Low Dose Methotrexate, Bleomycin, Doxorubicin, Cyclophosphamide, Vincristine, and Dexamethasone with Zalcitabine in Patients with Acquired Immunodeficiency Syndrome-Related Lymphoma

Effect on Human Immunodeficiency Virus and Serum Interleukin-6 Levels over Time

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BACKGROUND. Use of multiagent chemotherapy has been associated with complete remission (CR) in approximately 50% of patients with newly diagnosed acquired immunodeficiency syndrome (AIDS)-lymphoma, although additional AIDS-related complications may occur. Both chemotherapy and antiretroviral therapy were employed in an attempt to ascertain if the combination was safe, and associated with changes in human immunodeficiency virus (HIV) p24 antigen levels during the course of treatment.

METHODS. Low dose methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone(M-BACOD) chemotherapy and zalcitabine (ddC) were employed in 28 patients. Since both vincristine and zalcitabine may cause peripheral neuropathy, a Phase I/II study design was employed. Serum was analyzed for immune complex dissociated (ICD) HIV p24 antigen and interleukin (IL)-6 levels during therapy.

RESULTS. CR was achieved in 14 of 25 patients (56%), with partial response (PR) in 5 (20%). CRs were equivalent in patients with good or poor prognostic indicators, including a history of AIDS prior to lymphoma (CR = 60%); and/or CD4 lymphocytes $< 200/mm^3$ (CR = 53%). Five patients with a CR subsequently relapsed (36%); median survival of CR patients was 29.2 months (4.1–61+), whereas that of all of the treated patients was 8.1 months. No significant peripheral neuropathy or other toxicity was observed. Serum ICD p24 antigen levels either fell (7/14) or remained consistently negative (2/14) in 9 of 14 patients (64%), whereas 36% experienced an increase. Elevated serum IL-6 levels at diagnosis were associated with systemic "B" symptoms ($P = 0.023$), whereas changes in IL-6 correlated with response to therapy over time ($P = 0.006$).

CONCLUSIONS. Combination antineoplastic and zalcitabine antiretroviral therapy may be safely administered to patients with AIDS-related lymphoma, resulting in CR in 56%, lack of significant neurotoxicity, and favorable effect on HIV p24 antigen in 50%. Elevation of serum IL-6 is associated with systemic "B" symptoms, whereas changes in serum IL-6 may correlate with response. ***Cancer* 1996; 78:517–26.**

KEYWORDS: AIDS-related lymphoma, interleukin-6, Human immunodeficiency virus, zalcitabine, M-BACOD chemotherapy.

Lymphoma is a relatively late manifestation of human immunodeficiency virus (HIV) infection, comprising approximately 3% of newly diagnosed cases of acquired immunodeficiency syndrome

(AIDS)[1-3] and as many as 16% of all AIDS-related deaths.[4] The incidence of AIDS-associated lymphoma is expected to increase over time because survival is being prolonged for patients with HIV infection due to effective antiretroviral therapy, prophylaxis, and therapy for various opportunistic infections.[5,6] This delayed development of AIDS-lymphoma has recently been demonstrated in a cohort of 116 patients with symptomatic HIV disease. At 36 months of follow-up, 19% of the cohort had been diagnosed with lymphoma.[7]

Early in the AIDS epidemic, various regimens of dose-intensive chemotherapy were tested in patients with AIDS-related lymphoma, resulting in complete remission (CR) rates between 20 and 33%, and development of intercurrent opportunistic infections in as many as 78%.[8-10] Using an alternative approach consisting of a low-dose modification of the methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, and dexamethasone (M-BACOD) regimen with early central nervous system prophylaxis, a CR rate of approximately 50% was achieved in 35 patients who were studied as part of a prospective national trial.[11] These remissions were durable in 75% of the patients, while opportunistic infections developed in only 20%. However, patients with poor prognostic indicators of disease did not fare as well. Thus, patients with a history of AIDS prior to the lymphoma experienced a CR rate of only 25%.[11]

In the current trial, we employed the low dose M-BACOD regimen in 25 patients with newly diagnosed AIDS-related lymphoma.[11] In an attempt to improve upon earlier results, we added 2',3'-dideoxycytidine (ddC) (zalcitabine)[12] with the hypothesis that better control of the underlying HIV infection might be achieved with the potential for lower rates of opportunistic infection, higher rates of response, and a decrease in the HIV-1 induced cascade of cytokines which may be operative in the pathogenesis of lymphomatous disease.[13-16] We thus collected prospectively serum for measurement of interleukin-6 (IL-6) and HIV-p24 antigen levels over time. Zalcitabine was chosen as the antiretroviral agent in this study because of the known significant bone marrow toxicity of zidovudine (AZT) and because all patients were ddC-naive, thus obviating the potential for antiretroviral drug resistance at the outset. However, since both vincristine and zalcitabine are associated with peripheral neuropathy,[17,18] a Phase I/II study design was employed in which vincristine was initially withheld, and then added at increasing doses to sequential cohorts of patients.

MATERIALS AND METHODS

Patients

All of the patients were required to be HIV seropositive, with newly diagnosed, previously untreated, high or intermediate grade lymphoma, and presence of measurable tumor parameter(s). Any stage of lymphomatous disease was permissible. Karnofsky performance status of 50% or better was required, as was adequate hepatic (bilirubin < 2 mg/dl; serum glutamic-pyruvic transaminase (alanine aminotransferase) [SGPT] $< 3\times$ normal); renal (creatinine < 2 mg/dl); and bone marrow function (hemoglobin [Hb] > 9 gm/dl, granulocytes > 1000/dl, platelets $> 100{,}000$/dl), unless these values were abnormal secondary to lymphomatous involvement. Patients with primary central nervous system (CNS) lymphoma were excluded, as were those with active intercurrent infection. All patients signed an informed consent, which had been approved by the Human Research Committee of the University of Southern California (USC) School of Medicine.

A total of 28 patients were accrued, 25 of whom were evaluable. One patient was ineligible due to a Karnofsky performance status of <50%, and the presence of 2 active on-going opportunistic infections (CMV esophagitis and Torulopsis glabrata). He received <1 week of protocol therapy, and was removed from treatment due to the development of new neurologic symptoms confirmed as progressive multifocal leukoencephalopathy (PML) at autopsy. Two additional patients were considered inevaluable, one of whom received one cycle of therapy, improved clinically, and declined further therapy. The second patient received one cycle of therapy, and died of pre-existing pulmonary tuberculosis (TB) without further evaluation of tumor response.

Chemotherapy and Antiretroviral Regimen

The treatment schema employed was as follows[11]: bleomycin (4 mg/m^2); doxorubicin (25 mg/m^2); and cyclophosphamide (300 mg/m^2) administered on Day 1 of each cycle by intravenous (i.v.) injection. Dexamethasone (3 mg/m^2) administered by mouth (p.o.) on Days 1 to 5 of each cycle. Vincristine was withheld in the first cohort of 8 patients (Level 1), and then escalated to 0.125 mg/m^2 (Level 2); 0.25 mg/m^2 (Level 3); 0.5 mg/m^2 (Level 4); and 0.75 mg/m^2 (Level 5) in successive cohorts, also administered i.v. on Day 1 of each cycle. Methotrexate (200 mg/m^2) was given over 2 hours on Day 15 of each cycle, with folinic acid rescue (25 mg) administered either by vein or mouth beginning 24 hours after the completion of methotrexate, and repeated every 6 hours times 6 doses. Cytosine arabinoside (50 mg in sterile, preservative-free solution) was administered intrathecally (IT) once each week during the first 4 weeks of treatment for all patients. Additional IT chemotherapy (cytosine arabinoside, 50 mg 3 $\times$ weekly until CSF cleared, then once

each month for 12 mos) was employed in patients with known lymphomatous involvement of the CNS.

All patients received ddC (0.01 mg/kg) 3 times per day orally beginning on Day 1, and continued for 52 weeks.

Chemotherapy cycles were repeated every 4 weeks, for a maximum of 6 cycles, or 2 cycles beyond documentation of CR.

Supportive Care

Granulocyte-colony stimulating factor (G-CSF) (Neupogen, Amagen, Inc., Thousand Oaks, California) was administered at a dose of 5 ug/kg subcutaneously (s.c.) from Days 2 through 10 in patients who sustained absolute granulocyte counts of ≤500/dl in the preceding chemotherapy cycle.

All patients received prophylactic therapy against *Pneumocystis carinii* pneumonia consisting of pentamidine, 300 mg via an inhaler administered once each month.

Staging Procedures

All of the subjects underwent complete history and physical examinations; chest X-ray; computerized axial tomographic exam (CAT scan) of the chest, abdomen, and pelvis; CAT scan or magnetic resonance imaging (MRI) scan of the brain; lumbar puncture with cytologic and immunophenotypic evaluations for lymphoma cells; and bilateral bone marrow (BM) aspirate and biopsy. Upper and/or lower endoscopy with upper and/or lower gastrointestinal (GI) barium studies were performed in patients with GI symptoms or signs.

Patients with systemic "B" symptoms (e.g., fever, night sweats, weight loss) underwent extensive evaluation to exclude the presence of occult opportunistic infection(s).

Immunologic and HIV virologic studies included assessment of T cell subsets, IL-6 cytokine levels, and immune complex dissociated (ICD) p24 antigen levels in serum evaluated at baseline and every 2 months thereafter until completion of chemotherapy.

Restaging Procedures

After two cycles of chemotherapy, all of the patients were restaged, repeating all disease variables which were initially abnormal. These tests were also repeated 4 weeks after completion of all chemotherapy in patients who had initially attained CR or partial remission (PR) after the original restaging evaluation.

Definition of Response

CR was defined as the complete disappearance of all clinically detectable malignant disease without development of new lesions for at least 4 weeks.

PR was defined as tumor reduction which was >50% of pretreatment values, as measured by calculating the product of 2 perpendicular dimensions in each lesion, persisting for at least 4 weeks after completion of therapy.

Stable disease (SD) was defined as <50% reduction in tumor burden without progression.

Progressive disease (PD) was defined as the development of new lymphomatous disease, or ≥25% in the pretreatment tumor measurements.

Immunophenotypic Analysis of Peripheral Blood T Lymphocytes

Flow cytometric phenotyping of peripheral blood mononuclear cells (PBMC) was performed at the USC Flow Cytometry Laboratory using whole blood lysis and direct two-color staining (RD1/fluorescein isothiocyanate [FITC]) with fluorochrome conjugated monoclonal antibodies (MoAbs) (Coulter Cytometry, Hialeah, FL): CD14 (MO2)/CD45 (K56); immunoglobulin [Ig]G1/IgG2a; CD4/CD3; CD8/CD3.[19]

p24 Antigen Determinations

All of the samples were tested for polyclonal HIV p24 antigen using the standard p24 assay kit. Retrospectively, cryopreserved specimens were tested after immune complex dissociation (ICD) of the p24 antigen, using monoclonal ICD-p24 assay (Coulter Cytometry Hialeah, FL). Sera or plasma were pretreated with glycine HCL and the enzyme immunoassays were performed according to instructions (Coulter Cytometry, Hialeah, FL). All reactive samples were confirmed by neutralization. The cut-off for each assay was calculated by adding a predetermined factor of 0.055 to the mean optical density of 3 negative controls tested with each run. The minimum level for detection for the ICD p24 antigen was 5.4 pg/mL.

Determination of IL-6 Levels in Serum

Serial blood samples were collected at baseline and every 2 months thereafter during therapy. Serum was separated by centrifugation, and multiple 1 mL aliquots were stored at −70 °C until analysis. IL-6 levels were measured using a commercially available IL-6 enzyme-linked immunoadsorbent assay (ELISA) kit, employing the methodology recommended (R&D, Minneapolis, MN). Appropriate dilutions of serum were tested to measure IL-6 levels within the linear range of the standard curve. The lowest level for detection of serum IL-6 was 0.1 pg/mL. The assay does not crossreact with other cytokines.[20]

Statistical Methods

CR rate was calculated with 95% confidence intervals (CI), using a normal distribution table.[21] Fisher's exact

test was used for two-group comparison of response rates.[22] The geometric mean for IL-6 levels was compared using a two-sample Student's t test.[23] Median ICD p24 antigen levels were compared using the nonparametric Kruskal Wallis test.[23] To determine the relationship between IL-6 levels (log transformed) and HIV p-24 antigen, LDH, and infections during the course of study, a repeated measures linear regression model was used.[24] In this model, the HIV-p24 antigen levels and LDH values were also log transformed, and infection was coded as present or absent. The regression model included a single "random effect" term that allowed each subject to have their own baseline IL-6 level, constrained to come from a normal distribution rather than a fixed intercept value.

Lymphoma free survival was defined as the time from the documentation of CR to the first onset of relapse or the date of death. Overall survival was defined as the time from institution of therapy to the date of death or the last follow-up. The median lymphoma free and overall survivals were calculated using the method of Kaplan and Meier.[25]

RESULTS

Demographic Information

All of the patients were homosexual or bisexual males. Median age was 38 years (range: 22–63). There were 12 (48%) Latinos in the group, 10 (40%) whites, and 3 (12%) African-Americans.

HIV Disease Parameters at Study Entry

Five individuals (20%) had a history of AIDS prior to the diagnosis of lymphoma. This included opportunistic infection (OI) alone in three (*Pneumocystis Carinii* pneumonia; toxoplasmosis; and esophageal candidiasis), and OI plus Kaposi's sarcoma in two, one of whom had systemic cytomegalovirus (CMV) infection, while the other had history of extrapulmonary TB.

The median CD4 cell count at study entry was 164/mm^3 (range: 5–625/mm^3). Thirteen individuals (54%) had CD4 cells < 200/mm^3.

Full information concerning prior antiretroviral therapy was not collected routinely. However, no patient had received zalcitabine prior to receiving the drug as part of the current study.

Characteristics of Lymphomatous Disease

As demonstrated in Table 1, systemic "B" symptoms were present in 14 of 25 (56%), consisting of fevers, drenching night sweats, and/or weight loss. The majority of patients had advanced extranodal involvement found in 18 of 25 (72%), including 14 with Stage IV, 1 with Stage IIE, and 3 with Stage IE disease. Sites of extranodal involvement included GI tract in 10 of 25 (40%); CNS in 5 of 25 (20%); bone marrow in 2 of 25 (8%); and multiple other sites, including kidney in 3, liver in 2, and gingiva, adrenal, lung, epidural space, parotid, testis, and maxillary sinus in 1 patient each. Six patients had multiple sites of extranodal lymphoma.

Pathologic assessment of biopsy specimens revealed high grade lymphoma in 20 of 25 (80%), including 15 (60%) with small noncleaved cell lymphoma, 4 (16%) with immunoblastic, and 1 with high grade lymphoma which could not be further subclassified. The remaining 5 patients (20%) had intermediate grade, diffuse large cell lymphoma.

TABLE 1
Disease Characteristics at Study Entry 25 Patients with AIDS-Lymphoma

HIV characteristics	
Median CD4 cells	164/mm^3 (range: 5–625)
CD4 <200/mm^3	13/24 (54%)[a]
History of prior AIDS	5/25 (20%)
Lymphoma characteristics	
Systemic "B" symptoms	14/25 (56%)
Pathology	
B-immunoblastic	4/25 (16%)
small noncleaved	15/25 (60%)
large cell diffuse	5/25 (20%)
diffuse, high grade, nos	1/25 (4%)
Stage	
IE	3/25 (12%)
IIE	1/25 (4%)
II	2/25 (8%)
III	5/25 (20%)
IV	14/25 (56%)
Extranodal Involvement[b]	18/25 (72%)
GI	10
CNS	5
Bone marrow	2
Other, miscellaneous	9

AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus; nos: not otherwise specified; GI: gastrointestinal tract; CNS: central nervous system.
[a] One patient, CD4 not done.
[b] six patients with multiple extranodal sites of involvement.

Cycles of Chemotherapy Administered

The majority of patients (14/25, 56%) received 4 cycles of therapy, while 3 (12%) received 3 cycles; 5 (20%) received 2 cycles; and 3 (12%) received 5, 6, and 8 cycles, respectively. Those patients who received <3 cycles of therapy were those who were removed from protocol study early because of documented progressive lymphomatous disease or intercurrent toxicity.

Seven subjects were treated on Level I, which omitted vincristine; 4 on Level II; 4 each on Levels III and IV; and 6 on Level V, which included 0.75 mg/m^2 of vincristine along with zalcitabine which was given to all of the patients.

Response to Chemotherapy Plus ddC

Twenty-five patients were evaluable for response, having completed at least one cycle of chemotherapy, and returned for follow-up assessments. CR was attained in 14 of 25 patients (56%) (95% CI: 44–68%), while PR occurred in 5 (20%). SD was documented in 1 patient, while 5 of 25 (20%) had PD.

In evaluating response in terms of known prognostic factors for AIDS-lymphoma,[8,26] no significant differences were apparent between patients with good versus those with poor prognostic disease. Thus, CR was attained in 3 of 5 patients (60%) with a history of AIDS; versus 11 of 20 (55%) of those who had no such history ($P = 0.84$). CR was attained in 7 of 13 patients (53%) with CD4 cells < 200/dl versus 7 of 11 (64%) who had >200/dl CD4 cells ($P = 0.82$). CR was attained in 1 of 2 patients with initial bone marrow involvement, versus 13 of 23 (57%) without marrow infiltration ($P = 0.85$).

With a median follow-up interval of 29.2 months (range: 4.1–61+), 5 of the 14 complete responders relapsed at 1.4, 3, 3, 13, and 23 months from completion of chemotherapy; 4 of whom died. Four of the five complete responders who subsequently relapsed received four cycles of chemotherapy, whereas one received only two cycles because development of intercurrent Cryptococcal meningitis prevented the administration of further treatment. Sites of relapse included original sites (rectum, axillary node) in two, one of whom also developed leptomeningeal lymphoma; new sites (bone marrow [BM], paraspinal mass) in one; and CNS in two, one of whom had initial disease in the maxillary sinus and one of whom had gastric lymphoma. Four additional complete responders also died, while still in remission from lymphoma, of the following causes: AIDS-related wasting syndrome; pulmonary Kaposi Sarcoma (KS); adenocarcinoma of the prostrate and wasting; and AIDS, not otherwise specified, with death occurring 17 months after the institution of protocol therapy.

Toxicity of Combined Chemotherapy with Zalcitabine

All 28 patients were considered evaluable in terms of toxicity. BM compromise was the most common toxicity. Eight individuals developed nadir neutrophil counts between 500 and 1000/dl, occurring during Cycle 1 in 5 patients; Cycle 2 in 2; and Cycle 4 in 1. Four experienced granulocyte nadirs < 500/dl occurring in Cycles 1 or 2 in all. All four received G-CSF during subsequent cycles of chemotherapy. G-CSF was not required or administered to any of the remaining patients. Two patients experienced a drop in the platelet count below 25,000/dl, 1 of whom had extensive marrow involvement. One patient developed a nadir platelet count in the 25,000 to 50,000/dl range, while a fourth had a nadir platelet count in the 50,000 to 75,000/dl range.

Neurotoxicity, consisting of numbness and/or tingling of the distal extremities, was uncommon. Two patients developed Grade 1 paresthesias, including one subject on Level I who received no vincristine, and one subject on Level II who received 0.125 mg/m^2 vincristine. Two additional patients developed Grade 2 paresthesias, occurring in one during the first dosing level when concomitant vincristine was not employed, and in the second during Cycle 1 after receipt of 0.75 mg/m^2 vincristine with zalcitabine. Thus, a total of 4 of 25 (16%) who received more than 1 cycle of chemotherapy developed some evidence of peripheral neuropathy, occurring in only 2 of 20 patients who actually received both zalcitabine and vincristine. Further, neurotoxicity developed in none of the three patients who were invaluable for response, having received one cycle of chemotherapy or less.

Opportunistic and Other Infectious Complications

Opportunistic infections occurred in 3 (12%) individuals during or in the month following completion of chemotherapy. These included Pneumocystis Carinii pneumonia, cryptococcal meningitis, and mycobacterium avium intracellulare in one patient each.

During periods of neutropenia, four patients developed infections. These included one patient who developed a bacterial pneumonia; one who developed documented bacterial sepsis; and two who had neutropenic fevers requiring hospitalization. Two additional patients developed localized bacterial infections (Bacteroides fragilis from a loculated abscess in one; and perianal infection with E coli, α and β strep in the second). One patient developed Herpes simplex infection on the penis and mouth, requiring i.v. acyclovir therapy.

ICD HIV-p24 Antigen Levels

ICD HIV p24 antigen levels were available in 22 patients at baseline, and in 14 over the course of therapy, as depicted in Table 2. Seventeen patients (77%) had presence of elevated ICD p24 antigen levels at baseline, with a median level of 51.54 pg/mL (range: 8.2–548.9). Over the course of treatment, ICD p24 antigen levels increased in 5 of 14 patients (36%). p24 antigen levels remained negative over time in 2 of 14 patients, and decreased in 7 of 14. Thus, a total of 9 of 14 patients

TABLE 2
ICD p24 Antigen[a]

Patient	Level	Pre study	Restaging Cycle 2	Post study
1	I	306.09	60.21	23.31
2	I	41.95	0.00	0.00
3	I	0.00	0.00	0.00
4	I	45.30	35.05	25.17
5	I	407.50	548.99	70.28
6	I	24.61	51.64	1.49
7	I	0.00	ND	11.37
8	II	14.92	0.38	0.00
9	II	11.00	ND	84.82
10	II	548.99	ND	ND
11	II	332.38	ND	ND
12	III	44.93	ND	ND
13	III	0.00	ND	11.56
14	III	ND	ND	ND
15	III	2.80	0.00	ND
16	IV	40.46	ND	ND
17	IV	520.65	ND	ND
18	IV	15.10	0.00	ND
19	IV	8.21	ND	53.13
20	V	ND	ND	ND
21	V	ND	ND	ND
22	V	425.40	548.99	ND
23	V	2.24	ND	ND
24	V	200.58	ND	ND
25	V	57.79	ND	ND

ND: not done.
[a] ICD p24 antigen ≤ 5.4 pg/mL is considered within normal limits.

(64%) experienced either a significant decrease in ICD p24 antigen levels over time, or consistent absence of antigenemia, and 5 of 14 (36%) either became antigenemic or developed greater levels of antigenemia.

Serum IL-6 Levels at Baseline and over Time

Baseline levels of IL-6 are available for 22 patients, and are provided in Table 3. As shown, serum IL-6 levels varied widely. While the geometric mean serum IL-6 level did not differ significantly among patients with the various pathologic types of lymphoma ($P = 0.54$), stage of disease ($P = 0.96$), or LDH level ($P = 0.95$) at baseline, a correlation was apparent between presence of systemic "B" symptoms and elevated levels of serum IL-6. Thus, the baseline geometric mean IL-6 level among patients with fever, night sweats, and/or weight loss was 21.48 pg/mL (95% CI: 12.42–37.14), while that for patients without such symptoms was 7.09 pg/mL (95% CI: 3.07–16.37) ($P = 0.023$). Correlations were apparent between each of the "B" symptoms and elevated IL-6 values.

Changes in IL-6 values over time were analyzed with a repeated measures linear regression method.[24] There was a significant and strong correlation between serum IL-6 level and serum LDH ($P = 0.01$); and between serum IL-6 and serum ICD p24 antigen levels ($P = 0.0008$) over the course of therapy. While there was no evidence for a progressive trend in IL-6 level with time on study, there was evidence that the IL-6 levels were lower during treatment than at baseline. Further, although patient numbers were small, an apparent association was observed between serum IL-6 levels over time and response to therapy ($P = 0.006$). Changes in IL-6 values in 12 responders versus 3 nonresponders are depicted on Figures 1A and 1B. As shown, serum IL-6 levels remained relatively low and stable in the majority of responders, while four such patients with high baseline levels experienced a dramatic decrease in IL-6, coincident with tumor response. One of these responding patients demonstrated an increase in IL-6 after Cycle 2 at the time of a bacterial pneumonia; the IL-6 values decreased thereafter, as depicted. The IL-6 levels increased at the conclusion of chemotherapy in two patients, one of whom had extensive ulceration of the penis and the mouth due to herpes simplex; while the other developed fever and a perianal abcess (E. coli, α and β streptococci) at the time that this last IL-6 value was assessed. As also shown, two of three nonresponders for whom follow-up sera was available demonstrated a dramatic increase in IL-6 levels over time, coincident with tumor progression. One of these experienced a decrease in IL-6 levels at mid-study after Cycle 2 when a PR was documented. However, rapid tumor progression was demonstrated thereafter, at a time when IL-6 levels had increased dramatically (Fig. 1B).

Survival

The median survival for all 25 patients is 8.1 months (range: 2.9–61+), as shown in Figure 2. Of 14 complete responders, 5 subsequently relapsed, 4 of whom died, and the fifth is in a second CR. Four additional CR patients have died of other AIDS conditions without evidence of relapse of lymphoma. Thus, 6 of 14 CR patients (43%) remain alive and disease free at a median of 47.1 months (41.7+–61+ mos), and the median overall survival for complete responders is 29.2 months (range: 4.1–61+ mos).

Median survival times were also evaluated in terms of presence or absence of poor prognostic indicators at initial diagnosis. Thus, the median survival for those 16 patients (64% of the group) with a history of prior AIDS, CD4 cells $< 200/mm^3$, poor performance status, or BM involvement was 6.45 months (95% CI: 4.1–11.6) while that of the 9 patients who lacked such initial characteristics has not yet been reached (95% CI: 6.9-not reached) ($P = 0.025$). In

TABLE 3
Relationship between Characteristics of Lymphoma and Serum IL-6 in 22 Patients with Newly Diagnosed AIDS-Lymphoma

	Patient no.	IL-6 Geometric mean pg/mL	95% CI	*P* value
B symptoms				
No	11	7.09	3.07–16.37	0.023
Yes	11	21.48	12.42–37.14	
Stage				
I/II	6	12.22	4.05–36.83	0.96
III/IV	16	12.23	6.29–23.79	
Pathology				
SNC	13	14.53	6.12–34.47	0.54
IBL	3	16.56	8.83–31.04	
LC-diffuse	5	9.03	3.19–25.53	
HG-nos	1	2.89	—	
LDH				
Elevated	9	12.55	4.41–35.71	0.95
Normal	13	12.18	6.30–23.54	

IL-6: interleukin-6; AIDS: acquired immunodeficiency syndrome; SNC: small noncleaved; IBL: immunoblastic; LC: large cell; HG-nos: high grade not otherwise specified; CI: confidence interval; LDH: lactate dehydrogenase; CR: complete remission; PR: partial remission.

terms of relapse free survival, complete responders who presented with poor prognostic characteristics experienced a median relapse free survival of 11.6 months (95% CI: 6.6–27.5 mos), and all 5 CR patients presenting with good prognostic indicators remain alive and disease free ($P = 0.003$).

DISCUSSION

Results from the current trial employing chemotherapy with antiretroviral therapy are encouraging. CR was achieved in 56% of the patients, with a median overall survival in complete responders of 29.2 months (range: 4.1–61+ mos). Opportunistic infections occurred in 12% of the patients. In general, these results are similar to those obtained with low dose M-BACOD alone. Of interest, the addition of zalcitabine to low dose M-BACOD was not associated with undue toxicity. Further, with this combined modality regimen, CR rates and intercurrent opportunistic infection were statistically equivalent in patients with either favorable or poor prognostic indicators of disease,[8,26] although median survival was shorter in patients who initially presented with CD4 cells $< 200/mm^3$, Karnofsky performance status $< 70\%$; and/or presence of BM involvement. Since AIDS-related lymphoma may be encountered late in the course of HIV infection, when CD4 cells are typically low and prior AIDS-defining illnesses have occurred, it is apparent that chemotherapeutic regimens with efficacy in patients with poor prognosis will be required.

The toxicity of combined low dose M-BACOD and zalcitabine was acceptable, even in these frail patients with advanced HIV disease. BM compromise was the most common serious side effect, with development of nadir neutrophil counts $< 1000/mm^3$ in 12 patients (48%), of whom 4 (16%) developed nadir counts $< 500/mm^3$. Of interest, prior trials employing low dose M-BACOD alone, without antiretroviral therapy, have documented similar rates of neutropenia, with nadirs $< 1000/mm^3$ in 60%, and $<500/mm^3$ in 21%.[11] Zalcitabine is known to be suppressive to marrow progenitor cells.[27,28] One explanation for the lack of additional myelotoxicity in these patients, after receiving both chemotherapy and zalcitabine, may be that HIV p24 antigen was suppressed in 50% of them, allowing the possibility for amelioration of HIV-induced BM compromise.[29] We were also concerned about the potential for increased neurotoxicity with this combination, since both zalcitabine and vincristine are known to cause peripheral neuropathy.[17,18] In fact, only minor neurotoxicity was encountered in 16% of the patients, due primarily to the zalcitabine, and documented in only 2 of 20 who received both drugs. It is thus apparent from these data that zalcitabine may be given concomitantly with vincristine without concern for the increased occurrence of peripheral neuropathy.

The precise effect of cancer chemotherapy on patients with HIV viral burden has not yet been defined. Employing the regimen of cyclophosphamide, doxorubicin (vincristine), and prednisone (CHOP) chemotherapy, either with or without the addition of granulocyte-macrophage colony stimulating factor (GM-CSF), Kaplan et al.[30] demonstrated no real change in HIV p24 antigen levels in patients treated with chemotherapy alone, whereas those who received GM-CSF were shown to develop increasing levels of serum HIV p24

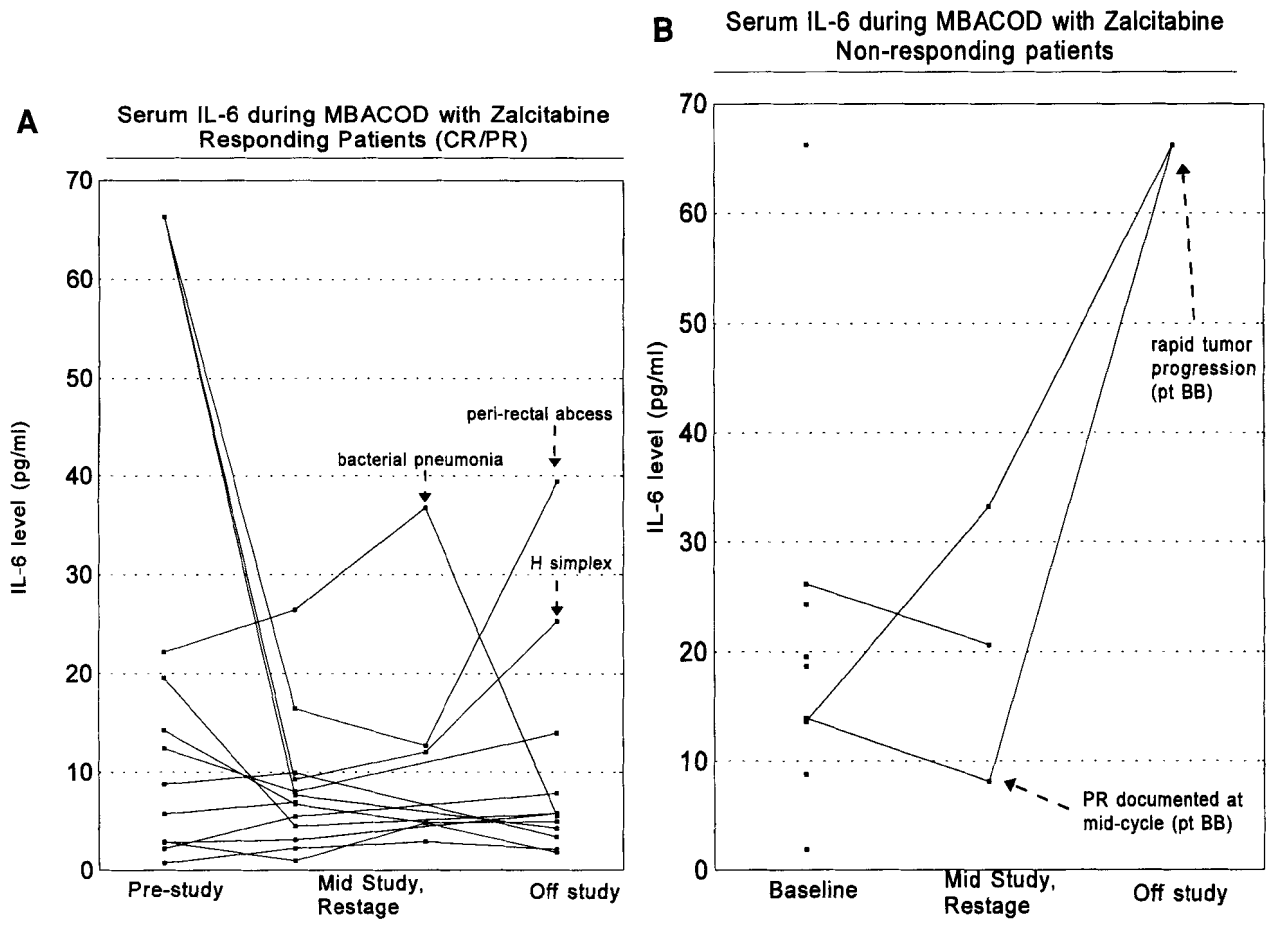

FIGURE 1. Relationship between serum interleukin-6 and response to methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and dexamethasone with zalcitabine is shown. Blood work was obtained after the second cycle of chemotherapy, at baseline, and at time of off study. (A) Complete and partial responders; (B) Non-responders.

antigen occurring only during the first cycle; the clinical significance of this finding could not be ascertained. Employing a more sensitive assay for ICD p24 antigen, 64% of our studied patients experienced either consistent lack of serum antigenemia or a significant fall in antigen levels over the course of treatment. It is apparent then, that viral activity, as measured by ICD p24 antigen, may be acceptably controlled during the course of cancer chemotherapy. However, with the recent advent of even more sensitive measures of HIV viral burden,[31] the precise relationship between HIV viral burden and use of chemotherapy should again be evaluated.

HIV, per se, has not been detected within the genome of AIDS-related B-lymphoma cells, and is not considered the direct etiology of this tumor.[32] However, HIV may be operative indirectly by inducing chronic B cell activation and proliferation in the setting of underlying immunosuppression. The induction of cytokine release from infected monocytes and lymphocytes provides one mechanism whereby HIV may induce this on-going B cell proliferation. Thus, as shown by Fauci et al., B cells from HIV-infected patients constitutively express tumor necrosis factor-α (TNF-α) and IL-6.[13] Of interest, IL-6 has been implicated in the development of diverse types of lymphoproliferative disorders,[14–16] and high levels of IL-6 expression have been demonstrated in both HIV-negative and positive B cell lymphoma tissues.[33] Of interest, elevated baseline levels of serum IL-6 were predictive of lymphoma in Pluda's cohort of 116 patients with symptomatic HIV infection.[7] The current prospective trial has confirmed an association between immune complex dissociated HIV p24 antigen levels (a measure of HIV viral burden), and serum levels of IL-6.

Although patient numbers are small, the current trial has also demonstrated an apparent relationship between chemotherapeutic response and serum levels of IL-6 over time. Thus, while the majority of subjects had baseline values which were only moderately ele-

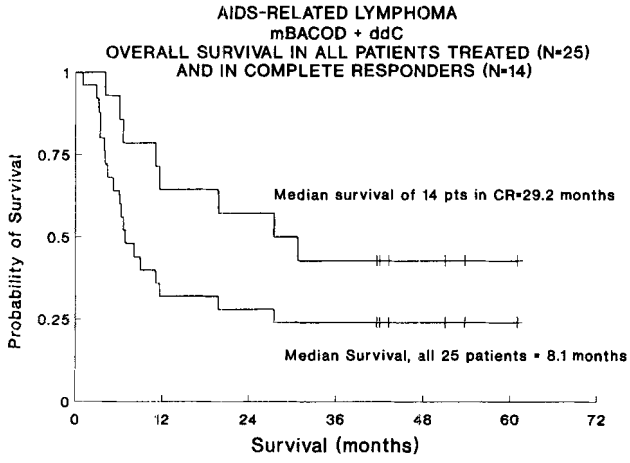

FIGURE 2. Overall survival in complete responders and in all treated patients is shown.

vated, clear decreases in IL-6 values were apparent in 6 of 7 responders with initial values ≥ 10 pg/mL (range: 12–66). Of interest, IL-6 levels increased during episodes of documented bacterial or viral infection in two of these. Such alterations in IL-6 production in the course of infection have been described previously.[34,35] Conversely, two of three patients with PD developed significant elevations of IL-6 levels over time. These results would be consistent with the hypothesis that IL-6 may be operative in the pathogenesis of AIDS-related lymphoma, derived either from the malignant cell and/or its surrounding stromal environment. By suppressing HIV p24 antigen expression in some patients with the use of zalcitabine either alone or with the addition of chemotherapy, the paracrine pathway of IL-6 production may have been impacted, while eradication of lymphoma by chemotherapy may have caused a fall in IL-6 produced by the tumor.

Of interest, the only clinical or pathologic characteristics which seemed to correlate with elevated serum IL-6 at diagnosis were the presence of systemic "B" symptoms ($P = 0.023$), whereas stage of disease and pathologic type of lymphoma were not associated. Systemic "B" symptoms have been associated with poorer prognosis in patients with "de novo" lymphoma, unrelated to HIV infection.[36] The reason for decreased survival in the presence of these symptoms has never been explained. As demonstrated herein, it is possible that the "B" symptoms observed in some patients with lymphoma may be induced by IL-6. Indeed, Emilie et al. have recently demonstrated the disappearance of systemic "B" symptoms after the use of a monoclonal antibody against IL-6 in patients with AIDS-related lymphoma.[37] Prompt alleviation of systemic "B" symptoms has also been reported in a patient with Castleman's disease, after receiving an anti-IL-6 MoAb.[38] Since IL-6 may function as a growth factor in various types of "de novo" as well as AIDS-related lymphomatous diseases,[14–16] the decreased survival experienced by patients with systemic "B" symptoms may reflect the propensity for cytokine-induced tumor growth, leading to an early demise; at the same time, IL-6 may actually cause the fever, night sweats, and/or weight loss which can be experienced.

It is apparent from this study that low dose M-BACOD may be given safely with concomitant zalcitabine antiretroviral therapy, yielding an overall CR rate of 56%. ICD p24 antigen levels remain undetectable or fall significantly in 64% of the patients, whereas 36% experience an increase during treatment. Changes in IL-6 levels over time are consistent with a possible role of this cytokine in the pathogenesis of disease. Future studies should evaluate the combined use of antiretroviral agents, chemotherapeutic drugs, and MoAbs, toxins, or antisense oligonucleotides against IL-6 in an attempt to define more optimal treatment for patients with AIDS-related lymphoma.

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