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A Phase II Trial of Single Agent Bevacizumab in Patients with Relapsed, Aggressive Non-Hodgkin Lymphoma: Southwest Oncology Group Study S0108

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Abstract

Purpose—In a phase II trial, SWOG S0108, patients with relapsed, aggressive non-Hodgkin lymphoma were treated with single agent bevacizumab. The primary endpoint was 6 month progression free survival (PFS) with secondary endpoints including response rate, toxicity, and laboratory correlative studies.

Patients and Methods—52 patients in first or second relapse with diffuse large B-cell or mantle cell lymphoma were enrolled. 45 patients were eligible. Patients were treated with bevacizumab at 10mg/kg every 2 weeks with disease assessments every 8 weeks. Biomarkers analyzed included plasma VCAM, urine VEGF, circulating endothelial cells (CEC), and VEGF and VEGF receptor expression in lymphoma specimens.

Results—Therapy was well tolerated with no unexpected toxicities observed. 6 month PFS was 16% with a response rate of 2%. 11 patients had prolonged stable disease. The median duration of response or stable disease was 5.2 months (range 3.5 – 72.7). VEGF and VEGF receptor expression was observed in 70% and 65% of specimens, respectively. There was excellent concordance between expression of VEGF and VEGF receptors. Baseline urine VEGF and VCAM levels correlated with survival. CEC levels decreased in patients with elevated baseline values.

Conclusion—Bevacizumab, as a single agent, is well-tolerated in patients with relapsed aggressive NHL, but has little single agent activity. Laboratory correlative studies and prolonged PFS in several patients suggest the VEGF pathway may play an important role in aggressive NHL. Future trials should test the hypothesis that antiangiogenic therapies will synergize with chemotherapy regimens in improving response rates and overall survival of patients with aggressive NHL.

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Introduction

Tumor-induced angiogenesis is necessary for tumor growth and metastasis. The overexpression of proangiogenic factors is frequently observed in malignancies and thought to be involved in tumor pathogenesis and progression. Of the many known inducers of tumor-induced angiogenesis, vascular endothelial growth factor (VEGF) plays the most important role.¹⁻³ VEGF promotes endothelial cell proliferation and migration and also acts as a survival factor required for maintenance of new vessels.⁴ Increased VEGF expression has been found in many tumor types, including NHL.⁵⁻⁹ Aggressive lymphomas often secrete vascular endothelial cell growth factor (VEGF) and express VEGF receptors.⁹⁻¹² In one study, 82% of aggressive histology lymphoma specimens expressed VEGF and VEGF receptor-1 (Flt-1) compared to only 22% of low grade or follicular lymphomas.¹²

Elevated levels of VEGF also correlate with worse overall survival and increased aggressiveness in patients with NHL.⁹⁻¹¹ Bevacizumab (Avastin) is a recombinant, humanized, monoclonal antibody that recognizes all known isoforms of VEGF.^{13,14} Bevacizumab, as a single agent, has shown antitumor activity in several tumor types, including breast cancer and renal cell cancer.^{15,16} In combination with chemotherapy, bevacizumab has shown substantial activity and has been FDA-approved for use in metastatic colorectal, lung, and breast cancers.¹⁷⁻¹⁹ Based on the antitumor activity of bevacizumab in other tumor types and the strong rationale for using anti-VEGF therapies in aggressive NHL, SWOG initiated the phase II study, S0108, to test biweekly intravenous bevacizumab as single agent therapy for patients with relapsed, aggressive NHL.

Patients and Methods

Study Design

S0108 was a non-randomized phase II clinical trial conducted through SWOG in patients with relapsed, aggressive non-Hodgkin lymphoma. All patients received bevacizumab at 10mg/kg every 2 weeks by intravenous bolus. Premedication with acetaminophen 650mg and diphenhydramine 25–50mg was allowed, but the use of steroids was prohibited. The first dose of bevacizumab was administered over a minimum of 90 minutes with further doses administered over a minimum of 30 minutes as tolerated.

Protocol therapy was continued until disease progression, the development of unacceptable toxicity, or until a maximum of 48 doses of bevacizumab was administered. Disease assessments were scheduled at week 8 of therapy and then every 3 months or as clinically indicated. Response criteria was per Cheson et al.²⁰ Toxicity assessments were per the NCI Common Toxicity Criteria (version 2.0).

All patients were informed on the investigational nature of the study and given written informed consent in accordance with institutional and federal guidelines.

Patient Selection

Patients in first or second relapse with NHL of the following aggressive subtypes were eligible: diffuse large B or T cell, mantle cell, anaplastic large cell, primary mediastinal, and high-grade Burkitt's or Burkitt-like. Patients with central nervous system involvement, or HIV infection were ineligible. Pre-induction chemotherapy followed by autologous bone marrow transplantation was considered one prior therapy. Prior rituximab therapy was allowed, but not within 12 weeks of registration. Other eligibility included Zubrod performance status 0–2, platelet count ≥ 75,000/ul, ANC ≥ 500, hematocrit ≥ 28%, PT ≤ 2 sec upper limit of normal (ULN), PTT within normal limits, Creatinine ≤ 1.5 ULN or Creatinine clearance ≥ 60 cc/ml, proteinuria ≤ 500 mg/24 hours, total bilirubin ≤ 2.0, and

liver transaminases <2.5 ULN and ≤ 5 ULN for patients with documented liver metastases. Other exclusion criteria included major surgery within 4 weeks, healing bone fractures, uncontrolled seizures or hypertension, receiving chronic steroids, pregnancy, history of transient ischemic attack, cerebral vascular accident, or myocardial infarction within 6 months, severe peripheral vascular disease, diabetic or venous stasis ulcers, or a history of deep venous or arterial thrombosis within the last 3 months.

Biomarker Methodology

Patient samples (whole blood, plasma, and urine) were collected at baseline, week 8, and at end of study. Tissue samples were preferentially obtained prior to registration, but also included initial diagnostic specimens if a biopsy was not deemed feasible. Bone marrow specimens were obtained from all patients initially and at progression of disease in patients with bone marrow involved.

Plasma vascular cell adhesion molecule-1 (VCAM) and urinary VEGF were quantified using enzyme-linked immunosorbent assay (ELISA) kits manufactured by R&D Systems (Minneapolis, MN).

Circulating endothelial cells (CEC) were measured by flow cytometry (FACScan Becton-Dickinson) on whole blood using a RBC lysis, no wash method. 100μL of ACD anticoagulated blood was incubated with anti-CD31-PE (BD PharMingen), anti-CD45-FITC (BD PharMingen), and LDS-751 (Molecular Probes). CECs were defined as the population of nucleated cells (LDS-751 positive), negative for the hematopoietic marker, CD45, and positive for the endothelial cell marker, CD31 (PECAM). The number of CEC/μL was calculated as: $(\text{WBC}/\mu\text{L}) \times (\% \text{CEC}/100)$.

Immunohistochemistry was performed on all diagnostic tissue samples for VEGF and its receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk/KDR) and correlated to histologic subtype. All immunohistochemical staining was performed using an automated in situ/immunostainer (Ventana Medical Systems, Tucson, AZ, Benchmark™, Discovery™) using a previously published method.¹² Antibodies to VEGF, VEGFR1, and VEGFR2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The degree of expression was judged at 400× magnification as 4+ (intense), 3+ (strong), 2+ (moderate), 1+ (faint), or 0 (negative) by one of the authors (WTB) blinded to the clinical results.

Statistical Analysis

The primary objective of the study was to estimate the 6-month progression-free survival (PFS) rate. Planned accrual was 60 eligible patients. However, due to a decreasing accrual rate, the study was closed with 52 patients (45 eligible). Forty-five eligible patients is sufficient to estimate the 6-month PFS rate, as well as response and toxicity rates, to within +/- 15% (95% confidence interval). Any adverse event occurring with at least 5% probability was likely to be seen at least once (90% chance). PFS was defined as the time from registration to the first observation of progressive disease or death due to any cause. Overall survival and PFS estimates were derived by the Kaplan-Meier method.²¹ Given historical data, the statistical design considered a 6-month progression-free survival estimate of 40% or greater to indicate that further investigation of this therapy at this dose and schedule would be warranted.

RESULTS

Clinical

Fifty-two patients were accrued to the study from 4/15/01 through 12/3/04. Seven patients were ineligible: 3 had > 2 prior relapses, 3 had follicular histologies after central pathologic review, and 1 patient had prestudy thrombocytopenia. Demographics for the 45 eligible patients are presented in Table 1. 58% had high-intermediate or high IPI risk scores on enrollment. Histologies treated included 30 diffuse and 15 mantle cell lymphomas.

All eligible patients have been evaluated for best response to protocol therapy. Two patients had inadequate disease assessment and were counted as non-responders. One patient with DLBCL had a partial response for a response rate of 2% (95% C.I.: <1 – 12%). Eleven additional patients had stable disease as their best response including 8 with DLBCL and 3 with mantle cell histologies. The median duration of response or stable disease was 5.2 months (range: 3.5–72.7 months) with one mantle cell patient having prolonged disease stability for over 72 months and one diffuse patient for 62 months. Forty-four of the 45 eligible patients have progressed or died. The primary endpoint of 6 month PFS was estimated at 16% (95% C.I.: 5% – 26%) with 6 month overall survival estimated at 53% (95% C.I.: 39% – 68%) (Figures 1A and 1B, respectively). There was no significant difference in the 6 month PFS (14 vs 14%) or OS (50 vs 55%) between mantle cell and DLBCL histologies, respectively.

All eligible patients were evaluated for toxicity. One patient died from a pulmonary embolism possibly treatment related and one patient died from a reportable adverse event not otherwise specified. One patient developed grade 4 fatigue, malaise, and lethargy. Grade 3 toxicities were observed in an additional 17 patients with fatigue being most common (Table 2). With regard to bevacizumab associated toxicities, there were no episodes of grade 3 or higher proteinuria, hemorrhage, or perforation. Grade 3 hypertension was reported in 2 patients and 3 patients had grade 3 dyspnea.

Immunohistochemistry Results

Immunohistochemistry (IHC) for VEGF and the receptors, VEGFR-1 (Flt-1) and VEGFR-2 (Flk/KDR), was evaluable in 20 tumor specimens (Table 3). VEGF and VEGFR-1 expression was found predominantly on lymphoma cells while VEGFR-2 staining was predominantly seen on endothelial cells in tumor related vessels (Fig 2). In approximately 50% of VEGFR-2 positive specimens, tumor cells exhibited some expression of VEGFR-2, but at a lower level of intensity compared to endothelial cells. Seventy percent of lymphomas tested positive for expression of VEGF including 7 of 11 DLBCL and 7 of 9 mantle cell specimens. There was excellent concordance between VEGF and VEGF receptor expression. All 7 DLBCL specimens that stained positive for VEGF also showed expression of one or both VEGF receptors. Similarly, all but one mantle cell specimen revealed concordant expression. Five patients had bone marrow involvement allowing for paired analysis pre and post therapy at time of progression. All but one patient had similar VEGF expression at both time points analyzed by IHC. In the exceptional patient, there was increased VEGF expression in the lymphoma cells after 32 weeks on bevacizumab therapy (expression increased from +1 to +3).

Biomarker Results

Urine VEGF and plasma VCAM were measured at baseline, week 8, and at conclusion of study. These angiogenic factors were chosen for analysis as they have been modulated or predictive of response to antiangiogenic therapies in prior clinical trials as well as in animal models.^{22–27} Urine, rather than plasma, VEGF was analyzed as bevacizumab interferes with

ELISA measurement of VEGF in plasma.^{28,29} Both urine VEGF and plasma VCAM are also stable for 24 hours at ambient temperature (data not shown).

Baseline values for all biomarkers showed a large range, but were higher than normal values suggesting an aggressive phenotype in our patients.^{9,10} Median and range values included 789 ng/ml for plasma VCAM (range 476 – 7601, n=28); 68 pg/ml for urine VEGF (range 4 – 401, n=32); and 33 for CEC/ μ l (range 2 – 552, n=32). There was a suggestion that baseline VEGF levels and plasma VCAM levels were associated with overall and progression free survival (Figures 3A–3B).³ Baseline biomarker values were not statistically different between histologic subtypes except for plasma VCAM which was significantly higher in mantle cell (median=1214) compared to DLBCL patients (median=750; p=0.04).

Over the course of study, plasma VCAM levels significantly increased (p=0.02). Urine VEGF levels decreased slightly from 67 to 51 pg/ml, but this did not reach statistical significance (p=.29). For CEC analysis, patients were divided into 2 groups based on their baseline CEC value (above or below the median). The rationale for this subset analysis was that only total CEC levels were measured. Several reports have now documented that levels of apoptotic CEC increase in response to antiangiogenic or metronomic therapies.^{30,31} Thus, patients with normal or lower levels of CEC increase their total CEC counts in response to therapy.³¹ In contrast, patients with elevated CEC levels should not see a rise in CEC number as the increase in apoptotic cells would be overshadowed by a larger decrease in the number of viable CEC induced by the antitumor or antiangiogenic therapy. In our patients (n = 10) with elevated CEC levels at baseline, a decrease in CEC was observed in all patients in response to therapy with median CEC levels falling from 81 at baseline to 22 CEC/ μ l at end of study. Conversely, patients with lower baseline CEC levels (n=12) increased or stabilized their CEC numbers in response to bevacizumab with their median levels increasing from 11 to 16 CEC/ μ l over the course of study.

Discussion

This is the first report of a phase II cooperative group trial of single agent bevacizumab as therapy for relapsed, aggressive histology NHL. While the overall response rate and 6 month PFS is disappointing, the results are not inconsistent with other trials of bevacizumab as a single agent.^{15,16} 27% of patients gained clinical benefit with partial response or stable disease for a median of 5 months, and in one patient with mantle cell lymphoma this benefit extended to almost 73 months. The completion of this trial in a cooperative group also strengthens the clinical observations as all pathology was confirmed by impartial review.

The laboratory correlative results support the findings of others that VEGF and its receptors are frequently expressed on lymphoma cells in tumors of aggressive histology.^{11,12,32–34} We also found high concordance between VEGF staining and expression of VEGF receptors in our specimens.^{11,32} Others have documented that VEGF expression in lymphoma specimens correlates with microvessel density suggesting an important role for VEGF in tumor-associated angiogenesis.^{11,34}

Our results regarding expression of VEGFR-1 primarily on lymphoma cells while expression of VEGFR-2 was primarily on lymphoma associated vessels is consistent with the role of these 2 receptors in physiologic and pathologic angiogenesis. While both receptors are expressed on endothelial cells, VEGFR-2 plays the primary role in endothelial cell proliferation, migration, and recruitment of precursor cells while VEGFR-1 acts as a decoy receptor to inhibit VEGF activation of the VEGFR-2 receptor and thereby modulate angiogenesis.³⁵ VEGFR-1 is also expressed on monocytes, macrophages, and hematopoietic

precursory cells and it is through activation of this autocrine pathway that growth factors and proteases are secreted that facilitate the capillary regression and neovascular formation characteristic of pathologic angiogenesis.^{35,36} In a xenograft model, Wang et al, implanted human lymphoma cell lines into NOD/SCID mice and then treated with neutralizing antibodies directed specifically at human or murine (host) VEGF receptors.³⁷ Only animals treated with antibodies directed at human VEGFR-1 or murine VEGFR-2 effectively inhibited the xenografts suggesting a role for VEGFR-1 in autocrine stimulation and VEGFR-2 in paracrine stimulation of the human lymphoma cells. Our immunohistochemistry results are consistent with these roles for VEGFR-1 and VEGFR-2.

Improved overall survival in DLBCL associated with increased expression of VEGF and VEGFR-1 receptors has also been described.³² The authors hypothesized that the VEGF-VEGFR-1 autocrine loop is important in lymphoma proliferation and survival, but that lymphomas dependent on this pathway are more susceptible to anthracycline based therapies.³² Our specimens were predominantly from relapsed patients who had failed prior anthracycline therapy. The high level of VEGF and VEGFR-1 expression found in these specimens suggests this pathway is still important in anthracycline treated patients.

Our biomarker results are limited by the small number of specimens analyzed, but confirm the poor prognosis associated with elevated circulating angiogenic factors.^{9,10} VCAM-1 is a glycoprotein expressed and secreted by endothelial cells in response to VEGF and inflammatory cytokines.²² Thus, it is a natural choice as a surrogate marker for angiogenesis.²² Others have documented elevated levels of VCAM in cancer patients and correlated elevated levels with tumor microvessel density, advanced stage, and non-response to therapy.^{22,40} The increase in plasma VCAM levels observed over the course of therapy is likely secondary to the low response rate and advanced stage of our patients. The high baseline levels of VCAM measured in our patients, up to 7600ng/ml, is consistent with increased levels of angiogenesis and/or elevated levels of inflammatory cytokines in relapsed NHL patients.

A decrease in elevated circulating CEC levels in response to therapy has been described by others, including in lymphoma patients treated with anti-angiogenic therapies.^{41,42} Measurement of CEC is non-standardized, and the best method and population of CEC to quantify has not been defined.⁴²⁻⁴⁴ The consistency of results seen in our population is encouraging and suggests that bevacizumab is effecting circulating endothelial cell survival. In addition, the number of apoptotic endothelial cells was not quantified and this may be a more relevant biomarker for monitoring antiangiogenic therapies.^{30,31}

The use of bevacizumab as a single agent was not effective in our population. Results from animal models and clinical trials suggest this agent is best utilized combined with effective antitumor therapies.^{17,18,36} High levels of VEGF in serum or by gene analysis on microarrays point to VEGF as a predictor of poor response to curative therapies or refractory/fatal disease in patients with aggressive NHL.^{34,45,46} Accumulating evidence suggests VEGF acts as an autocrine growth factor utilizing VEGFR-1 and a paracrine growth factor with VEGFR-2. Targeting these pathways earlier in lymphoma treatment and in combination with effective chemotherapy is a rationale next step. A clinical trial testing standard CHOP-rituximab therapy with bevacizumab in 13 patients with DLBCL has been published documenting the tolerability and feasibility of this treatment.³³ This trial measured the pharmacokinetics of rituximab and bevacizumab and found no interaction. We are currently enrolling patients in a SWOG phase II trial, S0515, of standard dose CHOP-rituximab with bevacizumab in patients with untreated, advanced DLBCL.

This is the first cooperative group study describing the use of a specific VEGF inhibitor in patients with relapsed, aggressive NHL. Our results confirm that VEGF and its receptors are frequently overexpressed on mantle cell and DLBCL. Circulating angiogenic markers including VEGF, VCAM, and CEC are elevated in these patients and high levels of urine VEGF and plasma VCAM predict worse outcomes. Whether these biomarkers can determine which subset of patients is most likely to benefit from antiangiogenic therapy awaits further analysis in larger clinical trials. Bevacizumab therapy was well-tolerated in this elderly and high risk population. The one fatal pulmonary embolism observed was not unexpected.⁴⁷ Additional trials testing the combination of bevacizumab with chemotherapy in newly diagnosed patients with aggressive NHL will determine the ultimate role of this agent in non-Hodgkin lymphoma.

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Figure 1A:

S0108 Progression-Free Survival

Figure 1B

Fig 1.
Kaplan-Meier curves: (A) progression free survival and (B) overall survival in all eligible patients

Fig 2. Immunohistochemical staining of a mantle cell lymphoma specimen showing (A) VEGF expression by lymphoma cells of +2 intensity ; (B) negative control; (C) VEGF-1 receptor expression on mantle cells of +2 intensity; (D) VEGF-2 receptor expression in tumor associated vessels of +2 intensity. (400X magnification)

Figure 3A

S0108 Progression-Free Survival By Baseline Urine VEGF

Figure 3B

S0108 Overall Survival By Baseline Urine VEGF

Figure 3C

Figure 3D

Fig 3.

Progression free and overall survival by Kaplan-Meier curves for patients based on their baseline level of urine VEGF (A, B) or plasma VCAM (C, D)

Table 1

Patient Demographics

Characteristic *	No. of patients* (%)
Age, yrs	
Median	70
Range	36–87
60 yrs of age	41 (91%)
Male	27 (60%)
White Race	44 (98%)
Stage II	4 (9%)
III	11 (25%)
IV	29 (66%)
Elevated LDH	24 (53%)
Extranodal disease	30 (67%)
Bone marrow involvement	15 (33%)
IPI Risk Factors	
0–1	6 (13%)
2	13 (29%)
3	21 (47%)
4–5	5 (11%)

* Counts may not sum to n=40 due to missing data

Table 2

Treatment – Related Toxicities *

Adverse Event	Toxicity Grade			
	1	2	3	4
Anemia	12	5	2	0
Anorexia	6	5	1	0
Fatigue	14	7	5	1
Dyspnea	0	3	3	0
Dehydration	0	2	2	0
Headache	6	3	1	0
Hypertension	3	0	2	0
Hyponatremia	3	0	2	0
Lymphopenia	0	3	2	0
Myalgia	6	2	0	0
Nausea	8	5	2	0
Neutropenia	3	0	2	0
Proteinuria	3	3	0	0
Stomatitis	4	3	0	0
Thrombocytopenia	6	0	3	0

* Includes grade 1 and 2 toxicities observed in > 10% of patients and grade 3 or 4 toxicities observed 2 events

Table 3

VEGF and VEGF receptor expression by Immunohistochemistry

	VEGF			VEGFR-1			VEGFR-2		
	+1	+2	+3	+1	+2	+3	+1	+2	+3
DLCL (N=11) *	3	1	3	3	3	1	2	3	2
Mantle Cell (N = 9)	3	0	4	2	3	1	2	3	1

Numbers correlate to the number of specimens staining positive at +1, +2, or +3 intensity. Counts may not sum to subgroup sample sizes due to missing data.

* includes 3 transformed specimens (2 expressed VEGF and Receptors)