreatment and posttreatment immunohistochemistry for CD31. As shown in Fig. 2, the proportion of cells expressing CD3 and S100 in the radical prostatectomy specimens was significantly greater than in the PBx specimens (medians: CD3, 20% versus 5%; S100, 5% versus 1.5%; \( P = 0.01 \) in both cases). There were no significant differences in the radical prostatectomy versus PBx tumor tissue expression of CD68 or Ki-67. Microscopically, however, an increased in the proportion of tumor-associated macrophages (CD68+) was observed in most of the posttreatment radical prostatectomy tissue analyzed (Figure 3).

In an effort to differentiate thalidomide-induced tumor tissue effects from those from GM-CSF, we attempted to look at several antiangiogenic tissue markers that agents such as thalidomide are known to target. We elected to analyze pretreatment and posttreatment expression of CD31, a transmembrane protein that plays a role in adhesive interactions between adjacent endothelial cells and leukocytes and is affected by the administration of thalidomide and not of GM-CSF. Although only a limited number of samples could be analyzed for CD31 expression, no difference between pretreatment and posttreatment tissue samples was observed.

Serum immune changes were evaluated in six patients. Serum levels of dendritic cell increased after 8 weeks of treatment with GM-CSF and thalidomide. Similarly, the mean amount of basic fibroblast growth factor decreased when compared with baseline. The mean amount of IL-8 and VEGF (pg/mL) increased after 8 weeks of treatment (data not shown). Likewise, despite a transient decline in the mean number of regulatory T cells (\( \times 10^6 \)) at 4 weeks, the mean count of regulatory T cells after treatment was increased. Levels of other cytokines measured, including IL-2, IL-4, IL-5, IL-10, IFN-γ, and tumor necrosis factor-α levels, seemed not to change after the administration of GM-CSF and thalidomide.

### Table 2. Tissue expression of immune and angiogenic markers (pretreatment versus posttreatment tissue specimens)

<table>
<thead>
<tr>
<th>Biopsy specimen</th>
<th>Radical prostatectomy specimen</th>
<th>Difference*</th>
<th>( P ) 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Ki-67</td>
<td>26</td>
<td>25</td>
<td>10.0 (1.0-80.0)</td>
</tr>
<tr>
<td>% CD3</td>
<td>25</td>
<td>26</td>
<td>5.0 (0.0-60.0)</td>
</tr>
<tr>
<td>% CD68</td>
<td>26</td>
<td>26</td>
<td>30.0 (2.0-80.0)</td>
</tr>
<tr>
<td>% S100</td>
<td>24</td>
<td>25</td>
<td>1.5 (0.0-15.0)</td>
</tr>
<tr>
<td>CD31*</td>
<td>22</td>
<td>24</td>
<td>8.0 (0.4-41.2)</td>
</tr>
</tbody>
</table>

*Prostatectomy specimen minus biopsy specimen.

1 Wilcoxon signed rank test.

**Discussion**

The results of our experience using GM-CSF and thalidomide as immunomodulatory and/or antiangiogenic agents to show that tumor microenvironment changes can translate into clinical benefit in prostate cancer patients can be dissected in two ways. Clinically, our study continues to show the ability of these two agents to induce PSA declines in patients with different states of prostate cancer. In the current study, only 1 of 27 patients achieved a PSA response (PSA decline ≥50% from baseline), whereas >78% of patients receiving therapy had some PSA decline ranging from 20% to 48% (\( n = 10 \)) and 6% to 16% (\( n = 11 \)) from baseline.

Although the clinical significance of having a PSA decline before radical prostatectomy remains unknown, our rate of PSA decline mirrored what has been reported when using neoadjuvant docetaxel-based chemotherapy (9–12).

Similar to other reported neoadjuvant trials using androgen deprivation and cytotoxic chemotherapy, the combination of GM-CSF and thalidomide failed to produce histologic evidence of pathologic responses. To date, five patients (19%) have manifested evidence of biochemical (PSA) failure. Although this rate of failure is relatively low when compared with other high-risk radical prostatectomy databases, no conclusions can be drawn from this observation. Although our sample size is too small for definitive analysis, pathologic changes observed
and surgical outcome was found. Similarly, no correlation between percentage of tumor-associated macrophage infiltration, PSA, postradical prostatectomy specimens. No correlation between posttreatment tissue samples, although microscopically greater not statistically different between our pretreatment and not allow us to define any potential contribution that within the tumor tissue (31). Additionally, the study design did not FACT restores recombinant epidermal growth factor expression and promotes androgen and epithelial growth factor regulation. Compelling data from Rubenstein et al. PSA decline observed was due to a decline of circulating testosterone levels. Despite of this, it is unlikely that the rate of "treatment effect" on the prostate epithelium and likely to be comparable with what is routinely observed in untreated men undergoing radical prostatectomy.

Biologically, our study has the strength of having matched pretreatment and posttreatment tumor tissue specimens used to determine immunohistochemical overexpression patterns of markers thought to be the target for GM-CSF and thalidomide. Among those markers tested, significant overexpression of T cells (CD3+) and activated dendritic cells (S100+) within prostatic tumor tissue was observed after 8 weeks of therapy. In contrast to the report of Shimura and colleagues (29), overexpression of tumor-associated macrophages (CD68+) was not statistically different between our pretreatment and posttreatment tissue samples, although microscopically greater tumor-associated macrophage infiltration was observed in all postradical prostatectomy specimens. No correlation between percentage of tumor-associated macrophage infiltration, PSA, and surgical outcome was found. Similarly, no correlation between CD3+ and S100+ overexpression and clinical outcome (PSA, pCR, and PSA failure) was observed. Despite of the lack of clinical correlation with the tissue end points of this study, we believe that our immunohistochemical findings correlate with the biological effects of GM-CSF on dendritic cells and T cells previously described in prostate cancer patients (30). Notwithstanding, consideration should be given to potential nonimmunologic mechanism(s) responsible for the PSA effects observed in several of our past and current clinical trials, such as GM-CSF stimulation of tumor-associated macrophage production of antiangiogenic proteins or effects on bone marrow stromal cells, which could influence the development of prostate cancer metastases.

In addition of this being a single-arm, single-institution trial, another limitation of this report is that testosterone levels were collected once during study; thus, we were unable to establish the effects of GM-CSF and thalidomide on posttreatment testosterone levels. Despite of this, it is unlikely that the rate of PSA decline observed was due to a decline of circulating testosterone levels. Compelling data from Rubenstein et al. using the Dunning prostate cancer model show that GM-CSF in fact restores recombinant epidermal growth factor expression and promotes androgen and epithelial growth factor regulation within the tumor tissue (31). Additionally, the study design did not allow us to define any potential contribution that thalidomide may have had to the tissue findings reported. Based on the proposed mechanism of action of this agent, it is conceivable that the observed CD3+ tumor tissue overexpression could be the result of synergistic activity of the combination of GM-CSF and thalidomide. However, there is no supporting data showing that thalidomide activates dendritic cells within the epithelial/stromal compartment. In fact, the treatment effects of single-agent thalidomide in prostate tumor tissue were recently reported by Efstathiou et al. (32). In their trial, 18 men with localized prostate cancer received different doses of single-agent thalidomide for 12 weeks before undergoing radical prostatectomy. TMA analyses indicated modulation of vascular marker expression (VEGF and IL-6) accompanied by a reduction in microvessel density in the treated group. Comparison of broader stromal-epithelial interaction markers (matrix metalloproteinase and E-cadherin ratio) between treated and control groups suggested a transition to a less aggressive phenotype as a result of thalidomide treatment.

In our trial, we also attempted to differentiate the activity of thalidomide from that of GM-CSF by evaluating immunohistochemical markers of angiogenesis, a process that would be mediated by thalidomide and not GM-CSF. Our immunohistochemical analysis for CD31+ did not reveal any differences in expression between pretreatment and radical prostatectomy specimens. Caution should be used when interpreting these data as we only had a small subset of samples available for testing. Similar to the experience of Efstathiou and colleagues, we did not observe any treatment effect in the proliferative index (Ki-67) or a change in pTEN status after treatment with GM-CSF and thalidomide.

Lastly, circulating levels of VEGF, basic fibroblast growth factor, IL-8, dendritic cells, regulatory T cells, and other cytokines seemed unaffected by the administration of GM-CSF and thalidomide. Elevation of mean VEGF levels during treatment suggests that thalidomide does not exert its function through inhibition of VEGF synthesis. Although provocative, spurious changes observed with IL-8, basic fibroblast growth after the administration of GM-CSF and thalidomide did not follow any specific pattern and were not correlated with PSA responses. These pathologic variations, which are also observed in other neoadjuvant studies, may be the result of "treatment effect" on the prostate epithelium and likely to be comparable with what is routinely observed in untreated men undergoing radical prostatectomy.

Biologically, our study has the strength of having matched pretreatment and posttreatment tumor tissue specimens used to determine immunohistochemical overexpression patterns of markers thought to be the target for GM-CSF and thalidomide. Among those markers tested, significant overexpression of T cells (CD3+) and activated dendritic cells (S100+) within prostatic tumor tissue was observed after 8 weeks of therapy. In contrast to the report of Shimura and colleagues (29), overexpression of tumor-associated macrophages (CD68+) was not statistically different between our pretreatment and posttreatment tissue samples, although microscopically greater tumor-associated macrophage infiltration was observed in all postradical prostatectomy specimens. No correlation between percentage of tumor-associated macrophage infiltration, PSA, and surgical outcome was found. Similarly, no correlation between CD3+ and S100+ overexpression and clinical outcome (PSA, pCR, and PSA failure) was observed. Despite of the lack of clinical correlation with the tissue end points of this study, we believe that our immunohistochemical findings correlate with the biological effects of GM-CSF on dendritic cells and T cells previously described in prostate cancer patients (30). Notwithstanding, consideration should be given to potential nonimmunologic mechanism(s) responsible for the PSA effects observed in several of our past and current clinical trials, such as GM-CSF stimulation of tumor-associated macrophage production of antiangiogenic proteins or effects on bone marrow stromal cells, which could influence the development of prostate cancer metastases.

In addition of this being a single-arm, single-institution trial, another limitation of this report is that testosterone levels were collected once during study; thus, we were unable to establish the effects of GM-CSF and thalidomide on posttreatment testosterone levels. Despite of this, it is unlikely that the rate of PSA decline observed was due to a decline of circulating testosterone levels. Compelling data from Rubenstein et al. using the Dunning prostate cancer model show that GM-CSF in fact restores recombinant epidermal growth factor expression and promotes androgen and epithelial growth factor regulation within the tumor tissue (31). Additionally, the study design did not allow us to define any potential contribution that thalidomide may have had to the tissue findings reported. Based on the proposed mechanism of action of this agent, it is conceivable that the observed CD3+ tumor tissue overexpression could be the result of synergistic activity of the combination of GM-CSF and thalidomide. However, there is no supporting data showing that thalidomide activates dendritic cells within the epithelial/stromal compartment. In fact, the treatment effects of single-agent thalidomide in prostate tumor tissue were recently reported by Efstathiou et al. (32). In their trial, 18 men with localized prostate cancer received different doses of single-agent thalidomide for 12 weeks before undergoing radical prostatectomy. TMA analyses indicated modulation of vascular marker expression (VEGF and IL-6) accompanied by a reduction in microvessel density in the treated group. Comparison of broader stromal-epithelial interaction markers (matrix metalloproteinase and E-cadherin ratio) between treated and control groups suggested a transition to a less aggressive phenotype as a result of thalidomide treatment.

In our trial, we also attempted to differentiate the activity of thalidomide from that of GM-CSF by evaluating immunohistochemical markers of angiogenesis, a process that would be mediated by thalidomide and not GM-CSF. Our immunohistochemical analysis for CD31+ did not reveal any differences in expression between pretreatment and radical prostatectomy specimens. Caution should be used when interpreting these data as we only had a small subset of samples available for testing. Similar to the experience of Efstathiou and colleagues, we did not observe any treatment effect in the proliferative index (Ki-67) or a change in pTEN status after treatment with GM-CSF and thalidomide.

Lastly, circulating levels of VEGF, basic fibroblast growth factor, IL-8, dendritic cells, regulatory T cells, and other cytokines seemed unaffected by the administration of GM-CSF and thalidomide. Elevation of mean VEGF levels during treatment suggests that thalidomide does not exert its function through inhibition of VEGF synthesis. Although provocative, spurious changes observed with IL-8, basic fibroblast growth

### Table 3. PTEN expression in patients with paired samples

<table>
<thead>
<tr>
<th>Prostate specimen</th>
<th>Positive</th>
<th>Mixed</th>
<th>Negative</th>
<th>$p^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biopsy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1 (17%)</td>
<td>4 (67%)</td>
<td>1 (17%)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>3 (27%)</td>
<td>5 (45%)</td>
<td>3 (27%)</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0 (0%)</td>
<td>4 (57%)</td>
<td>3 (43%)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

$^*$Test of Kendall’s $\tau$; percentages are the percents within each row.

![Fig. 2. Tumor tissue expression of Ki-67, CD3, CD68, CD31, and S100 in matched biopsy and prostatectomy specimens. Columns, mean; bars, SE. Proportion of cells staining positive for each specific marker, comparing pretreatment and posttreatment specimens. Only tissue expression of T cells and macrophages (CD3 and CD68, respectively) was increased by the administration of GM-CSF and thalidomide.](image-url)
factor, and dendritic cells may not necessarily reflect tumor tissue effects of GM-CSF and thalidomide.

**Conclusions**

Neoadjuvant administration of GM-CSF and thalidomide in patients with locally advanced prostate cancer induces a PSA decline in the vast majority of individuals. This combination, however, does not seem to affect pathologic and/or biochemical outcome. Although limited by the sample size, our tissue immunohistochemical and blood immune assays suggest that the clinical activity observed with these two agents in different prostate cancer states may be the result of immunomodulation within the prostate tumor microenvironment.

**Disclosure of Potential Conflicts of Interest**

J.A. Garcia has served research as a consultant for Colgene and currently receives research funding from Colgene. R. Dreicer receives research funding from Colgene.
GM-CSF and Thalidomide for High-Risk Localized Prostate Cancer

References

Clinical Cancer Research

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