The Prognostic Significance of Plasma Interleukin-6 Levels in Patients with Metastatic Hormone-Refractory Prostate Cancer: Results from Cancer and Leukemia Group B 9480

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ABSTRACT

Interleukin-6 signaling can activate androgen receptor in a ligand-independent manner and may play an important functional role in hormone-refractory prostate cancer (HRCaP) progression and patient survival. Plasma and serum IL-6 levels have been associated with prostate cancer progression in several small studies. In order to evaluate its prognostic significance in metastatic HRCaP patients, we measured IL-6 in plasma collected at baseline from patients in a large cooperative group study [Cancer and Leukemia Group B 9480 (CALGB 9480)].

Methods: **191 patients entered on CALGB 9480 had pretreatment plasma collected and centrally stored. Using a human IL-6 immunoassay, quantitative levels of IL-6 were measured in duplicate on 300 μL samples. The proportional hazard model was used to assess the prognostic significance of IL-6 in predicting overall survival.**

Results: **Median IL-6 level for the cohort of 191 patients was 4.80 pg/mL. Survival time among patients with IL-6 levels less than or equal to the median was 19 months (95% CI, 17-22) compared with 11 (95% CI, 8-14) months for patients above the median (P = 0.0004). In multivariate analysis, adjusting on performance status, lactate dehydrogenase, and prostate-specific antigen level, the hazard ratio was 1.38 (95% CI, 1.01-1.89; P = 0.043) using the median level as a cut point. Furthermore, a cut point of 13.31 pg/mL revealed robust prognostic significance with a hazard ratio of 2.02 (95% CI, 1.36-2.98; P = 0.0005).**

Conclusions: **Plasma IL-6 level has prognostic significance in patients with metastatic HRCaP from CALGB 9480. These findings support using IL-6 levels in prognostic models and support the rationale for IL-6-targeted therapy in patients with HRCaP.**

INTRODUCTION

Hormone-refractory prostate cancer (HRCaP) is a uniformly fatal disease accounting for an estimated 28,900 deaths annually in the United States, with a median survival slightly over 1 year (1–4) that can vary significantly based on known prognostic factors (5, 6). Currently established prognostic factors for patients with HRCaP qualitatively reflect either their overall tumor burden including serum levels of prostate-specific antigen (PSA), alkaline phosphatase, lactate dehydrogenase (LDH), and bone scan findings, or the overall condition of the patient as a host, including their performance status, plasma hemoglobin level, or history of weight loss. However, biological behavior of individual prostate cancers is not assessed directly by such factors (5). Plasma biomarkers produced by tumors may correlate with disease progression and predict a specific biological phenotype. Such markers might better characterize a subset of this heterogeneous patient population and might represent new biological targets for therapy. One such marker is interleukin-6 (IL-6).

IL-6 is a pleiotropic cytokine with a variety of effects on hematopoesis, immune system, and acute-phase responses. IL-6 is produced by androgen-independent prostate cancer cell lines and has been shown to act in an autocrine and/or paracrine manner to stimulate their growth but suppressing the growth of androgen-dependent cell lines (7–9). It has been implicated in the oncogenic process for a number of other tumors cell types including renal cell carcinoma, Kaposi's sarcoma, lymphoma, plasmacytoma/myeloma, and mammary cell carcinoma (10). Whereas the molecular actions of IL-6 in prostate cancer cells have not been completely elucidated, it is known that IL-6 binds to a transmembrane receptor (IL-6Rp80) which requires the association of a second glycoprotein (gp130; ref. 11). Signal transduction proceeds via three possible pathways: the ErbB3(2)-mitogen-activated protein kinase pathway, the phosphoinositide 3-kinase-Etk/Bmx pathway, and the Janus-activated kinase/signal transducers and activators of transcription pathway, all of which have been associated with androgen-independent prostate cancer (11). Recent studies suggest IL-6R activation may represent a dominant pathway for accessory activation of the androgen receptor (12).

IL-6 levels in patients with advanced prostate cancer may have important biological prognostic correlations. Twillie et al. (13) have shown that IL-6 is a candidate mediator of prostate

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cancer morbidity and have hypothesized that death in some patients may be caused or hastened by elevated levels of IL-6. Castleman's disease is a syndrome of high chronic levels of IL-6. Its symptoms, including fatigue, anemia, anorexia and weight loss, are experienced by many patients with symptomatic prostate cancer. Anti-IL-6 monoclonal antibodies have been used to effectively alleviate these symptoms in patients suffering from Castleman's disease (14, 15).

Four independent studies have examined serum IL-6 levels in controls and patients with differing stages of prostate cancer and benign prostatic hyperplasia (13–18). Three of these studies showed significantly higher median IL-6 levels in patients with metastatic disease (13, 16, 18) whereas the fourth associated significantly higher levels with HRCaP (17). Finally, Nakashima et al. (19) showed that elevated serum IL-6 levels were significantly associated with lower survival rates in patients with prostate cancer by univariate analysis. A small multivariate analysis adjusting for performance status, extent of disease, tumor histology, PSA, alkaline phosphatase, hemoglobin, and LDH revealed that only elevated IL-6 and extent of disease were significant prognostic factors. This study was limited by its small size—only 74 patients were examined in total, of these only 37 were stage D. Based on this preliminary evidence, we investigated the prognostic significance of IL-6 level in a large prospectively collected, multicenter data set.

METHODS

Patient Selection. An intergroup phase III trial [Cancer and Leukemia Group B 9480 (CALGB 9480)] of three different fixed doses of suramin was conducted. Between February 1996 and July 1998, 390 patients with metastatic HRCaP were enrolled on study after signing an Institutional Review Board–approved, protocol-specific informed consent in accordance with federal and institutional guidelines. Patients were then randomized with equal probability to receive one of three fixed dose regimens of suramin. Randomization was stratified by site (bone only versus soft tissue), performance status (0-1 versus 2), and number of prior hormonal manipulations (1-2 versus 3). Suramin was given on days 1, 2, 8, and 9 of a 28-day cycle for three cycles, with total doses cumulative of 3.19 g/m^2 (low dose arm), 5.32 g/m^2 (intermediate dose arm), and 7.66 g/m^2 (high dose arm). All patients received 40 mg/d of hydrocortisone whereas patients in the high dose arm also received 0.1 mg/d of fludrocortisone.

Patients were eligible if they had evidence of progressive metastatic adenocarcinoma of the prostate, a life expectancy of at least 3 months, a CALGB performance status of 0 to 2, and adequate hematologic, renal, hepatic and clotting function. Patients were allowed no more than three prior hormonal manipulations, no prior chemotherapy, immunotherapy, or nonhormonal therapy. If patients had been treated with strontium-89 or radiation therapy, it must have been completed at least 8 and 4 weeks before enrollment, respectively. The end points of the study were objective and PSA responses, progression-free survival, and overall survival.

Quality Control. Patient registration and data collection were managed by the CALGB Statistical Center. Data quality was ensured by careful review of data by CALGB Statistical Center staff and by the study chairperson. Statistical analyses were done by CALGB statisticians. As part of the quality assurance program of the CALGB, members of the Data Audit Committee visit all participating institutions at least once every 3 years to review source documents. The auditors verify compliance with federal regulations and protocol requirements, including those pertaining to eligibility, treatment, toxic effects, tumor response, and outcome in a sample of protocols at each institution. Such on-site review of medical records was done for a subgroup of 69 patients (17.6%) of the 390 patients treated under this study.

Pretreatment Blood Collection. During the accrual period, an amendment was added that allowed for a pretreatment blood sample to be drawn for correlative studies. Seven milliliters of blood drawn into glass vacutainer tubes containing EDTA were collected at various affiliated institutions and transferred to Dana-Farber Cancer Institute for plasma preparation and biomarker assessment. With 12 hours of arrival, samples were spun at 2,000 $\times$ g for 15 minutes. Plasma was removed, aliquoted into 500 μL microtubes, stored at −20 C, and thawed just before testing. In total, samples from 197 patients were received for these studies.

Assessment of Plasma Interleukin-6 Levels. Plasma IL-6 levels were measured at the Dana-Farber Cancer Institute. Using a microplate luminescence detection system (Dynex Technologies, Chantilly, VA) and a human IL-6 immunoassay Quantiglo kit (R&D Systems, Minneapolis, MN), quantitative levels of IL-6 can be accurately detected from 0.3 to 20,000 pg/mL using 300 μL of plasma. This solid phase ELISA system allows for high-throughput, rapid detection of IL-6 levels. By using luminescence detection, we are able to decrease the volume of plasma used from 400 to 300 μL per sample while improving intra-sample variation. In addition, quality control of a standardized kit, with internal controls and automated washes and detection, has lowered our coefficient of variance routinely below 10%.

Statistical Design and Data Analysis. Assuming that there are 250 deaths, the hazards in the two groups are proportional, and a two-sided type I error of 0.05, the log-rank statistics has 85% power to detect a hazard ratio of 1.5 between patients whose IL-6 levels are high (dichotomized at greater than the median level) and low (below or equal to the median). Survival time was defined as the time between randomization and death. Patients lost to follow-up were censored.

The Kaplan-Meier (20) product limit estimator was used to estimate the survival distribution by the two groups (low or high) of IL-6 levels based on the median value of IL-6 levels and the log-rank statistics was used to test for differences in the distribution of the survival times between the two groups of low and high IL-6 levels (21). Statistical methods based on exact asymptotic distributions were used to find a cut point (other than the median) for IL-6. The cut point corresponding to the largest discrepancy between the lower and higher risk groups was based on the log-rank statistics and the exact P value based on the maximally selected rank statistics was computed adjusting on multiple comparisons (22). In addition, the proportional hazard model was used to assess the prognostic importance of plasma IL-6 for survival adjusting for important baseline predictors, such as baseline PSA, performance status, alkaline phosphatase, LDH, and hemoglobin (23). All tests were done using a two-sided α level of 0.05.

RESULTS

Table 1 presents the baseline characteristics of the 191 patients with plasma IL-6 data. The median age of 191 patients in which plasma IL-6 levels were measured was 71 years; 82% of these patients were Caucasian. Eighty-eight percent of the patients had an Eastern Cooperative Oncology Group performance status of 0 or 1. The majority of patients had metastatic disease: 95% had bone metastases and 29% had bidimensionally measurable disease. The median baseline PSA and alkaline phosphatase were 135 ng/mL and 169 IU/L, respectively. The cohort was divided between the three different suramin treatment arms, however, none of the regimens resulted in a difference in survival. Furthermore, there was no association between baseline IL-6 level and prior therapy (antiandrogen, secondary hormonal therapy) or response to suramin. All recorded baseline characteristics of the 191 patients for whom plasma was available in this study were similar to the entire cohort of 390 patients enrolled to the CALGB 9480 (Table 1).

Table 1 Baseline characteristics of 191 patients with IL-6 data and entire CALGB 9480 population

	IL-6 data (N = 191)	CALGB 9480 (N = 390)
Demographics		
Age (y)*	71 (64-76)	70 (64-75)
Years since diagnosis†	3.7 (2.2-6.1)	4 (2-6)
Race, % white	82	81
Metastases[2]		
% Bone	95	93
% Lymph node	30	33
% Lung	7.9	7
% Liver	4.7	6
Gleason score		
% 0-4	9	10
% 5-7	54	46
% 8-10	37	44
Performance status		
% 0-1	88	88
% 2	12	12
Disease assessment		
% Measurable	29	36
% Evaluable	71	64
Laboratory data‡		
Hemoglobin (g/dL)	12 (11-14)	13 (11-14)
Creatinine (mg/dL)	1.0 (0.9-1.1)	1.0 (0.8-1.1)
PSA (ng/mL)	135 (45-347)	128 (49.0-338)
Alkaline phosphatase (IU/L)	169 (102-359)	163.5 (99.0-313.0)
LDH (units/L)	201 (165-345)	210 (168-411)
Prior therapy[3]		
% Antiandrogen	85	86
% Luteinizing hormone-releasing hormone analogue	61	63
% Surgical castration	46	42
% Estrogen	6.0	5.4
% Progesterone agent	2.7	3.6
Suramin dose		
% Low	32.98	33.59
% Intermediate	34.03	33.08
% High	32.98	33.33
IL-6		
Mean	15.76	N/A
Median	4.80	
SD	52.66	
Interquartile range	2.45-12.57	

*Median and inter-quartile range.
†Patients may have more than one metastases.
‡Patients may have more than one type of prior therapy.

Table 2 Overall survival based on IL-6 level

IL-6*	N	No. failed	Median (95% CI)	P
≤4.80	96	89	19 mo (17-22)	0.0004
>4.80	95	91	11 mo (8-14)	
IL-6†	N	No. failed	Median (95% CI)	P
≤13.31	145	135	17 mo (16-19)	<0.0001
>13.31	46	45	7.1 mo (4.3-10.9)	
IL-6‡	N	No. failed	Median (95% CI)	P
≤2.96	64	58	18 mo (17-25)	0.0005
2.96-8.41	63	61	17 mo (11-19)	
>8.41	64	61	11 mo (7-14)	
IL-6§	N	No. failed	Median (95% CI)	P
≤2.45	48	44	22 mo (17-27)	<0.0001
2.45-4.80	48	45	18 mo (16-19)	
>4.80-12.57	48	45	14 mo (8-18)	
>12.57	47	46	7 mo (5-11)	

*IL-6 level based on the median.
†IL-6 factor level based on maximally selected rank statistic.
‡IL-6 level based on the tertiles.
§IL-6 level based on the quartiles.

There was no statistically significant difference in survival when we compared the 191 patients in which a plasma IL-6 level was obtained to the entire cohort of 199 patients (log-rank test P = 0.560). The median survival time in the 191 patients was 16.5 (95% CI, 12.5-17.1) months versus 13.7 (95% CI, 11.3-15.6) for the remaining 199 patients, from whom no pretreatment plasma was available.

Of 191 patients, 180 deaths (94%) occurred and the median follow-up time among the 11 surviving patients was 33.90 months (95% CI, 4.43-54.17). Table 2 presents a univariate analysis of the plasma IL-6 levels based on the a priori cut point, the median, and other cut points based on the tertiles, quartiles, and the cut point that had the largest log-rank statistics. In the univariate analysis higher IL-6 levels were associated with shorter survival time. The median survival time was 19 months (95% CI, 17-22) among patients with low IL-6 levels (levels below or equal to 4.80) compared with 11 months among patients whose levels were greater than 4.80 (95% CI, 8-14; P = 0.00041; Table 2). Figure 1 shows the Kaplan-Meier survival distribution with IL-6 levels dichotomized by the median IL-6. Further, the median survival time was 17 months for patients whose IL-6 levels were ≤13.31 pg/mL compared with 7 months for patients whose IL-6 levels were >13.31 pg/mL, with an adjusted P value of <0.001 (Fig. 2). Roughly 24% of patients fell above this threshold.

The prognostic importance of IL-6 remained when adjusting for other potential prognostic factors, such as baseline PSA, LDH, and performance status. In multivariate analysis, IL-6 was an independent prognostic factor for overall survival, with elevated levels predictive of poor outcome at the median and Maxstat cut points. We calculated an adjusted hazard ratio of 1.38 (95% CI, 1.01-1.89; P = 0.0432) for patients with IL-6 levels greater than the median (4.80 pg/mL) compared with patients with IL-6 levels less than or equal to the median

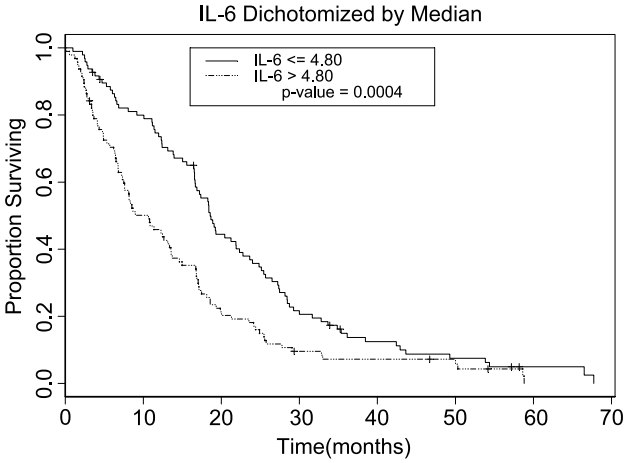

Fig. 1 IL-6 dichotomized by median. A Kaplan-Meier plot depicts the differences in survival seen in this patient population when separated by IL-6 level. Those patients with an IL-6 level above the median (>4.80 pg/mL) show a statistically worse survival (*dashed line*) compared with patients with an IL-6 level at or below the median (*solid line*).

(Table 3). The association of IL-6 level and survival became even stronger when we used a higher cut point (13.31 pg/mL). For this cut point the adjusted hazard ratio was 2.02 (95% CI, 1.36-2.98; $P = 0.0005$), which was the most significant factor in this multivariate model.

DISCUSSION

HRCaP remains a fatal disease with a median survival that varies between 7.5 and 27.2 months, depending on current prognostic factors (6). Such variation may also reflect the heterogeneous biology of this disease. Because of the nature of

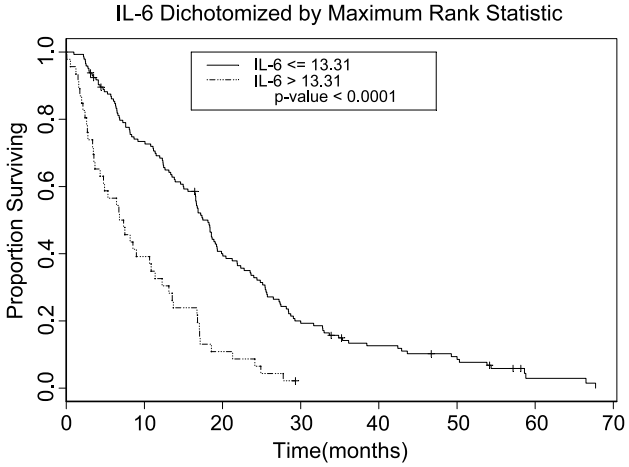

Fig. 2 IL-6 dichotomized by maximum rank statistics. The Kaplan-Meier plot here depicts the differences in survival seen in this patient population when separated by an IL-6 cut point of 13.31 pg/mL. This cut point was identified using the Maxstat program to select the statistical cut point with maximum significance. Those patients with an IL-6 level above 13.31 pg/mL show a statistically worse survival (*dashed line*) compared with patients with an IL-6 level at or below this cut point (*solid line*). Twenty-four percent of patients in this population were above this cut point.

Table 3 Multivariate proportional hazards analysis for predicting overall survival

Model 1*	HR (95% CI)†	*P*
IL-6 (>4.80 vs. ≤4.80)	1.38 (1.010-1.885)	0.0432
Performance status (2 vs. 0.1)	2.17 (1.340-3.498)	0.0016
PSA (>130 vs. ≤130)	1.74 (1.272-2.367)	0.0005
LDH (>199 vs. ≤199)	1.35 (0.995-1.818)	0.0543
Model 2‡	**HR (95% CI)†**	***P***
IL-6 (>13.31 vs. ≤13.31)	2.02 (1.362-2.982)	0.0005
Performance status (2 vs. 0.1)	1.81 (1.096-2.976)	0.0204
PSA (>130 vs. ≤130)	1.69 (1.233-2.306)	0.0011
LDH (>199 vs. ≤199)	1.28 (0.943-1.745)	0.1122

*IL-6 level based on the median.
†HR, hazard ratio.
‡IL-6 level based on maximally selected rank statistics.

clinical prostate cancer, metastatic tumor tissue is rarely obtained and difficult to assess. Consequently, little is known about the specific biological features of metastatic, hormone-refractory prostate tumors and the clinical relevance of tumor markers. Linking plasma levels of specific biological markers to prognosis may add insights to the biology of aggressive tumor phenotypes and identify rational targets for novel therapeutic approaches.

We report here results of a CALGB study that evaluated the prognostic significance of plasma IL-6 level. Prospectively collected plasma was collected in a multi-institutional study and linked to survival. We established cut points for evaluation prospectively (median, tertile, and quartile) and showed prognostic significance in both univariate and multivariate models using other known prognostic factors (LDH, PSA, and performance status). In addition, we identified retrospectively the optimal cut point (13.31 pg/mL) which represented 24% of the population.

One unexpected finding that emerged from this study is that IL-6 level did not show prognostic significance as a continuous variable. One explanation for this finding is that IL-6 levels showed a bimodal distribution that may reflect in some patients that other concomitant conditions, particularly those associated with inflammation, may result in mild elevations of IL-6. Such confounding factors might weaken the association of IL-6 with outcome, particularly at low levels. In contrast, we identified a subset of patients with elevated IL-6 levels by two cut points, the highest quartile and the Maxstat procedure, which show robust prognostic significance. Importantly, this prognostic association was independent of other known prognostic factors, which indirectly correlate with tumor burden (PSA level, LDH level, and performance status).

Our results raise the possibility that a subset of patients, representing the 25% highest IL-6 levels, may harbor a biologically more aggressive phenotype of prostate cancer. For instance, previous studies have shown that IL-6 can enhance the differentiation to a neuroendocrine phenotype that is thought to represent more aggressive biology (24–30). In addition, IL-6 has been identified as a relatively potent activator of androgen receptor in the absence of androgen, as well as synergistically in the presence of low levels of androgen (11, 12, 18, 31–37). As such, IL-6 acts as an autocrine growth factor for androgen-independent prostate cancer cell growth (7, 9, 38, 39). However,

some studies suggest that IL-6 could also induce in a more differentiated grade of cancer, resulting in decreased proliferation and apoptosis (40, 41). These studies suggest that the ultimate cellular effect of IL-6 may be dependent on other genetic and molecular features of the cancer including downstream pathways such as signal transducers and activators of transcription-3 and phosphoinositide 3-kinase.

Finally, the dysregulation of IL-6 may result in paraneoplastic morbidity and early mortality. Previous studies show that IL-6 is a potent mediator of acute-phase response to injury and infection (11). Chronically elevated IL-6 levels in Castleman's disease are associated with a constellation of symptoms analogous to many patients with end-stage prostate cancer (15). However, if IL-6 represented such biology solely, we would anticipate that its prognostic significance would more closely correlate with LDH, performance status, and other measures of the condition of the host.

Based on these preliminary results, further confirmatory investigations into the prognostic value of plasma IL-6 level are warranted to evaluate its biological implications and clinical importance in relation to other prognostic biomarkers, such as vascular endothelial growth factor (5, 42) and chromogranin A levels.[4] In particular, the robust preliminary findings using a cut point of 13.31 pg/mL should be confirmed using a separate, independent data set. Support of these findings would justify the clinical development of anti-IL-6-targeted treatment strategies in patients with prostate cancer.

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