Prognostic Significance of Plasma Vascular Endothelial Growth Factor Levels in Patients with Hormone-refractory Prostate Cancer Treated on Cancer and Leukemia Group B 9480

Daniel J. George, Susan Halabi, Timothy F. Shepard, Nicholas J. Vogelzang, Daniel F. Hayes, Eric J. Small, and Philip W. Kantoff

Lank Center for Genitourinary Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115 [D. J. G., T. F. S., P. W. K.]; Cancer and Leukemia Group B Statistical Center, Duke University Medical Center, Durham, North Carolina 27710 [S. H.]; Section of Hematology and Oncology, Department of Medicine, University of Chicago Cancer Research Center, Chicago, Illinois 60637-1470 [N. J. V.]; Lombardi Cancer Center, Georgetown University Medical Center, Washington DC 20007 [D. F. H.]; and Mount Zion Cancer Center, University of California San Francisco, San Francisco, California 94115 [E. J. S.]

ABSTRACT

Purpose: Plasma vascular endothelial growth factor (VEGF) levels are significantly elevated in patients with hormone-refractory prostate cancer (HRPC) compared with patients with localized disease and have been associated with disease progression in other cancer patient populations. Therefore, we measured VEGF levels in plasma prospectively collected from patients enrolled in Cancer and Leukemia Group B 9480, an intergroup study of suramin in patients with HRPC, to determine whether these levels had prognostic significance.

Experimental Design: Pretreatment plasma was collected from patients with HRPC enrolled in Cancer and Leukemia Group B 9480. In a subset of samples representative of the entire cohort, plasma VEGF levels were determined in duplicate using a Quantiglo chemiluminescent ELISA kit (R&D Systems, Minneapolis, MN). Statistical analyses were performed to determine the correlation between pretreatment plasma VEGF levels and time of overall survival. The proportional hazards model was used to assess the prognostic significance of various cut points in multivariate models.

Results: Plasma VEGF levels in this population ranged from 4–885 pg/ml, with a median level of 83 pg/ml. As a continuous variable, plasma VEGF levels inversely correlated with survival time (P = 0.002). Using various exploratory cut points, plasma VEGF levels appeared to correlate with survival. In multivariate models in which other prognostic factors (serum prostate-specific antigen, alkaline phosphatase, evidence of measurable disease, and hemoglobin) were included, plasma VEGF levels were significant at various cut points tested.

Conclusion: Although these data are exploratory and need to be confirmed in an independent data set, they suggest that VEGF may have clinical significance in patients with HRPC.

INTRODUCTION

HRPC1 is a uniformly fatal disease accounting for an estimated 31,900 deaths annually in the United States (1), with a median survival slightly over 1 year (2–5). Because of both selective and adaptive processes, HRPC is a heterogeneous disease. Factors that predict the outcome of HRPC patients qualitatively reflect either the overall tumor burden (e.g., elevated PSA levels, alkaline phosphatase, or bone scan findings) or condition of the host (e.g., performance status, plasma hemoglobin level, or weight loss). However, biological behavior of individual prostate cancers is not assessed directly by such factors (6). Circulating biomarkers produced by tumors may correlate with disease progression and predict a specific biological phenotype. Such markers might better characterize this heterogeneous patient population and might represent new biological targets for therapy. One such marker is VEGF.

VEGF is a homodimeric cytokine that was originally identified by its effects on endothelial cell proliferation and vascular permeability (7, 8). Since its discovery, VEGF has been demonstrated to bind to two tyrosine kinase receptors, FMS-like tyrosine receptor-1 and kinase domain receptor (or VEGFR-1 and 2, respectively), on the surface of endothelial cells to regulate physiological and pathophysiological angiogenesis (9). Microvessel density is a surrogate marker of tumor angiogenesis that correlates with disease progression and survival in patients with localized prostate cancer (10–13). However, in the metastatic setting there are no surrogate markers of tumor angiogenesis. Circulating levels of VEGF, which can be detected in patients with many solid tumor cell types including prostate cancer, may be a marker of the degree and activity of tumor angiogenesis.

1 The abbreviations used are: HRPC, hormone-refractory prostate cancer; VEGF, vascular endothelial growth factor; CALGB, Cancer and Leukemia Group B; PSA, prostate-specific antigen; HR, hazard ratio; CI, confidence interval.

Received 2/1/01; revised 4/20/01; accepted 4/23/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by a CapCURE Young Investigator Award [to D. J. G.] and the Gelb Center for Translational Research at the Dana-Farber Cancer Institute.

To whom requests for reprints should be addressed, at 1230 Dana Building, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115. Phone: (617) 632-3466; Fax: (617) 632-2165.
Initial studies (14, 15) evaluating circulating VEGF levels in various cancer patient populations were performed in serum; however, results were inconclusive, because VEGF is present in platelets and is released during the clotting process (16). One study (17) suggests that quantitative measurements of VEGF levels by ELISA in plasma offer the most reliable measure of circulating VEGF levels. Studies (18, 19) in patients with gastric and colorectal cancer have demonstrated an association between elevated plasma VEGF levels and advanced disease. However, the functional significance of these elevated VEGF levels is unknown.

Duque et al. (20) recently studied the relationship between plasma VEGF levels in patients with prostate cancer and demonstrated that median levels were significantly higher in patients with metastatic disease compared to patients with localized prostate cancer. To explore the use of circulating VEGF levels as a prognostic factor for survival, we measured plasma VEGF in patients with HRPC enrolled into a multicenter (intergroup) study and correlated these levels with outcomes, including duration of survival.

PATIENTS AND METHODS

Patient Selection. An intergroup Phase III trial (CALGB 9480) of three different fixed doses of suramin was conducted (21). Between February 1996 and July 1998, 390 patients with metastatic HRPC were 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 cumulative doses of 3.19 g/m² (low-dose arm), 5.32 g/m² (intermediate-dose arm), and 7.66 g/m² (high-dose arm). All of the patients received 40 mg/day of hydrocortisone, whereas patients in the high-dose arm also received 0.1 mg/day 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 0–2, and adequate hematological, renal, hepatic, and clotting function. Patients were allowed no more than three prior hormonal manipulations and no prior chemotherapy, immunotherapy, or non-hormonal therapy. If patients had been treated with strontium-89 or radiation therapy, it must have been completed at least 8 weeks and 4 weeks before enrollment, respectively. The end points of the study were objective and PSA responses, progression-free survival, and overall survival.

Pretreatment Blood Collection. During the accrual period, an amendment was added to that allowed for a pretreatment blood sample to be drawn for correlative studies. Blood (7 ml) drawn into glass vacutainer tubes containing EDTA was collected at various affiliated institutions and transferred to Dana-Farber Cancer Institute for plasma preparation and biomarker assessment. Within 12 h of arrival, samples were spun at 2000 × g for 15 min. Plasma was removed, aliquotted 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 VEGF Levels. Plasma VEGF levels were determined in duplicate using a QuantiGlo chemiluminescent ELISA kit (R&D Systems, Minneapolis, MN) following manufacturer’s instructions. A MLX Luminometer (DYNEX Technologies, Chantilly, VA) was used to measure light intensity correlating with VEGF binding.

Statistical Analysis. Survival time was defined as the time between study entry and death. Patients lost to follow-up were censored. Exploratory statistical methods were used to find different (than the median) cut points for VEGF. The Kaplan-Meier product limit estimator was used to estimate the survival distribution by the two groups of VEGF levels, and the log-rank statistic was used to test for differences in the distribution of the survival times between the two groups of low and high VEGF levels. In addition, the proportional hazards model was used to assess the prognostic importance of plasma VEGF for survival adjusting for important baseline predictors, such as baseline PSA, measurable disease, and alkaline phosphatase. The HR is the ratio of the failure probability among the high-risk group (above a certain cut point) compared with the low-risk group (less than or equal to a certain cut point); e.g., if for a given cut point, the HR is greater than one, then the failure rate for patients above the cut point is higher than the failure rate for patients below the cut point. All of the tests were performed using a two-sided α level = 0.05.

RESULTS

Table 1 presents the baseline characteristics of the 197 patients with plasma VEGF data. The median age of 197 patients in which plasma VEGF levels were measured was 68; 79% of these patients were Caucasian. Eighty-seven % of the patients had an Eastern Cooperative Oncology Group performance status of 0 or 1. The majority of patients had metastatic disease: 93% had bone metastases, and 29% had bidimensionally measurable disease. The median baseline PSA and alkaline phosphatase were 150 ng/ml and 170 IU/l, respectively. The baseline characteristics of the 197 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). The median follow-up time for the 197 patients was 14 months and included 124 deaths.

There was no statistically significant difference in survival when we compared the 197 patients in which a plasma VEGF level was obtained with the entire cohort of 390 patients (log-rank test, 2.36; df, 2; \( P = 0.308 \)). The median survival in the 197 patients was 14.3 months versus 12.6 months for the remaining 193 patients, from whom no pretreatment plasma was available.

Table 2 presents a univariate analysis of the plasma VEGF levels at various cut points and as a continuous covariate. When used as a continuous covariate, VEGF levels were significantly predictive of survival (\( P = 0.002 \)), whereas the unadjusted HR using the median VEGF level (83 pg/ml) as a cut point was not (HR = 1.3; \( P = 0.169 \)). To compare VEGF levels in a multivariate model using dichotomous factors, we identified other cut points that appeared significant using exploratory statistical analyses. Because these cut points were selected retrospectively, they require further prospective testing to confirm their significance. We selected several cut points above and below the
Plasma VEGF Levels in Prostate Cancer Patients

median that showed an association between high VEGF levels and decreased duration of survival; e.g., patients with VEGF levels \( \leq 64 \) pg/ml had a longer median survival (16 months; 95% CI, 13–21 months) compared to patients with VEGF levels > 64 pg/ml (14 months; 95% CI, 12–17 months; log-rank test, 3.85; df, 1; \( P = 0.05 \)). At higher cut points, the differences were even more striking. At a cut point of 260 pg/ml, differences in median survival were 17 months (95% CI, 14–18) versus 11 months (95% CI, 6–13) for patients below and above the cut point, respectively (log-rank test, 12.0; df, 1; \( P = 0.0005 \)).

The prognostic importance of VEGF remained when adjusting for other potential prognostic factors, such as measurable disease, baseline PSA, alkaline phosphatase, and baseline hemoglobin. On multivariate analysis, VEGF was an independent prognostic factor for overall survival, with elevated levels predictive of poor outcome even at the median cut point. We calculated an adjusted HR = 1.22 (95% CI, 1.01–1.50; \( P = 0.043 \)) for patients with VEGF levels of greater than the median (83 pg/ml) compared to patients with VEGF levels less than or equal to the median (Table 3). The association of VEGF levels and survival became even stronger when we used different cut points; e.g., the adjusted HR was 1.52 (95% CI, 1.03–2.24; \( P = 0.037 \); Table 3) for patients with VEGF levels greater than 64 pg/ml compared to patients with VEGF levels less than or equal to 64. Furthermore, as we increased the VEGF cut point, the HR increased, with a VEGF cut point of 260 pg/ml demonstrating the greatest HR (Table 3). Among other prognostic factors, measurable disease was the strongest predictor with a HR of 2.01 (95% CI, 1.36–3.00; \( P < 0.001 \)), followed by baseline PSA and alkaline phosphatase.

### DISCUSSION

HRPC remains a fatal disease with a median survival between 11–16 months. However, because of the long natural history of prostate cancer and the variable response to hormonal therapies, HRPC is a heterogeneous disease with a wide spectrum of clinical courses. Because of the nature of 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. Because of the limited availability of fresh metastatic tumor tissue, we began investigating the clinical significance of candidate biomarkers in plasma. Linking circulating levels of specific biological markers to prognosis may add insights into the biology of aggressive tumor phenotypes and identify rational targets for novel therapeutic approaches.

As a key angiogenic growth factor with various inhibitory strategies in clinical development (22–24), identifying the clinical relevance of VEGF in patients with HRPC could have direct therapeutic implications. Although it could be argued that circulating levels of a growth factor that is thought to function primarily in a paracrine manner may be not be relevant, circulating VEGF levels have been shown to predict for disease progression in gastric, colorectal, and small cell lung cancers (18, 19, 25, 26). In addition, plasma VEGF levels are significantly elevated in patients with a number of other malignancies, including HRPC (19). Therefore, we explored the prognostic significance of plasma VEGF levels to establish the clinical relevance of VEGF as a biological target in this patient population.

VEGF levels were measured in plasma collected from a cohort of patients with HRPC enrolling into a multicenter trial. Because the different treatment arms of this study did not result in a significant survival difference, pretreatment plasma VEGF levels were evaluated to determine whether they correlated with duration of survival. All of these samples were collected from patients before initiation of therapy; therefore, there was no effect of the drug treatments on the VEGF values. Moreover, we measured VEGF levels in a cohort of study patients that accurately represented the entire study population without bias to pretreatment characteristics or treatment arm (Table 1). Differ-

### Table 1 Baseline characteristics of 197 patients with plasma VEGF data and the entire sample of 390 patients on CALGB 9480

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Patients with VEGF data (n = 197)</th>
<th>Entire sample (n = 390)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, yrs</td>
<td>68 (62–75)</td>
<td>70 (64–75)</td>
</tr>
<tr>
<td>Race: white</td>
<td>79%</td>
<td>81%</td>
</tr>
<tr>
<td>Metastasis</td>
<td>93%</td>
<td>93%</td>
</tr>
<tr>
<td>With bone metastasis</td>
<td>28%</td>
<td>33%</td>
</tr>
<tr>
<td>With possible lymph nodes</td>
<td>5%</td>
<td>7%</td>
</tr>
<tr>
<td>With liver metastasis</td>
<td>4%</td>
<td>6%</td>
</tr>
<tr>
<td>Measurable disease</td>
<td>29%</td>
<td>34%</td>
</tr>
<tr>
<td>Median years since diagnosis</td>
<td>4 (2–6)</td>
<td>4 (2–6)</td>
</tr>
<tr>
<td>Performance status: 0–1</td>
<td>87%</td>
<td>87%</td>
</tr>
<tr>
<td>Laboratory data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median hemoglobin g/dl</td>
<td>13 (11–14)</td>
<td>13 (11–14)</td>
</tr>
<tr>
<td>Median PSA ng/ml</td>
<td>150 (48–418)</td>
<td>128 (49–338)</td>
</tr>
<tr>
<td>Median alkaline phosphatase IU/l</td>
<td>170 (103–330)</td>
<td>164 (99–313)</td>
</tr>
</tbody>
</table>

### Table 2 Results of the univariate, proportional hazard analyses with plasma VEGF predicting survival time among 197 patients

<table>
<thead>
<tr>
<th>Univariate analyses</th>
<th>HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF (continuous)</td>
<td>Not applicable</td>
<td>0.002</td>
</tr>
<tr>
<td>VEGF (&gt;83 pg/ml vs. ( \leq 83 ))</td>
<td>1.3 (0.9–1.8)</td>
<td>0.169</td>
</tr>
<tr>
<td>VEGF (&gt;64 vs. ( \leq 64 ))</td>
<td>1.4 (1.0–2.0)</td>
<td>0.050</td>
</tr>
<tr>
<td>VEGF (&gt;200 vs. ( \leq 200 ))</td>
<td>1.7 (1.0–2.7)</td>
<td>0.032</td>
</tr>
<tr>
<td>VEGF (&gt;260 vs. ( \leq 260 ))</td>
<td>2.6 (1.5–4.7)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Inter-quartile range.
* Patient may have more than one metastasis.
* Patient may have more than one type of prior therapy.
levels could define a specific biological phenotype. Finally, not simply be a marker of the extent of disease. Rather, elevated markers of disease burden, suggests that plasma VEGF might more significant in the multivariate setting, controlling for presence of measurable disease. The finding that these data were activated serum PSA and alkaline phosphatase levels and the presence of these samples at the various centers, may have introduced some variability. Nevertheless, despite variations in handling, the results were surprisingly consistent. As a continuous covariate, elevated plasma VEGF levels correlated with shorter survival. Although univariate analysis did not demonstrate a statistically significant difference at the median cut point, other cut points suggested that differences in VEGF levels could be prognostic. These cut points were selected using an exploratory statistical technique that gave the lowest possible “P(s)”; therefore, statistical significance should not be interpreted in the usual way. By this method, a chosen cut point is generally not unique because a range of values may provide similar results. Although the clinical relevance of cut points generated by this technique can be argued, they are nonetheless supportive of the hypothesis that VEGF could have a biological role in this patient population.

On multivariate analysis, the results were more compelling. At every cut point evaluated, including the median level, plasma VEGF levels were significantly prognostic for duration of survival. Moreover, at higher cut points (260 pg/ml), plasma VEGF level became the most powerful prognostic factor in a multivariate model, including markers of disease burden such as elevated serum PSA and alkaline phosphatase levels and the presence of measurable disease. The finding that these data were more significant in the multivariate setting, controlling for markers of disease burden, suggests that plasma VEGF might not simply be a marker of the extent of disease. Rather, elevated levels could define a specific biological phenotype. Finally, similar results (27) were reported using pretreatment urine VEGF levels measured in a different cohort of patients from this clinical trial. On the basis of these preliminary results, additional confirmatory investigations into the prognostic value of plasma VEGF levels are warranted to investigate the biological phenotype that may be associated with VEGF overproduction and the clinical importance of this parameter in patients with HRPC. Prospective confirmation of these cut points would support using plasma VEGF, urine VEGF, or a combination to create a multivariate prognostic model that better risk-stratifies patients and more accurately predicts survival time. In addition, support of these findings would help justify the continued clinical development of VEGF-targeted treatment strategies in patients with prostate cancer, especially if early clinical trials of single agent strategies are unremarkable.

ACKNOWLEDGMENTS
We thank the CALGB staff for their help in manuscript preparation, especially Ann Battershel.

REFERENCES


Prognostic Significance of Plasma Vascular Endothelial Growth Factor Levels in Patients with Hormone-refractory Prostate Cancer Treated on Cancer and Leukemia Group B 9480

Daniel J. George, Susan Halabi, Timothy F. Shepard, et al.


Updated version  Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/7/7/1932

Cited articles  This article cites 24 articles, 8 of which you can access for free at: http://clincancerres.aacrjournals.org/content/7/7/1932.full#ref-list-1

Citing articles  This article has been cited by 23 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/7/7/1932.full#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.