Serum Vascular Endothelial Growth Factor as a Predictive Factor in Metronomic (Weekly) Paclitaxel Treatment for Advanced Head and Neck Cancer

Miguel Caballero, MD, PhD; Juan José Grau, MD, PhD; Jose Luis Blanch, MD, PhD; Jose Domingo-Domenech, MD, PhD; Jose María Auge, MD, PhD; Wladimiro Jimenez, MD, PhD; Manuel Bernal-Sprekelsen, MD, PhD

Objective: To evaluate serum vascular endothelial growth factor (VEGF) as a prognostic factor in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck treated with metronomic (weekly) paclitaxel.

Design: Before-and-after trial.

Patients: A total of 33 consecutive patients were enrolled. Patients with recurrent and/or metastatic cancer of the head and neck refractory to platinum-based chemotherapy met inclusion criteria.

Setting: Tertiary referral center.

Intervention: Patients were treated weekly with 80 mg/m² of paclitaxel for 6 weeks.

Main Outcome Measures: Blood samples were collected after each dose and analyzed for serum VEGF using enzyme-linked immunosorbent assay kits. Non-parametric tests were used to analyze serum VEGF levels.

Results: In 33 patients, complete response was achieved in 1 (3%) and partial response in 20 (61%). No differences were found between responders and nonresponders with different levels of serum VEGF at any of the measurement times (baseline, after the first dose, and after the sixth dose). In responders, the median level of serum VEGF decreased after the first dose compared with baseline, but by the sixth dose, the median serum VEGF level had returned to baseline levels in all groups. The intensity of the serum VEGF level decrease (simple decrease, a decrease of at least 30%, or a decrease of at least 70%) was not related to response. The progression-to-disease time increased in the patients with a serum VEGF level reduction of at least 30% (P = .01) after 6 doses and decreased in patients with initially high levels, which remained high after the sixth dose (P = .03).

Conclusions: Serum VEGF levels after the first dose of paclitaxel may predict response to weekly paclitaxel in patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck. Initially high serum VEGF levels that persist after the sixth dose predict a shorter period until tumor progression.


Advanced head and neck cancers resistant to platinum-based chemotherapy in which no other local therapies can be administered have a very poor prognosis, with a low response rate and short survival rate.¹ Moreover, since many patients with these tumors are heavily pretreated, their physical condition is poor and their tolerance of standard doses of chemotherapeutic agents is reduced. Metronomic scheduling² (frequent or continuous low-dose chemotherapy) may be an option in these patients. Earlier studies have found that metronomic chemotherapy induces a long-standing high response rate, reducing acute toxic effects in some patients. Several anticancer agents such as cyclophosphamide and methotrexate have shown clinical activity in metronomic scheduling.³

Tumor growth depends on angiogenesis. Metronomic scheduling of certain chemotherapeutic agents exerts a marked antiangiogenic effect, generating greater antitumor activity than conventional chemotherapy scheduling using high doses at intervals of 2 to 4 weeks.⁴⁻⁶ These findings may herald a new era in chemotherapy; the ability to target genomically stable endothelial cells⁷⁻⁸ may reduce the risk of drug resistance while at the same time reducing toxic effects.⁵

Vascular endothelial growth factor (VEGF) is probably the most important angiogenesis stimulator⁹ and is believed to play a key role in the neovascularization of human tumors. Vascular endothelial
growth factor is overexpressed by cells of malignant solid tumors and hematological malignant tumors, including those of head and neck cancer. Moreover, it has been shown that there is an association between serum VEGF level and tumor stage and that circulating VEGF levels shown that there is an association between serum VEGF to be predictors of tumor progression.13

Because VEGF is the most potent angiogenic factor, control of its secretion probably constitutes the most important mechanism for achieving inhibition of angiogenesis-related processes. Paclitaxel appears to inhibit VEGF-induced angiogenesis, improving the prognosis of advanced head and neck cancer in patients with resistance to platinum-based chemotherapy.16,17

The aim of this study was to prospectively analyze the feasibility of testing serum level of VEGF as a surrogate marker of head and neck cancer angiogenesis and to correlate these levels with disease outcome and response to chemotherapy based on a low-dose weekly paclitaxel course in patients with advanced head and neck cancer.

### METHODS

#### PATIENT ENROLLMENT

A total of 33 consecutive patients with recurrent and/or metastatic squamous cell carcinoma of the head and neck with documented progression despite platinum-based chemotherapy were prospectively recruited. Ethical approval was obtained from the ethics committee of the Hospital Clinic, University of Barcelona, Barcelona, Spain, and informed consent was obtained from the patients who agreed to participate. Patient characteristics are given in Table 1.

Inclusion criteria for this study were age between 18 and 80 years; histological and radiological confirmation of advanced squamous cell carcinoma of the head and neck; no possibility of alternative treatment; Eastern Cooperative Oncology Group (ECOG) performance status score of 2 or less; Eastern Cooperative Oncology Group (ECOG) performance status score of 2 or less; appropriate hepatic, renal, and bone marrow function; and geographical proximity allowing adequate follow-up. Exclusion criteria were active infection, pregnancy, uncontrolled arterial hypertension, heart illness, symptomatic neuropathy, or other additional malignant tumors in other sites. The following clinical and pathological data were specifically obtained: demographic details, tumor site, clinical TNM stage and stage group, tumor grade, treatment given, toxic effects, follow-up, and survival or cause of death.

#### TREATMENT PLAN AND DOSE MODIFICATIONS

Patients received 80 mg/m² of paclitaxel with a 1-hour infusion schedule administered weekly as compassionate therapy. The National Cancer Institute Common Toxicity Criteria (NCI-CTC version 2.0) was used to grade toxicity. Patients remained enrolled in the study until an objective progression of disease or an unacceptable toxic effect was observed or if they asked to withdraw.

#### RESPONSE ASSESSMENT

Objective tumor response and toxic effects were evaluated using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Computed tomographic scanning or magnetic resonance imaging was used at baseline and as a reevaluation technique. Response was first evaluated after 6 doses, and confirmatory scans were obtained 4 weeks after the initial documentation of an objective response. Patients were seen weekly by a physician and reevaluated radiographically every 6 weeks.

Response to treatment was classified as progressive disease (PD), stable disease (SD), and partial response (PR), or complete response (CR). Based on the imaging results, CR was defined as the disappearance of the tumor; PR, a reduction of at least 30% (resulting from adding together the 2 maximum diameters of the tumor); SD, the tumor neither decreased enough
After the First Dose

Venous blood was obtained before the first dose and weekly until the completion of 6 doses of paclitaxel, as well as at all times of response assessment. Clotted and citrated samples were centrifuged (200g for 10 minutes) to yield serum and plasma samples, respectively. The samples were then aliquoted and stored in a freezer at −80°C until assay. A commercially available quantitative sandwich enzyme immunoassay technique (Quantikine; R&D Systems Inc, Minneapolis, Minnesota) was used for quantitative measurements of serum VEGF levels. Serum samples from all patients were incubated for 2 hours at room temperature in duplicate (100 L) on microtiter plates coated with a monoclonal antibody specific for VEGF. Next, any unbound substances were washed away, and an enzyme-linked polyclonal antibody specific for VEGF was introduced. This was allowed to incubate for 2 hours at room temperature, and the plates were washed to remove unbound antibody. A substrate solution was added, and color development was stopped after 25 minutes at room temperature. A microplate reader was then used to determine colorimetric densities at 570 nm and 450 nm for each sample. The optical density for each sample was determined by subtracting the readings at 570 nm from the reading at 450 nm.

Clinical benefit

CR + PR + SD

92.8 (53-167)

50.2 (35-114)

.91

Clinical benefit

CR + PR + SD

92.8 (53-167)

50.2 (35-114)

.91

Results were calculated from a standard curve generated by a form parametric logistic curve fit and expressed in picograms per milliliter of serum. The test sensitivity as determined by the manufacturer is less than 9.0 pg/mL.

STATISTICAL ANALYSIS

All analyses were performed with SPSS statistical package version 12.0 (SPSS Inc, Chicago, Illinois). The Kolmogorov-Smirnov test studied the distribution of serum VEGF levels. The result was found to be skewed to the right (asymmetry index, 2.675; kurtosis index, 8.382), so median and interquartile range were used to describe the central tendency and variation. The nonparametric Wilcoxon signed-rank test was used to compare changes in serum levels between baseline and different times of treatment. The Mann-Whitney test was used to compare serum VEGF levels at each time point between groups according to the response. Progression-free and overall survival probabilities were measured from the date of registration and estimated by the Kaplan-Meier product-limit method. Statistical significance in this study was set at P < .05.

RESULTS

PATIENTS' DESCRIPTIVE DATA

From October 2001 to June 2005, 33 patients were enrolled. Their data are presented in Table 1. There was a preponderance of men and patients with an ECOG performance status score of 1, N category of 0 or 3, and stage IV disease. Metastatic sites were skin (n = 14), lung (n = 10), bone (n = 6), liver (n = 4), and distant lymph nodes (n = 2). Only 3 patients had more than 1 metastatic site affecting bone and lung or liver, skin, and lung.

RESPONSE

Table 2 summarizes the results after treatment with 6 doses of paclitaxel, which were confirmed 4 weeks after the first response evaluation. The treatment was well tolerated, and there were few toxic effects. Six patients showed any type of toxic effects: after 6 months of treatment, 1 patient developed a skin reaction and paresthesia; another patient developed a grade 2 granulocytopenia; and the 4 other patients showed vascular toxic effects (1 ischemic heart stroke, 2 subclavala vein thrombosis, and 1 thrombophlebitis of the lower extremities).

SERUM VEGF LEVELS AND RESPONSE TO PACLITAXEL

No differences in serum VEGF levels were seen between responders vs nonresponders or between clinical benefiters vs clinical nonbenefiters at any of the measurements (baseline, after the first dose, and after the sixth dose) (Table 3). The median serum VEGF level after the first dose fell compared with baseline but then rose.
again and did not differ significantly from baseline levels after the sixth dose. When the levels were analyzed according to both objective response and clinical benefit, the decrease in serum VEGF level was only observed in responders (Figure 1A) and in clinical benefiters (Figure 1B) and only after the first dose of paclitaxel. By the sixth dose, no differences were found with respect to baseline in any of the groups (Table 3).

Having observed a correlation between response and fall in VEGF level after the first paclitaxel dose, we studied whether the extent of the decrease (rated as a simple decrease, a decrease of at least 30%, or a decrease of at least 70%) was related to response. No significant differences were found.

With the receiver operating characteristic curves, we obtained cutoff points for VEGF values (initial, second session, sixth session, and differences between the initial and the second week and between the second and the sixth week) that showed a higher sensitivity and specificity related to the response and clinical benefit. Using these cutoff points, we divided the patients into 2 groups, those with a high VEGF level and those with a low level. All comparisons between these 2 groups did not show differences related to response.

SURVIVAL AND PROGRESSION

Survival was higher in the responder group ($P = .04$). However, none of the (rating) classifications according to the VEGF values (initial values, difference between second and sixth session, or those according to the receiver operating characteristic curves) showed significant differences in survival. Patients with a reduction of at least 30% after the sixth dose showed a statistically significant increase in time of progression ($P = .01$) (Figure 2). Patients with initially higher serum VEGF levels that persisted after the sixth dose showed a shorter time of progression ($P = .004$), that is, in these cases the disease advanced faster.

Patients with advanced head and neck cancer resistant to platinum-based chemotherapy have a very poor prognosis, with a low response rate and short survival. The purpose of chemotherapy in these patients is to control potential symptoms, prevent serious complications, increase survival, and improve quality of life. It is very important to provide a treatment that can be well tolerated and has few toxic effects. Frequent or continuous low-dose che-
malignant tumors including head and neck cancer.\textsuperscript{10-12} Vascular endothelial growth factor is overexcretion may help to inhibit angiogenesis-related processes. Vascular endothelial growth factor is overexpressed by solid malignant tumor cells and hematological malignant tumors including head and neck cancer.\textsuperscript{10-12} It has been shown that there is an association between serum VEGF level and tumor stage, and that circulating VEGF levels decrease after curative resection in some solid tumors.\textsuperscript{13-15} High serum VEGF level has been proposed as a predictor of tumor progression.\textsuperscript{15,20}

Paclitaxel appears to inhibit VEGF-induced angiogenesis and may improve the prognosis of patients with resistance to platinum-based chemotherapy. We prospectively analyzed the feasibility of testing serum VEGF levels as a surrogate marker of head and neck cancer angiogenesis and correlated these levels with disease outcome and response to chemotherapy with low doses of weekly paclitaxel in a selected group of patients with advanced head and neck cancer.

Our results showed that VEGF serum concentrations fall in response to paclitaxel therapy during the initial phase of the treatment but rise again by the end of the 6-week period. This evolution is observed in all patients and in all response subgroups, but it was only significant in the groups that presented response and clinical benefit (SD or either PR or CR group). Therefore, these preliminary results suggest an association between serum VEGF level and response and disease stabilization.

In light of experimental data suggesting that repeated low doses of chemotherapeutic agents might induce a stronger antiangiogenic effect than single high doses,\textsuperscript{21,22} metronomic scheduling has been found to show high rates of efficacy in preclinical\textsuperscript{23,24} and clinical studies in breast cancer\textsuperscript{1,2} and head and neck cancer.\textsuperscript{26}

Our study presents clinical evidence that metronomic chemotherapy may control the angiogenic process in advanced head and neck cancer. The results are consistent with previous experimental studies\textsuperscript{21} and findings for in vivo breast cancer.\textsuperscript{27} Weekly paclitaxel therapy seems able to inhibit cancer-related angiogenesis, at least at initial stages of treatment.

The exact mechanism by which paclitaxel reduces serum VEGF level is unknown. It not only induces the destruction of abnormal VEGF–producing cancerous cells but may also influence normal cells physiologically involved in VEGF secretion, such as macrophages, monocytes, leukocytes, and platelets.\textsuperscript{27,28} In any case, even though the exact mechanism of action is still to be clarified, a chemotherapy-induced decline in VEGF level could constitute a clinically important biomarker for anticancer therapies, as Lisoni et al\textsuperscript{27} proposed. Those authors suggested that serum VEGF level predicts the response only in patients with high initial levels. In addition, since VEGF has been shown to induce immune suppression,\textsuperscript{29} the decline in VEGF concentrations could have a favorable prognostic significance in terms of host anticancer immunity.\textsuperscript{27} Our results show that high VEGF levels prior to treatment that persist after treatment seem to be associated with faster progression. Furthermore, VEGF variations during therapy may aid prognosis in different subgroups of patients with cancer characterized by a similar clinical response defined according to RECIST criteria. These results may encourage specialists to test other new antiangiogenic agents such as bevacizumab to intensify and prolong inhibition of the angiogenesis and to improve tumor response in patients with advanced head and neck cancer. However, further studies involving a larger number of patients and a longer follow-up are required to establish the predictive value of VEGF serum levels in head and neck cancer.

\textbf{Submitted for Publication:} February 14, 2007; final revision received April 23, 2007; accepted May 13, 2007.\textbf{Correspondence:} Miguel Caballero, MD, PhD, Department of Otorhinolaryngology, Hospital Clinic, University of Barcelona, Villarroel 170, 08036 Barcelona, Spain (mcaba@clinic.ub.es).

\textbf{Author Contributions:} Dr Caballero had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. \textit{Study concept and design:} Caballero, Grau, Domingo-Domenech, Jimenez, and Bernal-Sprekelsen. \textit{Acquisition of data:} Grau, Auge, and Jimenez. \textit{Analysis and interpretation of data:} Caballero, Grau, Blanch, Domingo-Domenech, and Bernal-Sprekelsen. \textit{Drafting of the manuscript:} Caballero, Grau, and Blanch. \textit{Critical revision of the manuscript for important intellectual content:} Grau, Auge, Jimenez, and Bernal-Sprekelsen. \textit{Statistical analysis:} Caballero, Grau, and Domingo-Domenech. \textit{Obtained funding:} Caballero. \textit{Administrative, technical, and material support:} Blanch and Jimenez. \textit{Study supervision:} Grau, Domingo-Domenech, Auge, Jimenez, and Bernal-Sprekelsen.

\textbf{Financial Disclosure:} None reported.

\textbf{REFERENCES}


\textbf{Financial Disclosure:} None reported.

\textbf{REFERENCES}


\textbf{Financial Disclosure:} None reported.


