C-reactive Protein as a Prognostic Marker for Men With Androgen-independent Prostate Cancer

Results From the ASCENT Trial

Tomasz M. Beer, MD1
Alshad S. Lalani, PhD2
Stella Lee, MS2
Motomi Mori, PhD3
Kristine M. Eilers, BS1
John G. Curd, MD4
W. David Henner, MD, PhD4
Christopher W. Ryan, MD1
Peter Venner, MD5
J. Dean Ruether, MD6
Kim N. Chi, MD7
and the ASCENT Investigators

1 Division of Hematology & Medical Oncology, Oregon Health & Sciences University, Portland, Oregon.
2 Preclinical & Transitional Oncology, Novacea Inc., South San Francisco, California.
3 Biostatistics Shared Resource, OHSU Cancer Institute, Oregon Health & Science University, Portland, Oregon.
4 Novacea Inc., South San Francisco, California.
5 Cross Cancer Institute, Alberta Cancer Board, Edmonton, Alberta, Canada.
6 Tom Baker Cancer Centre, Alberta Cancer Board, Calgary, Alberta, Canada.
7 Division of Medical Oncology, University of British Columbia, Cancer Agency-Vancouver Centre, Vancouver, British Columbia, Canada.

BACKGROUND. Studies of cancer risk and molecular carcinogenesis suggest a role for inflammation in cancer development and progression. The authors sought to determine whether specific blood proteins associated with inflammation predict for outcomes in men with metastatic androgen-independent prostate cancer (AIPC) who are initiating docetaxel-based chemotherapy.

METHODS. Baseline plasma samples were stored (~80°C) from 160 of 250 patients enrolled in the AIPC Study of Calcitriol ENhancing Taxotere (ASCENT) trial, a randomized, placebo-controlled trial comparing weekly docetaxel plus high-dose calcitriol with weekly docetaxel. Multiplex immunoassays measured 16 cytokine, chemokine, cardiovascular, or inflammatory markers. The Cox proportional hazards model was used to assess associations between baseline biomarkers, clinical characteristics, and survival. Logistic regression was used for analyses of associations with prostate-specific antigen (PSA) decline.

RESULTS. C-reactive protein (CRP) was found to be significantly predictive of a shorter overall survival (hazards ratio [HR] of 1.41 for each natural logarithm [ln] [CRP] increase; 95% confidence interval [95% CI], 1.20–1.65 [P < .0001]). When CRP (continuous) was entered into a multivariate model using 13 baseline clinical variables, only elevated CRP remained a significant predictor (P < .0001) of shorter overall survival. When categorized as normal (<8 mg/L) or abnormal (>8 mg/L), elevated CRP was found to be a significant predictor of shorter overall survival (HR of 2.96; 95% CI, 1.52–5.77 [P = .001]). When CRP was also associated with a lower probability of PSA decline (odds ratio of 0.74 for each ln(CRP) increase; 95% CI, 0.60–0.92 [P = .007]).

CONCLUSIONS. Elevated plasma CRP concentrations appear to be a strong predictor of poor survival and lower probability of PSA response to treatment in patients with AIPC who are receiving docetaxel-based therapy. Cancer 2008;112:2377–83. © 2008 American Cancer Society.

KEYWORDS: C-reactive protein, prostate cancer, prognostic factors, inflammation.

Docetaxel-containing chemotherapy prolongs survival for men with metastatic androgen-independent prostate cancer (AIPC) and has become the standard treatment in this setting.1,2 Considerable heterogeneity characterizes both the responses to treatment and the survival benefits of this therapy.3,4 The current study was designed to assess the potential of inflammatory markers to predict for response to docetaxel-containing chemotherapy in men with AIPC. The investigators aimed to identify specific blood proteins associated with inflammation that might predict for outcomes in men with AIPC who are initiating docetaxel-based chemotherapy.

The study included 250 patients enrolled in the AIPC Study of Calcitriol ENhancing Taxotere (ASCENT) trial, a randomized, placebo-controlled trial comparing weekly docetaxel plus high-dose calcitriol with weekly docetaxel. Baseline plasma samples were stored (~80°C) from 160 of the 250 patients enrolled in the trial. Multiplex immunoassays measured 16 cytokine, chemokine, cardiovascular, or inflammatory markers. The Cox proportional hazards model was used to assess associations between baseline biomarkers, clinical characteristics, and survival. Logistic regression was used for analyses of associations with prostate-specific antigen (PSA) decline.

The study found that C-reactive protein (CRP) was significantly predictive of a shorter overall survival (hazards ratio [HR] of 1.41 for each natural logarithm [ln] [CRP] increase; 95% confidence interval [95% CI], 1.20–1.65 [P < .0001]). When CRP (continuous) was entered into a multivariate model using 13 baseline clinical variables, only elevated CRP remained a significant predictor (P < .0001) of shorter overall survival. When categorized as normal (<8 mg/L) or abnormal (>8 mg/L), elevated CRP was found to be a significant predictor of shorter overall survival (HR of 2.96; 95% CI, 1.52–5.77 [P = .001]), as was hemoglobin (P = .007). Elevated CRP was also associated with a lower probability of PSA decline (odds ratio of 0.74 for each ln(CRP) increase; 95% CI, 0.60–0.92 [P = .007]).

The study concluded that elevated plasma CRP concentrations appear to be a strong predictor of poor survival and lower probability of PSA response to treatment in patients with AIPC who are receiving docetaxel-based therapy. Cancer 2008;112:2377–83. © 2008 American Cancer Society.

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and overall survival (OS) of patients treated with docetaxel-containing chemotherapy. The improved ability to more accurately predict individual survival and response to therapy would improve the ability to inform patients, clinical decision-making, interpretation of clinical trial results, and clinical trial design through proper stratification of patients by risk. To our knowledge to date, the most well-known efforts to develop prognostic models in AIPC have focused on multivariate analyses of clinically available variables such as age; performance status; Gleason score; the presence or absence of visceral disease; and of commonly available measurements of blood proteins such as lactate dehydrogenase (LDH), prostate-specific antigen (PSA) concentrations and kinetics, hemoglobin (HGB), and alkaline phosphatase. Such analyses have identified several independent clinical and biochemical predictors of survival and led to the development of useful nomograms.3,4

A growing body of evidence supports the hypothesis that both local and systemic inflammation play an important role in the development and progression of a variety of common solid tumors, likely through the paracrine actions of cytokines, chemokines, adhesion molecules, and mediators of angiogenesis generated by the inflammatory response to the tumor.5–8 Interleukin-6 (IL-6), tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF), and fibroblast growth factors (FGF) are among the inflammatory substances believed to contribute to the growth and progression of cancer.7–9 Indeed, the development of many cancers has been directly linked to specific infectious and inflammatory insults.6 The mechanism believed to be responsible for the effects of inflammation on cancer have recently been reviewed.10

To our knowledge the role of inflammation in prostate carcinogenesis and prostate cancer progression has not been fully elucidated, but evidence supporting this hypothesis is mounting. Prostatic inflammatory atrophy, a candidate early preneoplastic lesion, is associated with a prominent inflammatory component.11 Inflammatory T-cell infiltrates accompany prostate cancer.12 Autoantibodies to prostate cancer antigens are frequently detectable in prostate cancer patients. A panel testing for prostate cancer autoantibodies has recently been shown to be a sensitive and specific test with which to distinguish between men who do from those who do not harbor prostate cancer.13

Although not universally consistent, epidemiologic studies that examine prostate cancer risk and use of nonsteroidal antiinflammatory drugs (NSAIDs) suggest that inflammation may be a modifiable risk factor for the development of clinically evident prostate cancer.14–16

Specific inflammatory cytokines as well as non-specific measures of systemic inflammation such as C-reactive protein (CRP), albumin, and HGB have been found to be strongly correlated with prognosis in patients with a broad variety of common cancers.17

We hypothesized that inflammation is associated with shorter OS and a lower probability of PSA decline in AIPC patients initiating docetaxel-based chemotherapy. Furthermore, we hypothesized that the measurement of blood markers of inflammation would yield novel prognostic and predictive factors in advanced prostate cancer. We tested these hypotheses using samples and clinical outcomes data from the ASCENT (AIPC Study of Calcitriol ENHancing Taxotere) study, a double-blind randomized phase 2 study to evaluate the efficacy and safety of high-dose calcitriol plus weekly docetaxel compared with placebo plus weekly docetaxel in AIPC. Based on our exploratory study of 16 different cytokine, chemokine, or inflammatory markers from 160 patients participating in ASCENT, we report that CRP, a readily available measure of systemic inflammation in the blood, is a significant predictor of OS and PSA decline in response to docetaxel-based chemotherapy in men with metastatic AIPC.

MATERIALS AND METHODS

Patients

Detailed eligibility criteria and the treatment regimen have been previously described.18 Briefly, men with metastatic AIPC and no prior chemotherapy received DN-101 (45 μg of calcitriol) or placebo by mouth on Day 1 followed by docetaxel at a dose of 36 mg/m2 intravenously on Day 2 along with dexamethasone (4 mg administered orally 12 hours before, 1 hour before, and 12 hours after docetaxel administration). This regimen was administered weekly for 3 consecutive weeks of a 4-week cycle. Gonadotropin-releasing hormone (GnRH) agonists were continued in those patients who had not undergone orchiectomy. Institutional Review Board approval was obtained for all participating institutions and informed consent was obtained from all patients contributing samples.

Sample Handling and Assays

Blood samples were collected before therapy from 160 patients enrolled in the ASCENT study. Plasma was separated by centrifugation at 3000 revolutions per minute (rpm), stored at ~80°C, and shipped frozen to a central repository. Multiplex immunoassays (Linco Research, St. Charles, Mo) using uni-
quely labeled fluorescent microspheres conjugated to anticytokine capture antibodies were used to simultaneously measure IL-1α, IL-1β, IL-2, IL-6, IL-8, IL-10, TNF-α, monocyte chemotactic peptide-1 (MCP-1), epidermal growth factor (EGF), VEGF, plasminogen activator inhibitor-1 (PAI-1), matrix metalloproteinase-9 (MMP-9), soluble E selectin (sE-Selectin), soluble intracellular adhesion molecule (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1), and CRP from blinded plasma samples according to the manufacturer’s recommendation. After a conventional sandwich immunoassay, beads were washed of unbound or excess proteins and read on a Luminex100 system (Austin, Tex) to determine the concentration of the biomarker of interest. When appropriate, samples were diluted to be accurately measured within the dynamic range of the assay. Values below the lower limit of detection or above the upper limit of detection were set to the lower limit or upper limit, respectively. All samples were tested in duplicate and mean values were used for all analyses.

**Statistical Methods**

The efficacy endpoints of interest were OS and overall confirmed PSA decline.19 OS was defined as the time from randomization to death from any cause. PSA decline was defined as a ≥50% reduction from baseline PSA that was confirmed by another measurement at least 28 days later. OS was censored at 78 weeks for all subjects. Because of the skewed distribution of raw biomarker data, a natural logarithmic transformation was applied to all values to achieve a more normal distribution before inclusion in the models. A proportional hazards model was used to assess the correlation between the baseline biomarkers and clinical covariates on OS whereas logistic regression was used to assess the correlation between the baseline biomarkers and PSA decline. Variables in both models were selected using a backwards selection method with an α level of 0.05. Receiver operator curves (ROCs) for OS at 78 weeks were plotted using the method of Heagerty et al.20

**RESULTS**

**Patient Demographics**

Two hundred and fifty patients were randomized at 48 sites from the U.S. and Canada between September 2002 and January 2004. Samples adequate for analysis obtained before treatment were available from 160 of these patients. Baseline characteristics were similar to those of the 90 patients without samples, except for a somewhat lower age (mean of 68.0 years vs 70.6 years), higher baseline HGB (12.8 g/dL vs 12.2 g/dL), and race (African-American 7.5% vs 18.9%) in the patients with samples available. Baseline characteristics of the 2 groups are shown in Table 1. All remaining analyses refer to the 160 patients who provided blood samples.

**Biomarkers and OS**

Each of the measured biomarkers was included in a multivariate Cox proportional hazards model to determine the extent to which the level of the biomarker was predictive of OS. Of the 16 analyses tested, CRP was the only biomarker found to be in-
dependently associated with OS (hazards ratio [HR] of 1.41; 95% confidence interval [95% CI], 1.19–1.64; \( P < .0001 \)) (Table 2). This translates to a 41% increase in hazards of mortality for each 2.72-fold increase in CRP or a 27% increase in the hazards of mortality for each doubling of the CRP.

When categorized as normal (\(<8 \text{ mg/L}\)) or elevated (\(>8 \text{ mg/L}\)), an elevated CRP plasma level was determined to be a significant predictor of shorter OS (adjusted HR of 2.96; 95% CI, 1.52–5.77 \( P = .001 \)), as was HGB (\( P = .007 \)). The impact of an elevated baseline CRP on OS is shown in Figure 1.

It is interesting to note that the distribution of CRP concentrations in the current study patients, as shown in Figure 2, was broad and skewed. Quartile 1 ranged from 0.2 to 4.2 mg/L, quartile 2 ranged from 4.2 to 12.7 mg/L, quartile 3 ranged from 12.7 to 43.5 mg/L, and quartile 4 ranged from 43.5 to 500 mg/L (Fig. 2) and 64% of these men had elevated CRP values (\(>8 \text{ mg/L}\)).

CRP levels at baseline were correlated with Eastern Cooperative Oncology Group (ECOG) performance status (PS). The median CRP in patients with an ECOG PS of 0 was 6.9 mg/L and was 27.6 mg/L in patients with an ECOG PS \(>0 \) (\( P < .0001 \)). We therefore sought to determine whether CRP adds to established clinical prognostic factors. We performed a multivariate analysis that included 13 potential baseline prognostic markers including serum PSA, serum LDH, serum alkaline phosphatase, blood HGB concentration, ECOG PS, race, age, the presence or absence of bone metastases, the presence or absence of measurable disease, prior prostatectomy, opioid analgesic use, and prior skeletal-related events, which were examined in a Cox proportional hazards model in addition to CRP. In the absence of CRP from this model, baseline ECOG PS (0 vs 1 or 2) (HR of 1.94;
Biomarkers and Response to Therapy
PSA decline, defined as a ≥50% reduction that was confirmed ≥28 days later, was achieved in 65% of this group of 160 patients. From the 16 biomarkers analyzed in the baseline samples of these patients, CRP remained as the only predictor of a PSA decline, with statistical significance in the final model (odds ratio [OR] of 0.74; 95% CI, 0.60–0.92 [P = .007]) (Table 3). This translates to a 26% reduction in odds of a PSA decline for every 2.72-fold increase in baseline serum CRP. This effect can also be expressed as a 19% decrease in the odds of PSA decline for each doubling in baseline serum CRP. When patients were analyzed by quartile of CRP (n = 40 in each group), 80% of patients whose CRP was in the lowest quartile had a PSA decline in response to therapy, whereas only 53% of patients whose CRP was in the highest quartile met criteria for PSA decline. Quartiles 2 and 3 were associated with PSA decline rates of 68% and 60%, respectively.

DISCUSSION
CRP is a readily measurable blood marker of inflammation, proved to be a strong predictor of OS and PSA decline in this group of men with metastatic AIPC who were initiating a docetaxel-containing chemotherapy regimen. Indeed, it proved more predictive than conventional prognostic and predictive factors.

CRP has previously been described as being predictive of survival in several advanced malignancies including melanoma; non-Hodgkin lymphoma; and ovarian, colorectal, and pancreatic cancers as well as in multiple myeloma. In a recent study of 62 men with metastatic prostate cancer receiving androgen-deprivation therapy, elevated CRP concentrations were found to be independently predictive of shorter cancer-specific survival, although the association fell short of statistical significance in this small sample (HR of 1.97; 95% CI, 0.99–3.92 [P = .052]). Herein we demonstrated that in addition to predicting poor OS, elevated CRP levels (>8 mg/L) were associated with a lower probability of PSA decline during docetaxel-based therapy in men with AIPC. To our knowledge, this is the first clinical evidence linking systemic inflammation with poor OS as well as a lower probability of response to chemotherapy in men with AIPC.

To our knowledge, the biologic basis for the correlation between elevations in this general marker of systemic inflammation and disease risk and outcome are not completely understood. The liberation of multiple proinflammatory cytokines, including IL-1, IL-6, and TNF-α from the tumor microenvironment eventually results in the induction of CRP synthesis from the liver and other tissues. As a prototypical acute phase protein, CRP levels rise rapidly in response to inflammation and may regulate the innate immune response by activating complement, engaging fragment crystallizable region (Fc) receptors to activate phagocytosis, or in stimulating the pro-

**TABLE 3**

Logistic Regression of Baseline Biomarkers on Overall PSA Decline to Docetaxel-based Therapy

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Estimate (SD)</th>
<th>Chi-square statistic</th>
<th>P</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP, ng/mL</td>
<td>−0.39 (0.14)</td>
<td>8.44</td>
<td>.0037</td>
<td>0.676 (0.518–0.880)</td>
</tr>
<tr>
<td>EGF, pg/mL</td>
<td>−0.55 (0.25)</td>
<td>8.82</td>
<td>.0281</td>
<td>0.577 (0.354–0.943)</td>
</tr>
<tr>
<td>IL-10, pg/mL</td>
<td>−0.12 (0.22)</td>
<td>0.27</td>
<td>.6044</td>
<td>0.690 (0.577–1.375)</td>
</tr>
<tr>
<td>IL-1a, pg/mL</td>
<td>0.21 (0.10)</td>
<td>4.05</td>
<td>.0441</td>
<td>1.233 (1.006–1.512)</td>
</tr>
<tr>
<td>IL-1b, pg/mL</td>
<td>0.09 (0.34)</td>
<td>0.07</td>
<td>.7877</td>
<td>1.096 (0.561–2.143)</td>
</tr>
<tr>
<td>IL-2, pg/mL</td>
<td>−0.27 (0.26)</td>
<td>1.06</td>
<td>.3033</td>
<td>0.763 (0.455–1.277)</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
<td>0.35 (0.23)</td>
<td>2.30</td>
<td>.1295</td>
<td>1.421 (0.902–2.240)</td>
</tr>
<tr>
<td>IL-8, pg/mL</td>
<td>−0.60 (0.27)</td>
<td>4.86</td>
<td>.0274</td>
<td>0.549 (0.323–0.936)</td>
</tr>
<tr>
<td>MCP-1, pg/mL</td>
<td>−0.42 (0.40)</td>
<td>1.10</td>
<td>.2932</td>
<td>0.659 (0.303–1.434)</td>
</tr>
<tr>
<td>MMP-9, pg/mL</td>
<td>0.05 (0.29)</td>
<td>0.03</td>
<td>.8567</td>
<td>1.054 (0.598–1.855)</td>
</tr>
<tr>
<td>TNF-α, pg/mL</td>
<td>0.46 (0.38)</td>
<td>1.49</td>
<td>.2224</td>
<td>1.580 (0.758–3.297)</td>
</tr>
<tr>
<td>VEGF, pg/mL</td>
<td>0.57 (0.24)</td>
<td>5.69</td>
<td>.0170</td>
<td>1.775 (1.108–2.844)</td>
</tr>
<tr>
<td>sE-Selectin, ng/mL</td>
<td>−0.03 (0.50)</td>
<td>&lt;0.01</td>
<td>.9549</td>
<td>0.972 (0.366–2.582)</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
<td>0.08 (0.70)</td>
<td>1.52</td>
<td>.2172</td>
<td>0.432 (0.108–1.660)</td>
</tr>
<tr>
<td>sVCAM-1, ng/mL</td>
<td>−0.70 (0.86)</td>
<td>0.65</td>
<td>.4191</td>
<td>0.499 (0.092–2.694)</td>
</tr>
<tr>
<td>PAI-1, pg/mL</td>
<td>0.88 (0.44)</td>
<td>3.91</td>
<td>.0481</td>
<td>2.400 (1.007–5.718)</td>
</tr>
</tbody>
</table>

PSA indicates prostate-specific antigen; SD, standard deviation; OR, odds ratio; CI, 95% confidence interval; CRP, C-reactive protein; EGF, epidermal growth factor; IL, interleukin; MCP-1, monocyte chemotactic peptide-1; MMP-9, matrix metalloproteinase-9; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; sE-Selectin, soluble E selectin; sICAM-1, soluble intracellular adhesion molecule; sVCAM-1, soluble vascular cell adhesion molecule-1, PAI-1, plasminogen activator inhibitor-1.

**a** Logistic regression was used to assess the impact of baseline biomarkers on overall PSA response using a backward elimination method and an α of 0.05. PSA response was defined as a ≥50% reduction from baseline PSA that was confirmed ≥28 days later.

**1** Natural logarithm transformation was applied to all baseline biomarker measurements.
duction of additional proinflammatory cytokines to further enhance the inflammatory response.30,32

Others have previously shown that higher serum IL-6 concentrations were predictive of shorter survival and lower probability of response to docetaxel in patients with AIPC.33,34 In our analysis, IL-6 was not found to be an independent predictive or prognostic factor, although baseline IL-6 levels were found to be weakly correlated with baseline levels of CRP (R² = 0.029, P = .03, data not shown). The plasma half-life of CRP is approximately 19 hours,35 whereas the half-life of IL-6 is <6 hours.36 It is possible that differences in sample handling limited our ability to assess the more volatile cytokines without affecting the more stable CRP. It is also possible that CRP more reliably integrates the overall inflammatory state than the measurement of any individual cytokine. Therefore, CRP may not only be a stronger predictor of outcome, but a more practical measure that could be implemented into practice, even in settings in which immediate sample handling and freezing may not be realistic.

The current study has several limitations. A sample size of 160 is modest for an analysis of prognostic markers in this patient population. The study was an exploratory and retrospective effort and the results should be confirmed using an independent sample set from patients with AIPC. We are pursuing confirmatory analyses using samples and clinical data from prostate cancer studies performed within the Southwest Oncology Group. Lastly, because of the limited stability of cytokines, our analysis of soluble proteins from frozen plasma samples may not exclude the potential prognostic value of other circulating biomarkers.

If confirmed, CRP could prove to be a useful and readily measurable prognostic marker that could aid in clinical decision-making, patient counseling, and clinical trial design and interpretation. Furthermore, elevated CRP could provide us with vital insight into the fundamental role of inflammation in the progression of advanced prostate cancer. A better understanding of this process could provide us with novel therapeutic interventions for control of this disease and its symptoms.

REFERENCES


