High-Dose Celecoxib and Metronomic "Low-dose" Cyclophosphamide Is an Effective and Safe Therapy in Patients with Relapsed and Refractory Aggressive Histology Non – Hodgkin's Lymphoma

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Abstract

Purpose: Angiogenesis is increased in aggressive histology non – Hodgkin's lymphoma and may be a target with selective cyclooxygenase-2 inhibition and metronomic chemotherapy.

Experimental Design: We assessed response, toxicity, and biomarkers of angiogenesis to low-dose cyclophosphamide (50 mg p.o. o.d.) and high-dose celecoxib (400 mg p.o. b.i.d.) in adult patients with relapsed or refractory aggressive non – Hodgkin's lymphoma in a multicenter phase II prospective study.

Results: Thirty-two of 35 patients (median age, 62 years) are evaluable for response. Patients had primarily relapsed diffuse large B-cell lymphoma (63%) were heavily pretreated (median of three regimens) and high risk (79% international prognostic index, $\geq$2) and 34% were relapsed after autologous stem cell transplant. With a median follow-up of 8.4 months, the overall best response rate is 37% (2 complete clinical response/complete clinical response unconfirmed and 9 partial response), with 22% achieving stable disease. Median overall and progression-free survivals are 14.4 and 4.7 months, respectively. The median response duration was 8.2 months. The most common toxicity was skin rash (40%); myelosuppression and gastrointestinal side effects were uncommon. Three patients developed deep vein thromboses and two heavily pretreated patients developed treatment-related acute myelogenous leukemia or myelodysplasia after 3.7 and 12 months of therapy. Circulating endothelial cells and their precursors declined and remained low in responders, whereas plasma vascular endothelial growth factor trended to decline in responding patients but increase in nonresponders. Trough celecoxib levels achieved targeted "antiangiogenic" levels.

Conclusions: Low-dose cyclophosphamide and high-dose celecoxib is well tolerated and active in pretreated aggressive non – Hodgkin's lymphoma. Close surveillance for arterial and venous thrombotic events is recommended. The decline in circulating endothelial cells and their precursors suggests that this combination may be working by inhibiting angiogenesis but should be validated in a larger patient sample.

Despite the recent application of combination chemotherapy and recombinant chimeric antibody treatment with rituximab or radioimmunotherapy for B-cell lymphoma, treatment of relapsed aggressive histology non – Hodgkin's lymphoma is suboptimal and new approaches are needed. Recent data indicate that angiogenesis is important in the pathophysiology and prognosis of aggressive histologic subtypes of non – Hodgkin's lymphoma (1 – 5). Angiogenesis is a multistep process that leads to the formation of new blood vessels from existing vasculature and is associated with the growth and dissemination of malignant tumors.

Kerbel et al. (6) and others (7) have shown that the use of chronic, low-dose chemotherapy administered at close regular intervals with no prolonged breaks (termed "metronomic" chemotherapy) may inhibit angiogenesis by targeting genetically stable endothelial cells of the neovasculature and circulating proangiogenic bone marrow – derived cells, including circulating endothelial progenitor cells (CEPs) (8, 9). The antiangiogenic properties of low-dose chemotherapy are potentiated in preclinical models by the addition of a second agent targeting the vasculature, such as a vascular endothelial growth factor (VEGF) receptor antagonist (10, 11).

Cyclooxygenase 2 (COX-2) is a prostaglandin synthase enzyme that has been implicated in tumorigenesis (12, 13).

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One of the proposed mechanisms by which this may occur is by stimulating angiogenesis through the production of proangiogenic factors including VEGF, basic fibroblast growth factor, platelet-derived growth factor, transforming growth factor-β1, and endothelin-1. COX-2 inhibitors, such as celecoxib, have been shown to reduce the incidence of neoplastic lesions in familial adenomatous polyposis (14) and therefore may have a role in the treatment of established malignancies. Furthermore, COX-2 is overexpressed in some lymphomas and is of potential prognostic importance (15–17).

These preclinical data provide the rationale for using low-dose chemotherapy together with a selective COX-2 inhibitor in the treatment of aggressive histology non–Hodgkin's lymphoma. We chose to test cyclophosphamide based on available clinical and preclinical data (18, 19), in combination with high-dose celecoxib, because of the substantial clinical experience with this agent, indicating its tolerability. In addition to the clinical outcomes of response rate and toxicity, we evaluated several biomarkers of angiogenesis, including plasma VEGF and circulating endothelial cells (CECs) and their precursor subset (CEPs), to further define their ability to predict response in a population of patients with relapsed aggressive histology non–Hodgkin's lymphoma.

Patients and Methods

From July 2001 to December 2004, 35 patients with relapsed or refractory aggressive histology non–Hodgkin's lymphoma were entered on this phase II study conducted at two centers. The planned study design was dual stage (10 patients, stage 1; 19 patients, stage II) aimed at detecting an overall response rate of >20% (β, 0.2; α, 0.05) and allowing 10% for loss to follow-up. The primary objective was to determine the response rate to oral high-dose celecoxib and daily oral low-dose cyclophosphamide continuous administration in this patient population. The secondary objectives were to define the toxicity and tolerability (compliance) of treatment and identify biomarkers that might indicate an antiangiogenic effect of this therapy.

Patients were considered eligible if they had relapsed after any number of preceding therapies (as long as one had included an anthracycline) and had a projected life expectancy of ≥4 months. Patients who relapsed following autologous hematopoietic stem cell transplant were eligible. Patients with T cell, transformed or mantle cell lymphomas were also included. Patients were considered ineligible for the study if they were transplant eligible, receiving concurrent chemotheraphy or radiotherapy (including corticosteroids) or had received any other antineoplastic therapy within the preceding 2 weeks. Patients with Eastern Cooperative Oncology Group performance status of >3, uncontrolled hypertension, and unstable cardiovascular or significant renal disease were also excluded. Because celecoxib is a sulphonamide, patients with a proven allergy to sulfa drugs were excluded, and patients with the triad of acetylsalicylic acid intolerance, asthma, and nasal polyposis were also excluded. Adequate hematologic (hemoglobin count, >85 g/L; absolute neutrophil count, >1,000/mm^3; platelet count, >75,000/mm^3), renal (serum creatinine, ≤125 μmol/L), and hepatic (total bilirubin, <35 μmol/L; alkaline phosphatase, <2× normal; alanine aminotransferase/aspartate aminotransferase, ≤2× normal) functions were mandatory. All patients had to have at least one bidimensionally measurable target lesion. The protocol was reviewed and approved by the institutional review boards of Sunnybrook and Women's College Health Sciences Center and Princess Margaret Hospital and informed consent was obtained from all patients. Separate institutional review board approvals were obtained during the trial for a substudy to carry out CEC/CEP assays and pharmacokinetics.

Pretreatment evaluations included a complete history and physical examination, routine laboratory evaluation, and computed tomography of chest, abdomen, and pelvis. Bone marrow biopsy was done if it was previously involved. Patients were clinically assessed by physical exam and blood work monthly for 6 months and then every 2 months until progression. Computed tomography scans were repeated for response evaluation every 3 months or sooner if clinically indicated. Patients were followed off study until death.

Treatment plan. All patients received celecoxib 400 mg p.o. b.i.d. (with food) and cyclophosphamide 50 mg p.o. daily. Celecoxib could be reduced on two occasions (200 mg p.o. b.i.d. then 100 mg p.o. bid) for grade ≥3 nausea and vomiting, dyspepsia, or abdominal pain or a 50% increase in serum creatinine or liver enzymes. Greater than two dose reductions or any gastrointestinal bleed grade ≥2 resulted in study discontinuation. Similarly, cyclophosphamide could be reduced twice (to 25 mg p.o. daily then 25 mg p.o. alternate days) for grade ≥3 neutropenia or thrombocytopenia. No dose escalations were permitted.

Response. Response to treatment was assessed according to criteria of Cheson et al. (20). An objective response consisted of complete clinical response, complete clinical response unconfirmed, or a partial response. Progression of disease was determined by an increase of 50% in the product of perpendicular diameters of the index lesions or the appearance of new lesions. All other patients were considered to have stable disease. Because antiangiogenic therapy is cytostatic, and in many preclinical models of metronomic chemotherapy, tumors may initially grow before they stabilize and sometimes regress (10, 19), we allowed patients to remain on study for up to 4 months if radiologic or clinical tumor progression was asymptomatic and not a threat to vital organ function. If no stabilization was observed by 4 months or if patients were symptomatic at any time with progression, they were removed from study.

Pharmacokinetics of celecoxib. Celecoxib pharmacokinetics were measured over a 12-hour period in patients who gave separate consent for these assays. Five milliliters of blood were collected in EDTA tubes just before and at 0.5, 1, 3, 6, and 12 hours after the first dose of celecoxib was administered. All samples were immediately placed on ice and centrifuged at 2,000 rpm for 10 minutes at 4°C within 15 minutes. The plasma was aliquoted and frozen at −80°C until analysis.

Celecoxib was analyzed using specific and sensitive high-pressure liquid chromatography assays. Estimates of pharmacokinetic variables for each drug studied were derived from individual concentration-time data sets by noncompartmental analysis using the software package WinNonlin (version 4.0.1, 1998-2002; Pharsight Corp., Mountain View, CA) as described previously (21).

Evaluation of CECs and CEPs by flow cytometry. The analysis of patient CECs and CEPs commenced on November 2003. Samples were collected at baseline, monthly for 6 months and then every 2 months thereafter. Enumeration of CECs and CEPs was carried out on peripheral blood collected in EDTA tubes followed by enumeration using four-color flow cytometry as described previously with some modifications (22). A panel of monoclonal antibodies, including anti-CD45, to exclude hematopoietic cells, anti-CD31, anti-CD146, and anti-CD133 (23, 24) and appropriate analysis gates were used to enumerate CECs and CEPs and exclude hematopoietic cells. Nuclear staining (Procount, BD Biosciences, San Jose, CA) was conducted to exclude the possibility of platelets or cellular debris interfering with the accuracy of CEC and CEP enumeration. After red cell lysis, cell suspensions were evaluated by a FACSCalibur cell analyzer and Cellquest Pro acquisition and analysis program (BD Biosciences) using analysis gates designed to exclude dead cells, platelets, and debris. Acquisition of 100,000 events per sample was obtained to analyze the percentage of CECs/CEPs. The absolute number of CECs/CEPs was then calculated as the percentage of the events that were collected in the CEC and CEP enumeration gates, multiplied by the total white count. Percentages of stained cells were determined and compared with appropriate negative controls. Positive staining was defined as being

greater than nonspecific background staining, and 7-aminoactinomycin D was used to enumerate viable versus apoptotic and dead CECs (25).

VEGF plasma concentrations. Blood samples were collected at baseline, monthly for 6 months, and then every 2 months in plasma separating tubes (Becton Dickinson, Franklin Lakes, NJ) and centrifuged at 4°C and plasma was immediately frozen and stored at −20°C until assayed. All the samples were thawed at 4°C only at the time of the ELISA analysis. The ELISA was done according to the protocol provided by the manufacturer (R&D Systems; Minneapolis, MN).

Statistics. The study sample size of 32 patients was powered to detect a response rate >20% with 95% confidence intervals (95% CI) of 3.2% to 36.8%. Results were expressed as the mean ± SD for quantitative variables and as proportions for categorical findings. Estimate of tumor response rate and its 95% CI was obtained based on binominal probability. Time-to-event distributions for the overall survival and progression-free survival were estimated using the Kaplan-Meier estimate. The general linear mixed model, also termed the hierarchical linear model, was used to determine whether there was an intervention effect on the biomarkers over time (26). The general linear mixed model is a flexible statistical procedure that is widely used for analyzing continuous longitudinal data and easily accommodates missing values and mistimed data. This approach models a curve across time, where time is included in the model as a continuous explanatory variable. It accounts for the correlation present across the repeated measures within each subject. We used PROC MIXED in SAS version 9.1 (SAS Institute, Cary, NC) to conduct this analysis. Results were considered significant at the 5% critical level ($P < 0.05$) after adjusting for multiple testing.

Results

Thirty-five patients were enrolled, and three were subsequently excluded from analysis: one due to incorrect diagnosis (small lymphocytic lymphoma), one due to early protocol violation, and one due to withdrawal of patient consent. Patient characteristics are listed in Table 1. The median age was 63 years (range, 22-83) and the majority of patients were male. Most patients were heavily pretreated, with a median of three prior chemotherapy regimens (range, 1-7) and a median of 10 prior cycles of chemotherapy (range, 3-21). Eleven (34%) had relapsed after autologous hematopoietic stem cell transplant. Seventy-seven percent had two or more international prognostic index risk factors at enrollment (27). The median times from diagnosis, last treatment, and last relapse/progression were 30, 8, and 1 months, respectively.

Table 1. Patient characteristics

Characteristics	No. patients (%)
Sex	
Male	20 (62)
Female	12 (38)
Age (y)	
Median	64
Range	22-83
Diagnosis	
Diffuse large cell	20
Mantle cell	1
Peripheral T cell	2
Transformed follicular	4
Transformed small lymphocytic	1
Anaplastic large cell	3
Follicular large cell	1
Prior no. chemotherapy regimens (no. cycles)	
Median	3 (10)
Range	1-7 (3-21)
Progressive disease to preceding therapy	6 (19)
Prior autograft	11 (34)
International prognostic index risk factors at enrollment	
0	3 (9)
1	4 (12)
2	13 (40)
3	9 (28)
4	3 (9)
Time from diagnosis (mo)	
Median	30
Range	6-113
Time from last treatment (mo)	
Median	8
Range	0.7-50
Time from last relapse or progression date (months)	
Median	1
Range	0-15

Response rates. Twelve patients (37.5%; 95% CI, 20.7-54.3) had an objective response to therapy. Two patients achieved complete clinical response and complete clinical response unconfirmed at 10 and 6 months, respectively, and 10 achieved partial response at a median time of 2.9 months (range, 2.6-9.8). Seven patients had stable disease and 13 patients progressed on treatment at a median time of 1.6 months (range, 0.2-3.1). Of the responders, eight ultimately progressed while on therapy. Twenty patients have died at a median time of 6 months (range, 0.8-38) from study enrollment, all of progressive disease. Six patients were withdrawn without disease progression for the following reasons: two with partial response at 21 and 16.5 months because of physician concern about cumulative cyclophosphamide exposures, one with partial response at 12.5 months for patient withdrawal of consent, and one with partial response at 2.6 months for asthenia and anorexia. Two patients with stable disease were withdrawn at 3 and 3.7 months for grades 3 and 4 neutropenia or thrombocytopenia. Nonresponders (60%) had an elevated lactate dehydrogenase at baseline compared with 16.7% of responders. All 12 responders showed chemosensitivity to their preceding chemotherapy regimen, whereas none of the patients with progressive disease on therapy before study enrollment responded to cyclophosphamide and celecoxib.

Overall and progression-free survival. With a median follow-up of 8.4 months (range, 0.9-38), the median actuarial progression-free was 4.7 months (95% CI, 2.5-9.2) and median overall survival was 14.4 months (95% CI, 6.2-23; Fig. 1). Median actuarial progression-free survival, overall survival, and response durations for responders were 13.6 (95% CI, 2.6-34.6), 31.6 (95% CI, 18.9-38), and 8.2 (95% CI, 0-29) months, respectively (Fig. 1).

Adverse events. The adverse events experienced by patients on this study are summarized in Table 2. There were few grade 3 and 4 toxicities. The most common adverse events were grades 1 and 2 skin rashes developing early on therapy, which were attributed to celecoxib. Skin rashes were treated symptomatically with agents that included topical corticosteroid and oral antihistamines. Celecoxib was discontinued for up to

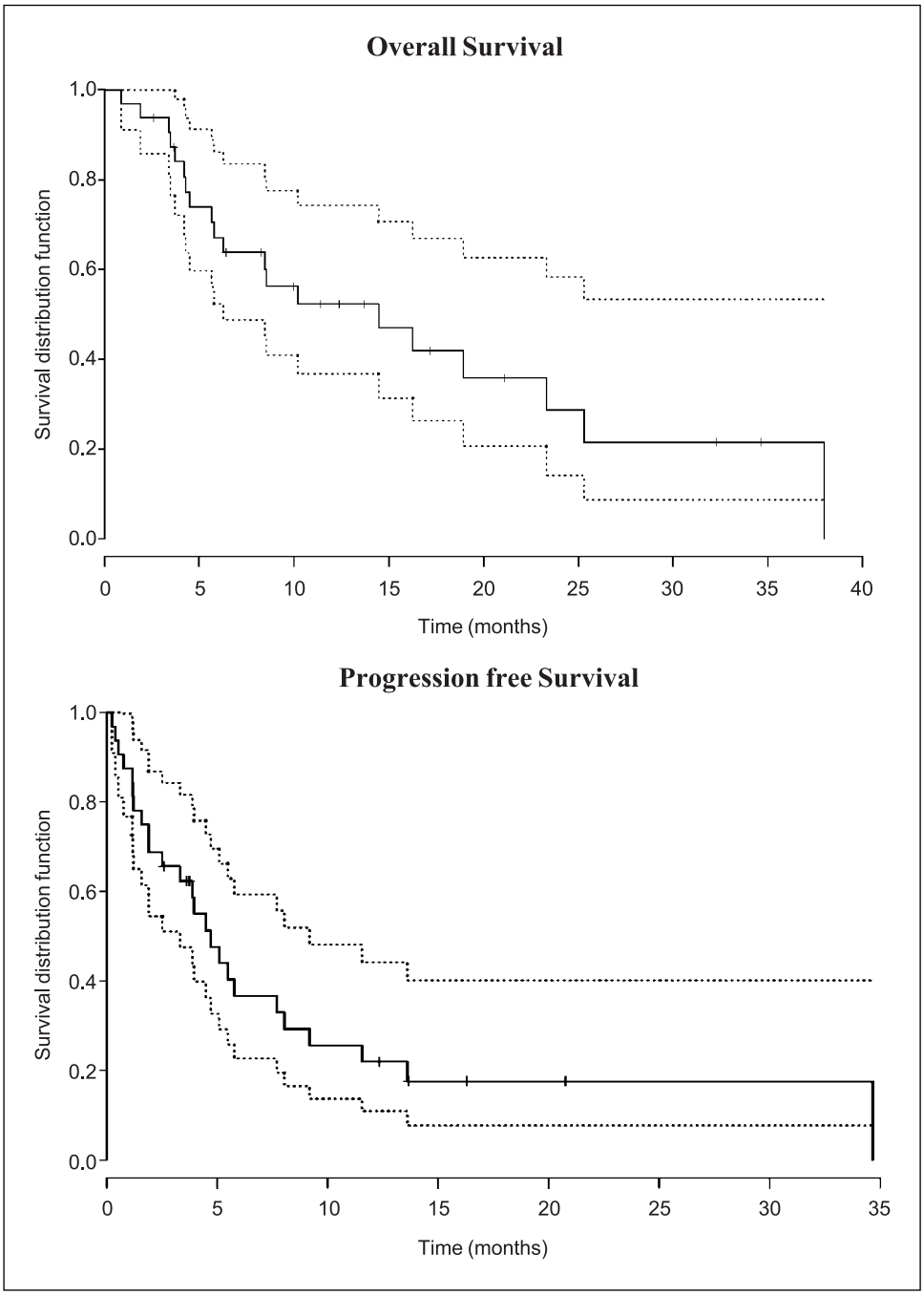


Fig. 1. Kaplan Meier survival curves with 95% CIs.

28 days in three patients and then reinstituted without difficulty.

Adverse events led to the discontinuation of therapy in three patients, two being cytopenia related. Gastrointestinal toxicity was minimal and did not affect compliance. Cardiovascular toxicity consisted primarily of grades 1 to 3 hypertension in three patients, deep vein thromboses (two patients) and one pulmonary embolism (one patient). Twelve patients underwent dose reductions of either celecoxib, cyclophosphamide, or both for 1- to 2-month durations due to either grades 2 to 3 rash (n = 4), grades 3 to 4 neutropenia (n = 2) and thrombocytopenia (n = 2), or grades 2 to 3 elevated liver enzymes (n = 2). A 51-year-old woman who had received three prior regimens [CHOP, ESHAP, mobilization chemotherapy, and high-dose chemotherapy (HDT)/autologous stem cell transplantation (ASCT)] and who withdrew from study after 12 months after achieving a partial response to treatment, developed myelodysplasia with monosomy 7 nine months later and 60 months from diagnosis. A second patient, a 51-year-old woman, who was withdrawn from study with stable disease after 3.7 months due to thrombocytopenia developed acute myelogenous leukemia with monosomy 7 nine months later and 72 months from diagnosis. She had been treated previously with chlorambucil CVP, CHOP, ESHAP, MiniBeam, and mobilization chemotherapy preceding HDT/ASCT.

Table 2. Adverse events without regard for causality

Description	Grade (*n*)				Total
	1	2	3	4	
Rectal bleeding	1				1
Dyspepsia (heartburn)	5	1			6
Diarrhea	5	1			6
Nausea	2	2			4
Vomiting	1	1			2
Anorexia	2	4			6
Abdominal pain		3			3
Anemia	2	4	1		7
Neutropenia	2		1	1	4
Thrombocytopenia	2	2	5		9
Skin rash	4	7	2		13
Urticaria		1			1
Serum creatinine elevation		2	1		3
Elevated alanine aminotransferase	1	2	1		4
Hypertension	1	1	1		3
Edema	4	3			7
Headache	2		1		3
Upper respiratory tract infection	3	1			4
Pneumonia			1		1
Cough	5	1			6
Dyspnea		2	2	1	5
Fatigue or weakness	12	5	6		23
Weight loss	2	2			4
Deep vein thromboses			2		2
Pulmonary embolism		1			1
Supraventricular tachycardia			2		2

After studies evaluating celecoxib for cancer prevention Adenoma Prevention with Celecoxib trial and the Alzheimer's Disease Anti-Inflammatory Prevention Trial (ADAPT) were halted in December 2004 due to the observed increased risk of strokes and heart attacks in participants taking celecoxib compared with placebo, four patients had their celecoxib doses reduced to 200 mg p.o. daily for a period of 1 to 2 months. These doses were then reescalated to 400 mg p.o. bid in all patients after Research Ethics Board approved a revised consent form and we repeated consent of all patients.

Celecoxib pharmacokinetics. Single-dose celecoxib pharmacokinetic data are available for six patients and are summarized in Fig. 2. These data were calculated using the plasma concentrations obtained during the 12 hours after the first dose of celecoxib. In a preclinical model, a plasma concentration of celecoxib >500 μg/L was antiangiogenic.[6] Peak concentration (C_{max}) of celecoxib after a single dose of 400 mg was 2,369 ± 1,586 μg/L at a median time of 3.2 hours (range, 1.1-6.1) after dosing. The area under the plasma concentration time curve extrapolated to infinity ($AUC_{0-\infty}$) was 15,203 ± 10,031 μg/L h, the apparent volume of distribution (V_d/F) was 3.6 ± 2.2 L/kg, and the apparent clearance (Cl/F) of the drug was 0.6 ± 0.4 L/h/kg, with an elimination half-life ($t_{1/2}$) of 4.1 ± 0.9 hours. The 12-hour trough concentration (C_{min}) after a single dose was 539 ± 335 μg/L. The mean celecoxib plasma concentration time curve is shown in Fig. 2.

Plasma VEGF. The plasma VEGF concentrations over time with linear regression lines are shown in Fig. 3 for both responders and nonresponders. The median VEGF level at baseline was 60.4 pg/mL (range, 0-669). At 2 months, 56% of patients had a reduction in plasma VEGF, whereas 44% had an increase. By logistic regression and Wilcoxon nonparametric tests, there were no statistically significant differences in baseline median VEGF levels between responders and nonresponders. By the linear mixed model analysis, VEGF trended to decline in responders (3.4 pg/mL monthly; $P = 0.34$) and increase in nonresponders (5.3 pg/mL monthly; $P = 0.61$).

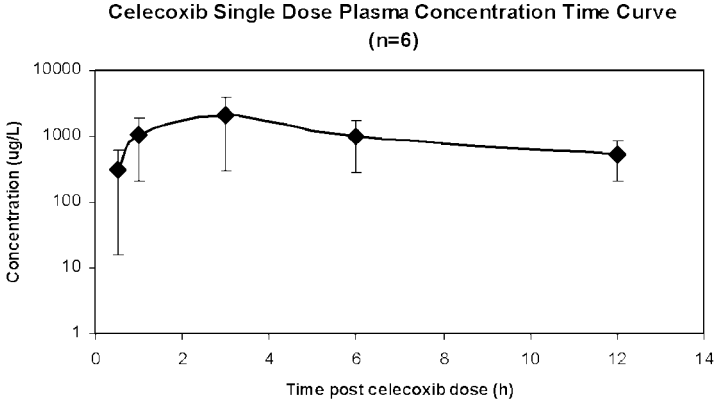
CEC and CEP assays. We quantified overall CECs and CEPs. The profiles of total CECs and over time with linear regression lines are outlined in Fig. 4 for both responders and nonresponders. The median baseline CECs were 20.1 (range, 1.8-204.9). For reference, healthy control CECs and CEPs levels are 7 to 10 cells/μL and <2 cells/μL (22). At 2 months, 80% of all patients had a reduction and 20% of patients had an increase in CECs. The overall trend was for the CECs to decline by 48% and 68% at 2 and 6 months of treatment, respectively. A similar decline was seen with overall CEPs (51% and 69%) at the same time points. By 4 months, median CEC (6.3 cells/μL) levels fell to healthy control levels. By logistic regression and Wilcoxon nonparametric tests, there were no statistically significant differences between median baseline CEC readings in responders (50.1 cells/μL) and nonresponders (16.9 cells/μL). From the general linear mixed model analysis, CECs significantly decreased over time in responders (CECs, 6 cells/μL monthly; $P = 0.0065$). Similarly, CECs tended to decrease in nonresponders over time but this was not statistically significant ($P = 0.15$).

Discussion

We designed a study to evaluate the efficacy and safety of low-dose continuous (metronomic) chemotherapy with high-dose celecoxib in a population of non–Hodgkin's lymphoma patients that were relapsed or refractory to previous chemotherapy. We combined cyclophosphamide with celecoxib based on the postulated importance of angiogenesis in non–Hodgkin's lymphoma (1–5), the *in vitro* and *in vivo* antiangiogenic and antineoplastic properties of celecoxib (12, 28), the "antivascular" properties of chemotherapy delivered continuously in mouse xenografts, especially when given in combination with anti-VEGF therapy and not as monotherapy (7, 10, 29), and early clinical experience in other cancers when standard maximally tolerated dose chemotherapy has failed (18, 30–33).

In this study, we have shown that the combination of continuous low-dose cyclophosphamide with high-dose celecoxib resulted in a response rate of 37.5% in a group of heavily pretreated patients with aggressive histology lymphoma. Twenty five percent of the patients were refractory to their preceding conventional-dose therapy. Most notable is the overall response of 37% in the 11 patients who had progressed after HDT. The combination was well tolerated, with a low incidence of hematologic, gastrointestinal, and renal toxicity

[6] J. Masferrer, personal communication.



#	T_{max} (h)	C_{max} (μg/L)	$AUC_{0-\infty}$ (μg/L·h)	V_d/F (L/kg)	Cl/F (L/h/kg)	$t_{½}$ (h)	T_{min} (h)	C_{min} (μg/L)
Celecoxib Mean ± S.D.	3.2 (median)	2369 ± 1586	15203 ± 10031	3.6 ± 2.2	0.6 ± 0.4	4.1 ± 0.9	11.2 (median)	539 ± 335

Fig. 2. Single-dose plasma celecoxib concentration-time curve and first-dose pharmacokinetic variables. Blood samples were drawn after the first dose of 400 mg was administered orally. The data are based on the samples collected 12 hours after the first dose of celecoxib. Points, mean; bars, SD.

despite the high dose of celecoxib used. Skin rash was encountered relatively frequently (43%) but responded to topical therapy and generally did not recur following a short period of discontinuation of celecoxib. Two cases of myeloid neoplasia were observed, but both patients had previously received HDT and ASCT and had received three and six prior chemotherapy regimens. Secondary myelodysplastic syndrome and acute myelogenous leukemia is a recognized consequence of ASCT, especially in heavily pretreated patients, such as those in our study (34). Nevertheless, one cannot dismiss the possibility that additional alkylating agent exposure with cyclophosphamide played a role in the development of these myeloid neoplasms. Other hematologic toxicity was mild and there were no admissions for febrile neutropenia, clinically significant bleeding, or arterial thrombotic events. Three patients developed venous thrombosis, but this is a recognized complication in the treatment of non–Hodgkin's lymphoma, especially at an advanced stage (35, 36). The progression-free (4.7 months) and overall survivals (14.4 months) seen in this cohort are favorable in comparison with similar patient populations treated with other palliative chemotherapy regimens(37–41). Several responses seen were quite durable: in five patients, response lasted for >9 months (9.4-29 months).

We evaluated changes in soluble and cellular markers of angiogenesis in an attempt to correlate changes in these variables with clinical and radiological response. The overall trend in VEGF is a modest decline in responders and an increase in nonresponders. Baseline and 3-month VEGF levels were not predictive of response in multivariable analysis. This may be a function of the small sample size, although many others have reported an inconsistent relationship between soluble markers of angiogenesis (specifically VEGF) and response to antiangiogenic therapy. Nevertheless, some studies of metronomic chemotherapy have shown their usefulness (18).

CECs and CEPs have been found to increase in the blood of cancer patients (22) and may serve as more reliable surrogate markers of angiogenesis than soluble circulating angiogenic growth factors and microvessel density (42). Recently, several investigators have shown that CEPs (measured by flow cytometry) can serve as biomarkers for angiogenic responsiveness to VEGFR-2 blocking antibody or a thrombospondin mimetic peptide in several mouse strains (43). In preclinical models of melanoma, breast cancer and erythroleukemia, the optimal biological dose of different chemotherapies, including cyclophosphamide, was tested by Shaked et al. (9) In that study, each optimal biological dose correlated strongly with the maximum reduction in $VEGFR\text{-}2^+$ CEPs. These studies suggest that CEPs may be able to serve as pharmacodynamic biomarkers to determine the optimal biological dose of metronomic chemotherapy regimens. Bertolini et al. (8) found that maximally tolerated dose and low-dose metronomic cyclophosphamide have opposite effects on the mobilization and viability of CECs and CEPs in a mouse model of lymphoma. Metronomic cyclophosphamide was associated with a consistent decrease in CEP numbers and viability and with more durable inhibition of tumor growth than maximally tolerated dose dosing.

In our study, the majority of evaluated patients had an early decline in their CECs and CEPs with therapy, and significant declines in CECs and CEPs were noted in responding patients. Despite this, only 37% of patients responded to treatment. Although not statistically different, it is noteworthy that the median baseline CECs and CEPs were higher in responders compared with nonresponders. The observation that the majority (83%) of responding patients had normal serum lactate dehydrogenase levels at study entry and responses were uncommon in those with elevated lactate dehydrogenase suggests that a therapy actively inhibiting angiogenesis in aggressive histology lymphomas may be insufficient to guarantee clinical responses in all patients. It may be that rapidly proliferating lymphomas cannot be controlled with cytostatic therapy. Alternatively, it could be hypothesized that the primary mode of action of this treatment in responding patients is only in part or not at all antiangiogenic.

Our analysis is limited by the small number of patients enrolled and the small numbers tested, particularly beyond 6 months.

The optimal *in vivo* "antiangiogenic" doses of celecoxib and cyclophosphamide are currently unknown. We selected the 400 mg p.o. b.i.d. dose of celecoxib based on the familial adenomatous polyposis study (14) because our aim was to maximize the potential *in vivo* antiangiogenic and "COX-2 inhibitory" drug levels (17). Whether lower or higher doses may be more efficacious is unknown. Celecoxib trough levels measured in this study are achieving preclinical target levels of 500 μg/L required to inhibit angiogenesis,[6] but further assessment over longer periods of treatment is needed to correlate drug levels (celecoxib and 4-hydroxycyclophosphamide) with outcome.

In recent years, numerous clinical (44, 45) and preclinical studies have been published explaining possible mechanisms of action. For example, low-dose cyclophosphamide may inhibit angiogenesis and hence tumor growth by up-regulating the endogenous inhibitor thrombospondin-1 in tumor, perivascular (11), or tumor-associated stromal cells (46). Thrombospondin-1 in turn promotes endothelial cell apoptosis (46) and can suppress the mobilization of CEPs (43). COX-2 has been found to be a rational target in non–Hodgkin's lymphoma. In an immunohistochemical study of 52 human lymphoma biopsies, COX-2 was detected in 57% of non–Hodgkin's lymphoma and 70% of Hodgkin's lymphoma samples. Furthermore, COX-2 expression correlated with prognostic factors, increased with stage (15), p53 accumulation and cellular proliferation (16). The COX-2 protein was found to be overexpressed (2.2- to 4.3-fold) in several lymphoma cell lines compared with primary B cells. Treatment with celecoxib decreased proliferation and induced apoptosis in a dose-dependent fashion and promoted apoptosis in 85% of lymphoma cell lines tested at 50 μmol/L celecoxib (17).

Recently, selective COX-2 inhibitors in cancer have come under scrutiny because of reports suggesting an increased cardiovascular risk associated with their use (47). This culminated in the early halting of two government-sponsored studies: the Adenoma Prevention with Celecoxib trial sponsored by the National Cancer Institute, and ADAPT sponsored by the National Institute on Aging (48). In our study, celecoxib at high doses was well tolerated and no excess cardiovascular toxicity was observed. Nevertheless, because of concerns of arterial thrombotic events with COX-2 inhibitors and the increased arterial and venous thrombotic complications associated with angiogenesis inhibitors, we recommend close monitoring of cancer patients treated with these agents. We also recommend the exclusion of patients with active cardiovascular disease from trials testing COX-2 inhibitors. In the era of enormously expensive targeted therapies (49, 50) in cancer with inconvenient administration schedules and unproven cost effectiveness, celecoxib is a drug that should be given serious consideration for further study.

In conclusion, we provide evidence that low-dose cyclophosphamide given continuously in combination with celecoxib may be effective in the treatment of some patients with aggressive lymphoma. Given the small sample size of this study, these results should be validated in a larger more homogeneous population of relapsed lymphoma, perhaps earlier in the disease course. The cellular mechanisms of action and predictors of response should be further studied. Measuring COX-2 in non–Hodgkin's lymphoma samples by immunohistochemistry may help predict for response to

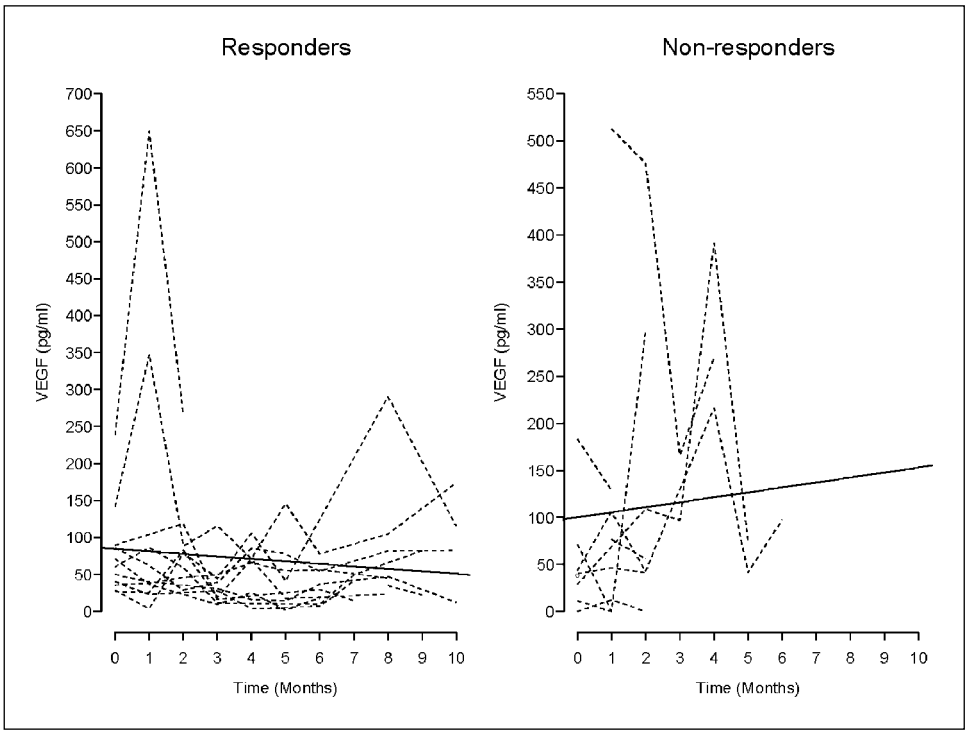


Fig. 3. Individual profile of VEGF over time with a linear regression line in responders and nonresponders.

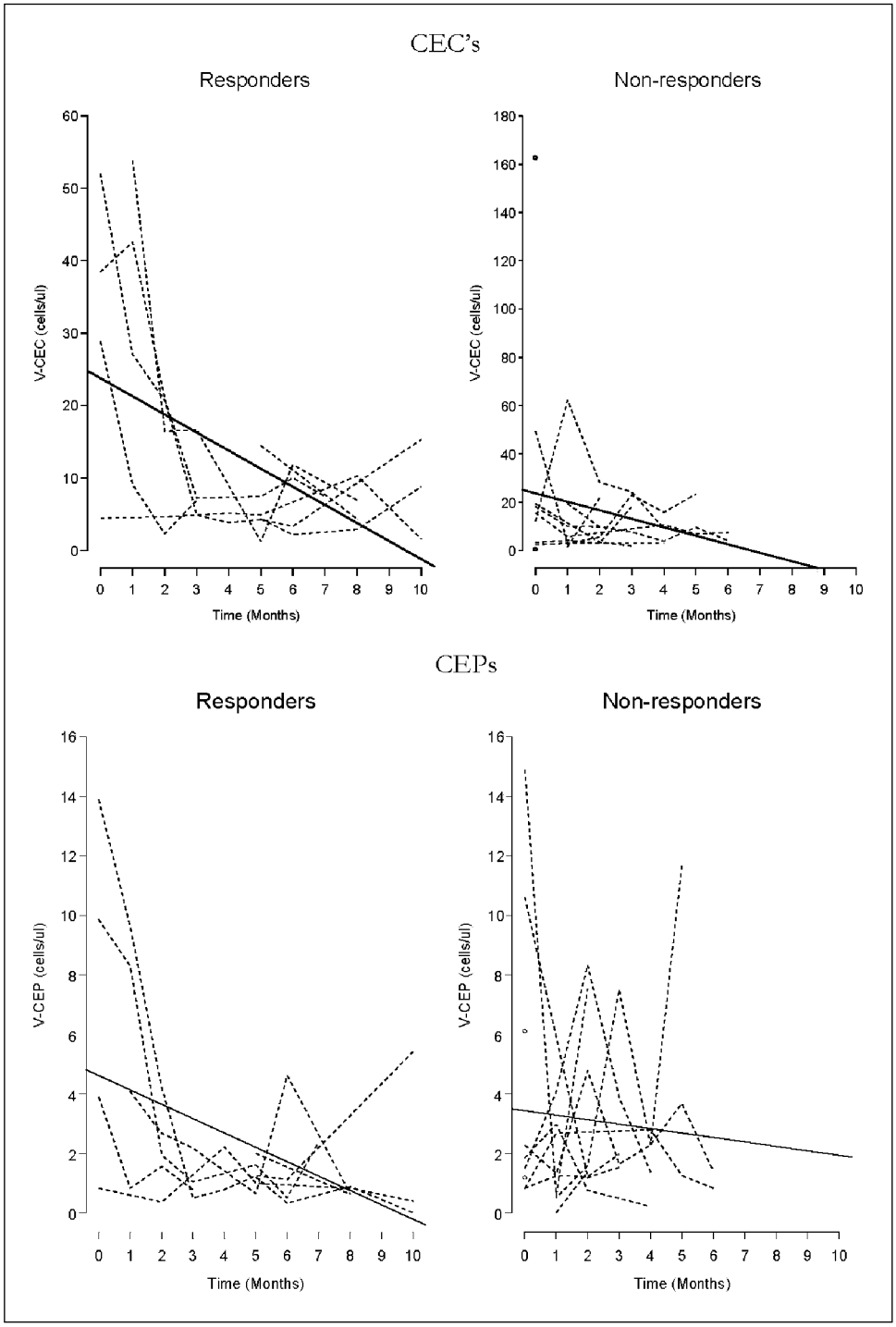


Fig. 4. Individual profile of total CECs and CEPs over time with a linear regression line in responders and nonresponders.

celecoxib. Additional techniques (such as dynamic magnetic resonance imaging or computed tomography) may be useful for evaluaing the effect of this therapy on angiogenesis. Further evaluation of this combination as maintenance therapy in patients at high risk of relapse after response to initial chemotherapy seems justified.

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