Phase II Trials of Single Agent Anti-VEGF Therapy for Patients with Chronic Lymphocytic Leukemia (CLL)

Tait Shanafelt, M.D.[1], **Clive Zent, M.D.**[1], **John Byrd, M.D.**[2], **Charles Erlichman, M.D.**[3], **Betsy LaPlant, M.S.**[4], **Asish Ghosh, Ph.D.**[1], **Timothy Call, M.D.**[1], **Miguel Villalona-Calero, M.D.**[2], **Diane Jelinek, Ph.D.**[5], **Deborah Bowen, F.N.P.**[1], **Kristina Laumann, B.A.**[4], **Wenting Wu, Ph.D.**[4], **Curtis Hanson, M.D.**[6], and **Neil Kay, M.D.**[1]

[1]Department of Medicine, Mayo Clinic, Rochester, MN, USA

[2]Department of Medicine, Ohio State University, Columbus, OH, USA

[3]Department of Oncology, Mayo Clinic, Rochester, MN, USA

[4]Department of Health Sciences Research, Mayo Clinic, Rochester, MN, USA

[5]Department of Immunology, Mayo Clinic, Rochester, MN, USA

[6]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

Abstract

Between 2005 and 2008, we conducted separate phase II clinical testing of 3 distinct anti-VEGF therapies for patients with relapsed/refractory CLL. Collectively, 46 patients were accrued to trials of single-agent anti-VEGF antibody (bevacizumab, n=13) or 1 of 2 receptor tyrosine kinase inhibitors (AZD2171, n=15; sunitinib malate, n=18). All patients have completed treatment. Patients received a median of 2 cycles of bevacizumab, AZD2171, or sunitinib malate. All 3 trials were closed early due to lack of efficacy. No complete or partial remissions were observed. Individually and collectively, these studies indicate single-agent anti-VEGF therapy has minimal clinical activity for patients with relapsed/refractory CLL.

Keywords

chronic lymphocytic leukemia; angiogenesis; vascular endothelial growth factor (VEGF); therapy; bevacizumab; receptor tyrosine kinase inhibitor

INTRODUCTION

Despite significant improvement in response rates and progression-free survival with chemoimmunotherapy approaches, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) is uncurable with conventional treatment and novel non-cross resistant agents are needed. Angiogenesis and signaling via angiogenic cytokines have increasingly been recognized as an important process in the growth of both solid tumors[1] and hematologic malignancies[2] including CLL[3]. Early work in CLL demonstrated the CLL

Corresponding Author: Tait D. Shanafelt, M.D., 200 First Street, Rochester, MN 55902, Phone: 507 266-1154, FAX: 507 266-4972, shanafelt.tait@mayo.edu.

B-cell synthesizes and secretes pro-angiogenic molecules.[4] In addition, bone marrow microvessel density, a marker of angiogenesis, correlates with CLL disease stage[5,6] and identifies patients with a shorter progression free survival.[7] Other reports also suggest serum and urine levels of pro-angiogenic factors are increased in CLL.[8]

Based on this work, several preliminary investigations have explored the relationship between vascular endothelial growth factor (VEGF) and survival in CLL. VEGF is a physiologic stimulator of angiogenesis and is also believed to be a significant mediator of tumor angiogenesis.[8] CLL B-cells express VEGF receptors (R1 and R2)[9–11], and these receptors are constitutively phosphorylated.[12] Culture of CLL B-cells with exogenous VEGF is associated with increased levels of the anti-apoptotic proteins MCL1 and XIAP as well as a reduction in both spontaneous and drug induced apoptosis.[12,13] Furthermore, inhibition of VEGF receptor signaling with receptor tyrosine kinase (RTK) inhibitors *in vitro* decreases levels of the anti-apoptotic proteins MCL-1 and XIAP[12] and blocks both the cytoprotective effect of the CD40-CD40 ligand interaction as well as CD40-CD40 ligand induced increases in survivin and NF-kB.[14] VEGF has also been implicated in CLL B-cell migration [15,16] and can modulate expression of B-cell receptor signaling through effects on protein kinase C (beta) II.[17] In addition clinical studies found that patients with early stage CLL who had higher serum VEGF levels had significantly shorter progression free survival[8] and that pre-treatment plasma VEGF levels were associated with response to chemoimmunotherapy treatment in CLL patients.[18]

In aggregate, these results suggest that signaling via the VEGF pathway may be an important process in the pathogenesis of CLL and could provide an important therapeutic target for patients with this disease. This postulate also builds on the clinical trial data in solid tumors where anti-VEGF therapy has already been found to improve clinical outcome in patients with colon, lung, and renal cell carcinoma.[19–21] To test the efficacy of anti-VEGF therapy in CLL patients, we initiated and completed separate phase II clinical testing of 3 different anti-VEGF therapies for patients with relapsed/refractory CLL: AZD2171 (a potent, oral, pan VEGF receptor inhibitor), bevacizumab (a recombinant humanized monoclonal antibody to VEGF) and sunitinib malate (a multi-targeted, small molecule inhibitor of RTKs involved in tumor proliferation and angiogenesis including VEGFR-1, VEGFR-2, VEGFR-3, and platelet-derived growth factor receptor [PDGFR]).

MATERIALS and METHODS

We performed separate phase II trials evaluating the safety and efficacy of three distinct anti-VEGF or RTK inhibitor therapies each targeting a different aspect of VEGF receptor signaling (Figure. 1). Each trial was registered with the National Cancer Institute at clinicaltrials.gov (NCT00290810; NCT00398112; NCT00321724). All patients were required to have a confirmed diagnosis of CLL or SLL by standard criteria.[22,23] All three trials accrued relapsed or refractory CLL patients with an indication for treatment as defined by marrow failure, measurable and progressive lymphadenopathy, measurable and progressive lymphocytosis, and/or clinically significant constitutional symptoms due to CLL. All patients had received prior treatment (the AZD2171 trial required prior purine nucleoside analogue treatment; the trials of sunitinib malate and bevacizumab trials required prior purine nucleoside analogues and/or alkylating agent treatment). Mantle cell lymphoma was excluded in all patients by fluorescence *in situ* hybridization (FISH) assessment for the presence of the t(11;14) or immunophenotypic analysis (CD23 expression and intensity of surface immunoglobulin expression).

The eligibility criteria of all three trials required participants to have an Eastern Cooperative Oncology Group (ECOG) performance status of 0–2 along with adequate renal and hepatic

function. Bone marrow biopsy and baseline echocardiogram were required at study entry in all 3 trials. Concurrent chemotherapy, immunotherapy, radiotherapy, or steroid treatment was not allowed and patients who had received recent chemotherapy (< 4–6 weeks), antibody (4–8 weeks), or other experimental therapy (< 4 weeks) were not eligible. Patients with uncontrolled hypertension, significant proteinuria, severe thrombocytopenia, recent myocardial infarction or stroke were excluded from all 3 studies. Patients with QTc prolongation (> 500 msec), other arrhythmia, or on potentially pro-arrhythmic drugs were excluded from the trials of AZD2171 and sunitinib malate. Patients with recent gastrointestinal fistula, gastrointestinal perforation, a serious non-healing wound, ulcer or bone fracture were explicitly excluded from the trials of sunitinib malate and bevacizumab. Patients with known bleeding diathesis or with pathologic conditions carrying a high risk for bleeding (e.g. varices) were excluded from the bevacizumab trial, while those with pulmonary embolus in the last 12 months were excluded from the sunitinib malate trial. Those who had received prior anti-VEGF therapy were explicitly excluded from the sunitinib malate trial.

All three protocols were reviewed and approved by the Mayo Clinic Institutional Review Board and registered with the National Institute of Health (clinicaltrials.gov). All patients provided written informed consent prior to study enrollment in accordance with the Declaration of Helsinki. Toxicity was graded using NCI Common Terminology Criteria for Adverse Events version 3.0, except for hemoglobin and platelets which were graded according to the Grading Scale for Hematological Toxicity in CLL Studies.[22] Response was evaluated using the NCI-WG criteria.[22]

PROTOCOL TREATMENT FOR TRIAL 1: AZD2171

Protocol 1 was a multi-center phase II trial of AZD2171 conducted in the North Central Cancer Treatment Group (NCCTG). Patients received AZD2171 45 mg orally once daily on days 1–28 as part of 28 day cycles. Patients were assessed by physical examination, CBC, and chemistries prior to each cycle. Blood pressure (BP) was assessed prior to each cycle as well as assessed by patients twice daily using a home BP device with results recorded in a BP diary. Urinalysis evaluating for proteinuria was assessed prior to each cycle. Patients with significant proteinuria underwent 24-hour urine protein analysis. ECG was obtained at baseline and prior to each cycle to evaluate QTc prolongation. The dose of AZD2171 was reduced to 30 mg daily (dose level minus 1), 20 mg daily (dose level minus 2), or 10 mg daily (dose level minus 3) as indicated by adverse events. In general, for grade 3 adverse events AZD2171 was reduced by 1 dose level while for grade 4 adverse events AZD2171 was held and restarted at next lower dose level when symptoms had resolved to grade 2. Blood samples were obtained for research purposes at study entry, after every other cycle, and at time of progression. Patients continued on treatment until they experienced disease progression or prohibitive toxicity.

PROTOCOL TREATMENT FOR TRIAL 2: BEVACIZUMAB

Protocol 2 was a multi-center phase II trial of bevacizumab conducted through the Mayo Clinic and Ohio State University Phase II Consortia. Patients received bevacizumab 10 mg/kg intravenously, every 14 days (+/− 3 days) as part of 28 day cycles. Patients were assessed by physical examination, CBC, and chemistries prior to each cycle. BP and urine protein:creatinine ratio were assessed prior to each dose. Patients who developed a urine protein:creatinine >3 underwent 24-hour urine protein analysis. Bevacizumab was held in patients with grade 3 proteinuria. Bevacizumab was discontinued in patients with grade 4 proteinuria or those with bowel perforation, wound dehiscence, or arterial thromboembolic events (any grade). Blood samples were obtained for correlative research purposes at study

entry and prior to each cycle. Patients continued on treatment provided they did not experience disease progression or prohibitive toxicity.

PROTOCOL TREATMENT FOR TRIAL 3: SUNITINIB MALATE

Protocol 3 was a multi-center phase II trial of sunitinib malate conducted in the NCCTG. Patients received sunitinib malate 37.5 mg orally once daily on days 1–28 as part of 28 day cycles. Patients were assessed by physical examination, CBC, and chemistries prior to each cycle. BP was assessed weekly during cycle 1 and every 2 weeks during cycles 2–12. ECG was obtained at baseline and prior to cycle 2 to evaluate QTc prolongation. The dose of sunitinib malate was reduced to 25 mg daily (dose level minus 1) or 12.5 mg daily (dose level minus 2) as indicated by adverse events. In general, sunitinib malate was held for grade 3 adverse events and restarted at the next lower dose level when symptoms had resolved to   grade 1. Blood samples were obtained for correlative research purposes at study entry, after 8 weeks of treatment and at the time study treatments were discontinued. Patients continued on treatment for up to 12 cycles unless they experienced disease progression or prohibitive toxicity.

PLASMA LEVELS OF ANGIOGENIC CYTOKINES

To test the effect of anti-VEGF therapies on plasma levels of VEGF, we measured VEGF prior to treatment and after 8 weeks of therapy (for patients still on active treatment). VEGF (isoform 165) was measured using Quantikine kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions as previously described.[18]

STATISTICAL ANALYSIS

Response to treatment based on intent to treat analysis was the primary outcome for all three trials. Confirmed clinical responses (NCI-WG complete or partial remission[22,24]) on 2 consecutive evaluations at least 4 weeks apart were summarized by simple descriptive statistics. The same one-stage design with an interim analysis was used for each trial independently. A total of 32 evaluable patients were required to test the null hypothesis that the true response rate is at most 5% vs. the alternative hypothesis that it is at least 20%. Assuming that the number of responses is binomially distributed, each study had 90% power, with a 7% Type I error rate. In all 3 trials, an interim analysis was planned/conducted after 16 evaluable patients were enrolled, where at least one response was required to continue accrual. A patient was considered evaluable for response if they were eligible and received treatment. Progression-free survival (PFS) was defined as the time from registration to progression or death due to any cause. The distribution of PFS was estimated using the Kaplan-Meier method.

RESULTS

PATIENT DEMOGRAPHICS

Fifteen patients were accrued to the AZD2171 trial between May 2006 and June 2007, 13 patients to the bevacizumab trial between December 2005 and March 2009, and 18 patients to the trial of sunitinib malate between August 2007 and December 2008. One patient was deemed ineligible for the AZD2171 trial after receiving 1 cycle or treatment because he/she had not received prior purine nucleoside analogue treatment. One patient on the bevacizumab trial never received protocol treatment due to a high protein/creatinine ratio and high 24-hour urine protein excretion. Accordingly 14, 12, and 18 eligible patients were included in the outcome analysis for the AZD2171, bevacizumab, and sunitinib malate trials, respectively. Patient characteristics for the 3 trials are shown in Table 1.

The results of prognostic testing with ZAP-70, IGHV mutation status, CD38, and cytogenetic analysis by FISH generally demonstrated biologically aggressive disease typical of patients with relapsed/refractory CLL. Thus, with respect to high risk cytogenetic abnormalities, 39–50% of patients on the 3 trials had deletion 17p13 and/or deletion 11q23. Patients in all three trials had received a median of 2 prior therapies (range 1–10) and nearly all patients in each of the three trials had received prior purine nucleoside analogue based therapy and/or alkylating agent-based therapy.

TOXICITY AND TOLERABILITY

At the time of this report, all patients in all 3 trials have completed active treatment. Overall, 10 (71%) patients in the AZD2171 trial, 4 (33%) in the bevacizumab trial, and 16 (89%) in the sunitinib malate trial experienced a grade 3 or higher adverse event attributed to study medication. The frequency of specific side effects attributed to study therapy differed for the 3 agents tested and are shown in Table 2. In the AZD2171 trial, the most frequent grade 3 adverse events were thrombocytopenia (5/14 patients), fatigue (5/14 patients), diarrhea (3/14 patients), muscle weakness (3/14 patients) and hypertension (3/14 patients). In the bevacizumab trial, the most frequent grade 3 adverse events were proteinuria (2/12 patients) and fatigue (2/12 patients). In the sunitinib malate trial, the most frequent grade 3 adverse events were thrombocytopenia (10/18 patients), fatigue (6/18 patients), neutropenia (5/18 patients), and anorexia (4/18 patients).

Patients received a median of 2 cycles of AZD2171, 2 cycles of bevacizumab, and 2 cycles of sunitinib malate. In the AZD2171 trial, 5 patients discontinued treatment due to adverse events (grade 4 fatigue; grade 3 infection and fatigue; grade 3 peripheral neuropathy of the hands; grade 3 hypertension; grade 3 reversible posterior leukoencephalopathy syndrome), 7 due to disease progression, 1 due to patient refusal, and 1 at their physician's discretion. In the bevacizumab trial, 3 patients discontinued treatment due to an adverse event (grade 2 hypertension; potential retinal detachment and subsequently grade 3 fatigue; worsening performance status and grade 3 fatigue), 7 due to disease progression, 1 due to patient refusal, and 1 at their physician's discretion. In the sunitinib malate trial, 4 patients discontinued treatment due to an adverse event (low platelets; grade 3 rash; neutropenia; grade 3 platelets), 9 progressed, 1 received alternative treatment (rituxan and fludarabine), 3 due to patient refusal, and 1 died on study.

RESPONSE TO THERAPY

All three trials were closed early due to lack of efficacy. No complete or partial remissions were observed (Table 3). Although no complete or partial responses were obtained, 5/14 patients on AZD2171, 10/12 patients on bevacizumab, 10/18 patients on sunitinib had stabilization of disease for a median duration of 2.7, 2.9, and 4.4 months respectively. The ALC values declined by at least 10% during treatment for 5/14 patients on AZD2171, 3/12 patients on bevacizumab, and 6/18 patients on sunitinib malate.

CORRELATIVE STUDIES

To evaluate the effect of treatment on plasma levels of VEGF at baseline and 8 week follow-up, plasma samples were collected from patients on the trials of bevacizumab and sunitinib malate. Unfortunately, since few patients completed at least 8 weeks of active treatment, baseline and 8 week follow-up serum specimens while still on active treatment were only available for 5 patients on the bevacizumab trial and 3 patients on the trial of sunitinib malate. A decline in plasma VEGF levels after 8 weeks of treatment was observed in 4 of the 5 patients on the bevacizumab trial as compared to 1 of the 3 patients on the sunitinib malate trial (Figure 2). Leukemia cell specimens intended to allow assessment of VEGF receptor phosphorylation status were also collected and cryopreserved at baseline and after 8

weeks of therapy in all 3 trials. However, on thawing of cells to make lysates, the high level of spontaneous cell death precluded meaningful analysis.

DISCUSSION

We report here the first 3 trials evaluating anti-VEGF therapy for patients with CLL. The VEGF pathway was inhibited using either anti-VEGF anti-bodies or by blocking VEGF receptor tyrosine kinase. Despite the strong biologic basis for testing anti-VEGF therapy in patients with CLL, minimal clinical activity (stabilization of disease; declines in ALC) was observed and all 3 trials were closed early due to lack of efficacy. Individually and collectively, the results of the 3 studies show that single agent anti-VEGF therapy has minimal clinical efficacy for patients with relapsed/refractory CLL.

These findings should be interpreted with a number of important caveats. First, all 3 trials enrolled heavily pretreated CLL patients with a high proportion of patients having del 17p13 and del 11q23, a very difficult group of patients to treat. Second, although single agent anti-VEGF therapy has clear clinical activity in some human malignancies, [21,25–28] in others the benefits are primarily observed when anti-VEGF therapy is combined with chemotherapy.[19,20] In this regard, the anti-VEGF therapies tested were generally well tolerated and combination with other CLL treatment approaches appears feasible. Since pre-treatment VEGF levels have been found to predict response to chemoimmunotherapy treatment in CLL patients,[18] and to upregulate anti-apoptotic proteins known to relate to chemotherapy resistance[12,13] testing of anti-VEGF therapy in combination with chemotherapy appears warranted. Third, the lack of efficacy in these 3 trials could reflect either i) failure to inhibit the intended biologic target (VEGF signaling) or ii) effective targeting but a lack of biologic effect. Although the doses of bevacizumab and AZD2171 tested were consistent with the established doses for treatment of solid tumors, we were unable to definitively determine whether the doses used effectively inhibited the VEGF pathway in the limited correlative studies performed because too few patients were still on active therapy at the time the follow-up samples were collected. The dose of sunitinib malate (37.5 mg daily, continuous) was slightly different from the standard dose in solid tumors (50 mg daily for 4 weeks followed by 2 weeks off); however, this was the dose recommended by the NCI for testing in hematologic malignancy at the time the trial was initiated and the total dose over a 6 week interval (daily, continuous: 1575 mg; 4 weeks on/2 weeks off: 1400 mg) is similar in both schedules. Fourth, aberrant angiogenesis in human malignancy is now recognized as a multi-step process that is regulated at least by a complex balance of multiple pro and anti-angiogenic molecules[29]. It is possible that targeting VEGF signaling alone may be inadequate to kill CLL cells.

Despite the lack of clinical activity observed in these trials, scientific insights continue to indicate that VEGF and other related angiogenic events play a role in CLL biology.[3] These include recent studies indicating that marrow vascular density is significantly higher in CLL patients with high risk FISH and CD38 positivity,[30] a pro-angiogenic profile favors disease progression,[31] circulating endothelial cells correlate with more advanced disease stage,[32] pro-angiogenic molecules such as angiopoietin-2 and matrix metalloproteinase 9 are associated with progressive CLL,[33,34] and that use of combination chemoimmunotherapy may work in part via anti-angiogenic effects.[35] Newer VEGF receptor RTK inhibitors have also recently demonstrated activity against CLL B-cells *in vitro* as well as in a xenograft model and appear to increase the efficacy of purine nucleoside analogues against CLL on *in vitro* testing.[36] These observations suggest VEGF inhibition remains a potential therapeutic target in CLL and suggest combining anti-VEGF therapy with more traditional therapeutic agents may be a useful strategy for patients with this

disease. Indeed we and others have already initiated clinical trials exploring the benefits of this approach as part of efforts to improve outcomes for patients with CLL.

Acknowledgments

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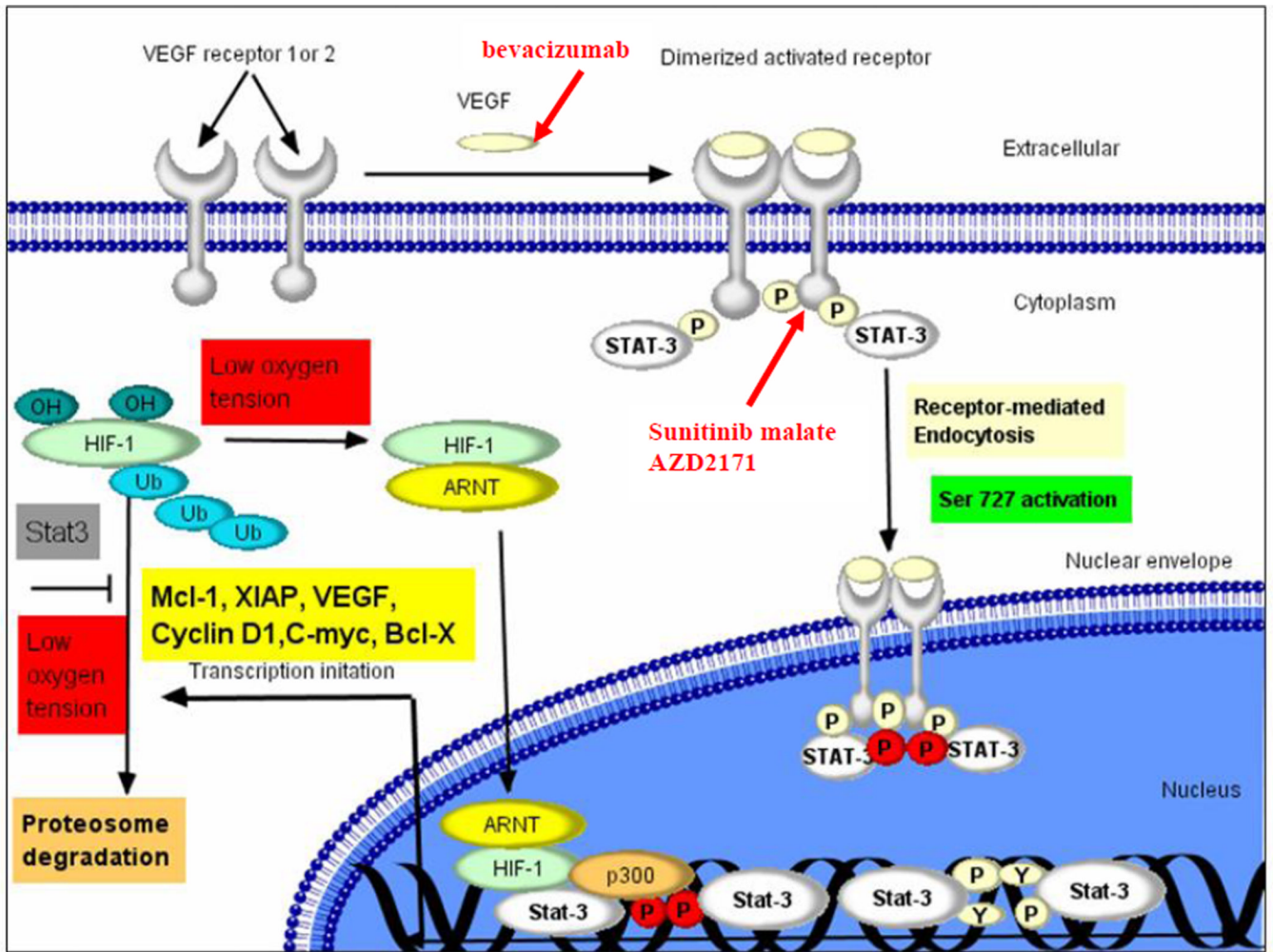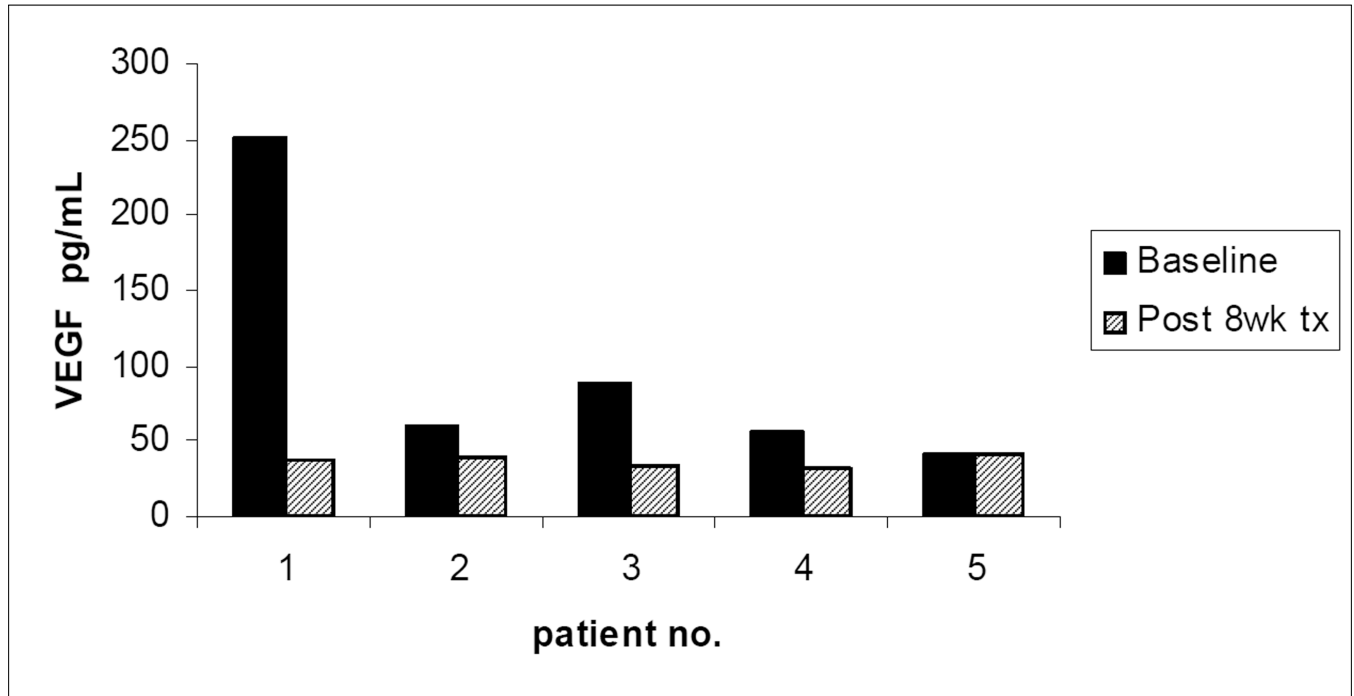
Figure 1. VEGF Pathway in CLL
Figure shows scheme of VEGF receptor signaling pathway in CLL as well as aspects of pathway targeted by bevacizumab, AZD2171, and sunitinib malate.
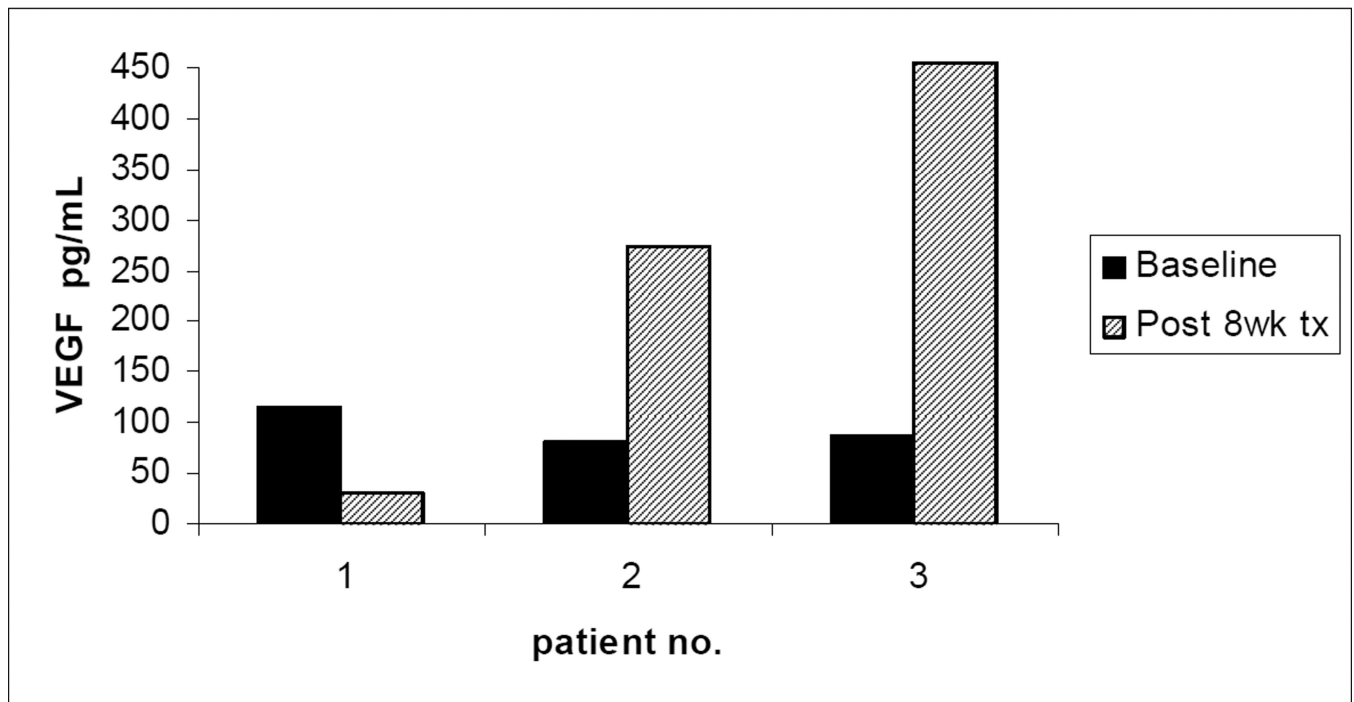
A.

B.

Figure 2. Plasma VEGF Levels Before and After 8 Weeks of Treatment
A. Plasma levels of VEGF before and after 8 weeks of bevacizumab therapy (n=5)
B. Plasma levels of VEGF before and after 8 weeks of sunitinib therapy (n=3)

Table 1

Patient Characteristics

	AZD2171 N=14	Bevacizumab N=12	Sunitinib malate N=18
DEMOGRAPHIC CHARACTERISTICS			
AGE (Median; range)	64.5 (44.0–77.0)	73.0 (60.0–80.0)	67.5 (51.0–89.0)
MALE	10 (71.4%)	5 (41.7%)	9 (50%)
ALC (Median; range)	46.5 (8.3–225.1)	125 (9.3–760.9)	35.1 (0.4–134.7)
STAGE			
Low (Rai 0)	0	0	0
Intermediate (Rai I–II)	6 (42.9%)	2 (16.6%)	8 (44.4%)
High (Rai III–IV)	8 (57.1%)	10 (83.3%)	10 (55.6%)
ZAP-70			
Positive	7 (50%)	5 (41.7%)	10 (55.6%)
Negative	7 (50%)	7 (58.3%)	8 (44.4%)
CD38			
Positive	10 (71.4%)	3 (25%)	9 (50%)
Negative	4 (28.6%)	9 (75%)	9 (50%)
FISH			
Del (17p13)	0 (0%)	4 (33.3%)	6 (33.3%)
Del (11q23)	6 (46.2%)	2 (16.7%)	1 (5.6%)
Trisomy 12	1 (7.7%)	2 (16.7%)	5 (27.8%)
Del 13q14	3 (23.1%)	2 (16.7%)	2 (11.1%)
Other	1 (7.7%)	1 (8.3%)	0 (0%)
Normal	2 (15.4%)	1 (8.3%)	4 (22.2%)
Missing	1	0	0
IGHV			
Unmutated	3 (75%)	3 (50%)	1 (100%)
Mutated	1 (25%)	3 (50%)	0
Missing	10	6	17
PRIOR TREATMENT:			
# prior therapies (Median; range)	2.0 (1.0–10.0)	3.0 (1.0–10.0)	2.5 (1.0–7.0)
Prior purine analogue treatment	14 (100%)	10 (83%)	14 (78%)
Prior alkylating agent treatment	13 (93%)	10 (83%)	17 (94%)
Prior rituximab	13 (93%)	12 (100%)	15 (83%)
Prior alemtuzumab	2 (14%)	1 (8%)	3 (17%)
Time (days) from last prior treatment (Median; range)	365.5 (39–1085)	249.5 (47–1410)	386.5 (62–1816)

Table 2

Grade 3–4 Adverse Events Attributed to Study Treatment

CTCAE Classification	AZD2171 N=14	Bevacizumab N=12	Sunitinib malate N=18
Platelet Count Decreased	5	0	10
Fatigue	5	2	6
Neutrophil Count Decreased	2	0	5
Anorexia	1	0	4
Diarrhea-No Colostomy	3	0	2
Headache	2	1	1
Hypertension	3	0	0
Muscle Weakness	3	0	0
Hyperkalemia	1	0	1
Nausea	2	0	0
Proteinuria	0	2	0
Clostridial Infection	0	0	1
Dehydration	0	0	1
Dyspnea	0	1	0
Edema Limbs	0	0	1
Hyponatremia	1	0	0
Hypothyroidism	1	0	0
Neuro	1	0	0
Petechiae	1	0	0
Rash	0	0	1
Rectum Infection	0	0	1
Skin Reaction-Hand/Foot	0	0	1
Urinary Tract Infection	1	0	0
Vision	0	1	0
Voice Change	1	0	0

Table 3

Number of Cycles Received and Response to Treatment

	AZD2171 N=14	Bevacizumab N=12	Sunitinib malate N=18
# Cycles administered (median)	2	2	2
BEST RESPONSE			
Complete remission	0	0	0
Partial remission	0	0	0
Stable disease	5	10	10
Progression	6	2	6
PROGRESSION FREE SURVIVAL (median)	1.8 months (95% CI: 1.0–3.4)	2.9 months (95% CI: 1.8–3.7)	2.7 months (95% CI: 1.8–4.6)