Clinical Cancer Research

Predictive Value of Cytokines and Immune Activation Biomarkers in AIDS-Related Non-Hodgkin Lymphoma Treated with Rituximab plus Infusional EPOCH (AMC-034 trial)

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Abstract

Purpose: The aims of this study were to determine whether pretreatment plasma levels of cytokines and immune activation-associated molecules changed following treatment for AIDS-NHL with rituximab plus infusional EPOCH, and to determine whether pretreatment levels of these molecules were associated with response to treatment and/or survival.

Experimental Design: We quantified plasma levels of B-cell activation-associated molecules (sCD27, sCD30, and sCD23) and cytokines (IL6, IL10, and CXCL13) before and after the initiation of treatment in persons with AIDS-NHL ($n = 69$) in the AIDS Malignancies Consortium (AMC) 034 study, which evaluated treatment of AIDS-NHL with EPOCH chemotherapy and rituximab.

Results: Treatment resulted in decreased plasma levels of some of these molecules (CXCL13, sCD27, and sCD30), with decreased levels persisting for one year following the completion of treatment. Lower levels of CXCL13 before treatment were associated with complete responses following lymphoma therapy. Elevated levels of IL6 pretreatment were associated with decreased overall survival, whereas higher IL10 levels were associated with shorter progression-free survival (PFS), in multivariate analyses. Furthermore, patients with CXCL13 or IL6 levels higher than the median levels for the NHL group, as well as those who had detectable IL10, had lower overall survival and PFS, in Kaplan–Meier analyses.

Conclusions: These results indicate that CXCL13, IL6, and IL10 have significant potential as prognostic biomarkers for AIDS-NHL. *Clin Cancer Res; 22(2); 328–36. ©2015 AACR.*

Introduction

The risk for developing B-cell non-Hodgkin lymphoma (NHL) is significantly and markedly increased in persons living with HIV infection (1–5). The introduction of combination antiretroviral therapy (HAART) has had a significant impact on overall survival of persons living with HIV infection (6–10). The incidence of AIDS-NHL has decreased in the HAART era, but not to the same extent as that of Kaposi sarcoma or other AIDS-defining conditions. In addition, the widespread availability of HAART appears to have had a differential effect on the incidence of AIDS-NHL subtypes, with a marked decrease in the incidence of primary central nervous system lymphoma (PCNSL), while that of other forms of AIDS-NHL, such as Burkitt lymphoma or diffuse large B-cell lymphoma (DLBCL), either has not decreased or has remained unchanged (7, 11). Therefore, lymphoma remains a significant clinical problem in the HAART era. In fact, NHL appears to be the most common AIDS-related cancer in populations with ready access to HAART, and remains a significant cause of morbidity and mortality in HIV^+ persons in the post-HAART era (12, 13).

B-cell hyperactivation, as well as loss of regulation of Epstein-Barr virus (EBV)-infected B cells, are believed to play important roles in the development of AIDS-NHL (14–17). We and others have shown elevated serum/plasma levels of several B-cell–stimulatory cytokines, including IL6, IL10, and CXCL13, are present over a period of several years before the diagnosis of AIDS-NHL (18–29). Elevated levels of circulating IL6 or IL10 also are seen after AIDS-NHL diagnosis (30–31). AIDS-NHL cell lines also are known to produce cytokines, including IL6 and IL10 (32). In addition, we saw elevated levels of the expression of activation-induced cytidine deaminase (AICDA), a DNA-mutating enzyme, in circulating mononuclear cells, preceding the diagnosis of AIDS-NHL (33).

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Translational Relevance

HIV infection greatly increases the risk for non-Hodgkin lymphoma (NHL), an AIDS-defining cancer. In fact, NHL is now the most common AIDS-related cancer in populations that have access to treatment with effective combination antiretroviral drug treatment regimens (HAART). Although elevated serum/plasma levels of several cytokines and immune activation-associated molecules have been seen to precede AIDS-NHL diagnosis, little is known about their prognostic value. Defining new prognostic biomarkers is of importance, as common techniques for assessing NHL prognosis (i.e., PET) have significant limitations when used in HIV-infected patients. The results presented here indicate that CXCL13, IL6, and IL10, B-cell–stimulatory cytokines, have the potential to serve as prognostic biomarkers in AIDS-NHL.

AICDA expression has also been reported to be elevated in B cells and lymphoma cells infected with HCV (34). HIV can directly induce B-cell *AICDA* expression, as well as their secretion of several cytokines (IL6 and IL10) and surface molecules (CD23), *in vitro* (17, 35).

In the present study, we evaluated plasma levels of several B-cell activation-associated molecules (sCD23, sCD27, sCD30, and IgE) and B-cell–stimulatory cytokines (IL6, IL10, and CXCL13), in persons who had an AIDS-NHL diagnosis, before and after the initiation of treatment, with the aim of better defining postdiagnosis, pretreatment levels of these molecules in AIDS-NHL, and to determine how levels of these immune system stimulatory molecules are affected by treatment for AIDS-NHL. We found that AIDS-NHL patients had high pretreatment plasma levels of several B-cell activation-associated molecules (IL6, IL10, CXCL13, sCD27, and sCD30). In addition, treatment of NHL resulted in a rapid decrease in plasma levels of most of these molecules, with decreased levels persisting for one year following the completion of treatment. Importantly, pretreatment levels of some of these molecules were associated with response to lymphoma therapy, as well as overall survival.

Materials and Methods

Study population

Of the 106 AIDS NHL patients enrolled in an AIDS Malignancy Consortium (AMC) trial, AMC protocol #034 (AMC-034), which compared infusional combination chemotherapy (EPOCH: etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) with concurrent or sequential rituximab (36), plasma specimens were available from 69 patients with intermediate- or high-grade HIV-associated B-cell NHL (50 patients had DLBCL, 17 Burkitt lymphoma, and 2 were classified only as lymphoma). The median age of lymphoma patients was 42.6 ± 8.8 years. Lymphoma patients had a median HIV plasma level of 9908 [inter-quartile rage (IQR) = 492.5–45,660], and a median CD4 number of 187 cells/mm^3 (IQR = 82–333). Plasma was collected before the initiation of therapy, at the end of the first cycle (within a week or less of treatment), and at 6 months and one year following the completion of treatment. Clinical responses were defined as described in the report detailing the AMC-034 trial results (36).

Rituximab, EPOCH, supportive care, and clinical evaluation

Details regarding the treatment protocol can be found in Sparano and colleagues (36). Clinical responses were defined by the International Response Criteria for Non-Hodgkin Lymphoma (which uses anatomic but not functional imaging). Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (Version 2.0). Response was evaluated after every two cycles of EPOCH therapy (with computerized tomography of the chest, abdomen, and pelvis) and continued for two cycles beyond achieving a CR (for a minimum of four and maximum of six cycles), including after completion of R-EPOCH in the concurrent arm, and after completion of EPOCH alone and by rituximab alone in the sequential arm. No patients received rituximab when not approved as part of the study protocol. All patients were required to have bone marrow biopsy and lumbar puncture for cerebrospinal fluid cytologic examination at baseline. A repeat bone marrow biopsy was required if the original study demonstrated lymphomatous marrow involvement, and if the physical examination and imaging studies were consistent with a complete response (36).

Determination of cytokines and soluble receptor molecules and IgE in plasma samples

Plasma levels of B-cell stimulatory cytokines and molecules associated with immune system activation were assessed by ELISA. IL6 was measured using an ultrasensitive assay (Biosource/Invitrogen), with color development time extended to 40 minutes to ensure consistent low-level detection (detection limit = 0.2 pg/mL). IL10 was measured using a human IL10-specific assay (Biosource/Invitrogen) that does not cross-react with EBV viral IL10 (21), modified to increase sensitivity by extending the standard curve (detection limit = 2 pg/mL), increasing sample incubation time to 3 hours, and performing all incubations on a microtiter plate rotator (500 rpm). CXCL13/BCA-1 was measured using the R&D Systems ELISA kit according to the manufacturer's protocol, with a 1:2 dilution (detection limit = 7.8 pg/mL). sCD27 was determined using the PeliKine-compact ELISA kit and Toolset according to the manufacturer's protocol (CLB/Sanquin, the Netherlands), with 1:20 dilutions on all HIV+ samples (detection limit = 32 U/mL, taking dilution into account). Assays for sCD23 (detection limit = 13 U/mL) and sCD30 (detection limit = 6 U/mL) were performed according to the manufacturer's protocols (Bender MedSystems USA). Total plasma IgE was determined utilizing the CIA-7.12 and CIA-4.15 monoclonal antibodies (37) as previously described (38), with the following modifications: IgE ELISA plates were blocked with 10% fetal bovine plasma in PBS-Tween buffer, and all plasma samples were diluted 1:10 using PBS-Tween buffer before addition to the ELISA plate (19). Diluted sera and all subsequent reagents were added at 50 μL per well, and all incubations were performed on a microtiter plate rotator (500 rpm). The IgE standard was pooled normal plasma (generously provided by Drs. Andrew Saxon and Ke Zhang); when referenced to the WHO IgE standard NIBSC 75/502 (which is also pooled human sera), the mean conversion factor was 0.67 ng per IU. The concentration of the lowest IgE standard was 0.8 ng/mL; taking the dilution into account, the lower limit of detection in plasma samples was 8 ng/mL. Plasma samples used for the measurement

of IL6, IL10, sCD27, sCD30, and IgE were frozen and thawed once. Samples used for the measurement of CXCL13 and sCD23 were frozen and thawed twice.

Statistical analysis

The Wilcoxon rank-sum test was used to compare biomarker levels of complete responders with patients who did not achieve a complete response with treatment for lymphoma. To assess their association with outcome measures (complete response, overall survival and progression-free survival; PFS), levels for CXCL13, CD23, CD27, CD30, IL6, and LDH were dichotomized at their median value. Fisher exact test was used to compare those who achieved complete response and those who did not with respect to international prognostic index (IPI) score (age-adjusted) and each biomarker. Those biomarkers that were associated with complete response at the 0.05 significance level were incorporated into a stepwise logistic regression model.

Proportional hazards models were used to evaluate the association of each individual biomarker with overall survival and PFS. Those factors associated with overall survival and PFS at the 0.05 level were incorporated into a stepwise proportional hazards model.

Results

Plasma levels of B-cell–stimulatory cytokines and immune activation molecules were detected in persons with AIDS-NHL

Plasma levels of B-cell–stimulatory cytokines (IL6, IL10, CXCL13), and of molecules associated with B-cell activation (sCD23, sCD27, sCD30, IgE), were measured by ELISA in specimens collected, pre- and posttreatment, from persons enrolled in an AIDS Malignancy Consortium trial comparing infusional combination chemotherapy (EPOCH: etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) with concurrent or sequential rituximab (AMC protocol #034). There was a negative correlation between CD4 counts and CXCL13 levels ($P = 0.042$); correlations of CD4 with the other biomarkers were not significant.

Plasma levels of CXCL13 decreased following NHL treatment

A central aim of this study was to determine whether there is a change in the levels of these cytokines and immune activation molecules following treatment for AIDS-NHL. In order to study this, we measured plasma levels of these molecules before (Pre-Rx), during lymphoma treatment (Post-Rx), as well as 6 months (6mo FU) and one year (1 year FU) after lymphoma treatment. Treatment was seen to result in a marked decrease in plasma CXCL13 levels following the initiation of treatment ($P = 0.005$, Wilcoxon signed rank test; Fig. 1); this decrease was a consequence of treatment and not of survival. This decrease was maintained over time, with decreased plasma CXCL13 levels seen at 6 months and 1 year after the completion of treatment ($P = 0.016$ and 0.031, respectively, Wilcoxon signed rank test).

In contrast, plasma levels of IL6 or IL10 were not seen to decrease significantly following treatment ($P = 0.421$ and 0.492, respectively), nor at 6 months ($P = 0.688$ and 0.625, respectively) or 12 months posttreatment ($P = 1.00$ and not evaluable, respectively; Fig. 1).

Plasma levels of some immune stimulatory molecules decreased following NHL treatment

Compared with pretreatment, sCD30 plasma levels were seen to decrease significantly ($P < 0.004$, Wilcoxon signed rank

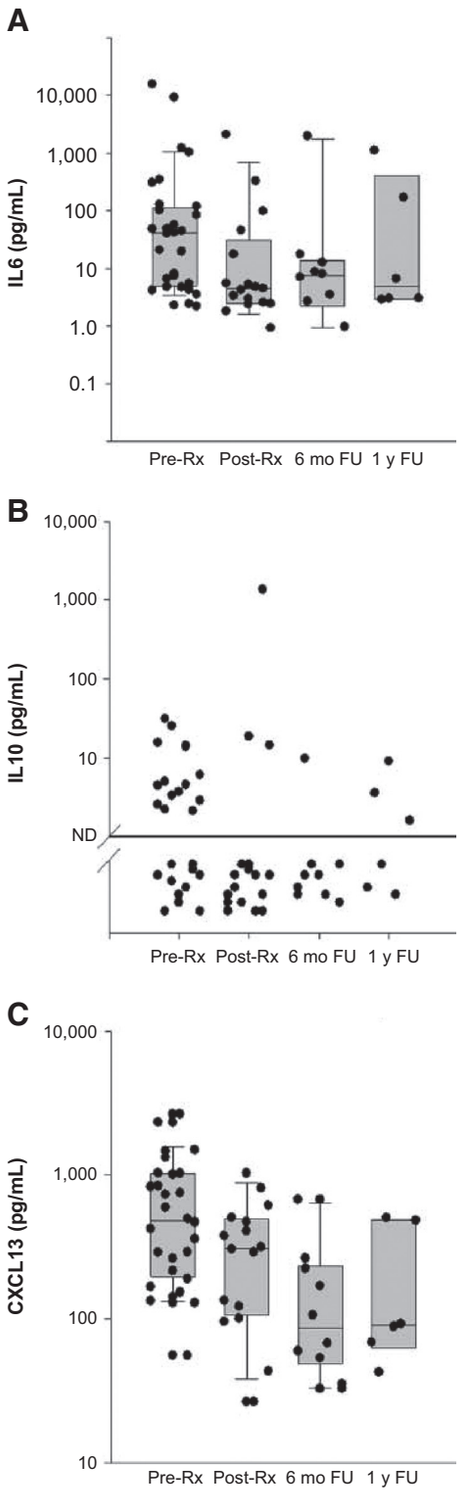

Figure 1.
Levels of B-cell–stimulatory cytokines pre- and post-NHL treatment. Plasma IL6 (A), IL10 (B), and CXCL13 (C) levels were measured in samples from persons who had been diagnosed with NHL, in samples from the same patients collected after lymphoma treatment (at the end of the first cycle of treatment, within a week after treatment initiation), 6 months after treatment and 1 year after treatment. Each filled circle represents a sample from a single patient.

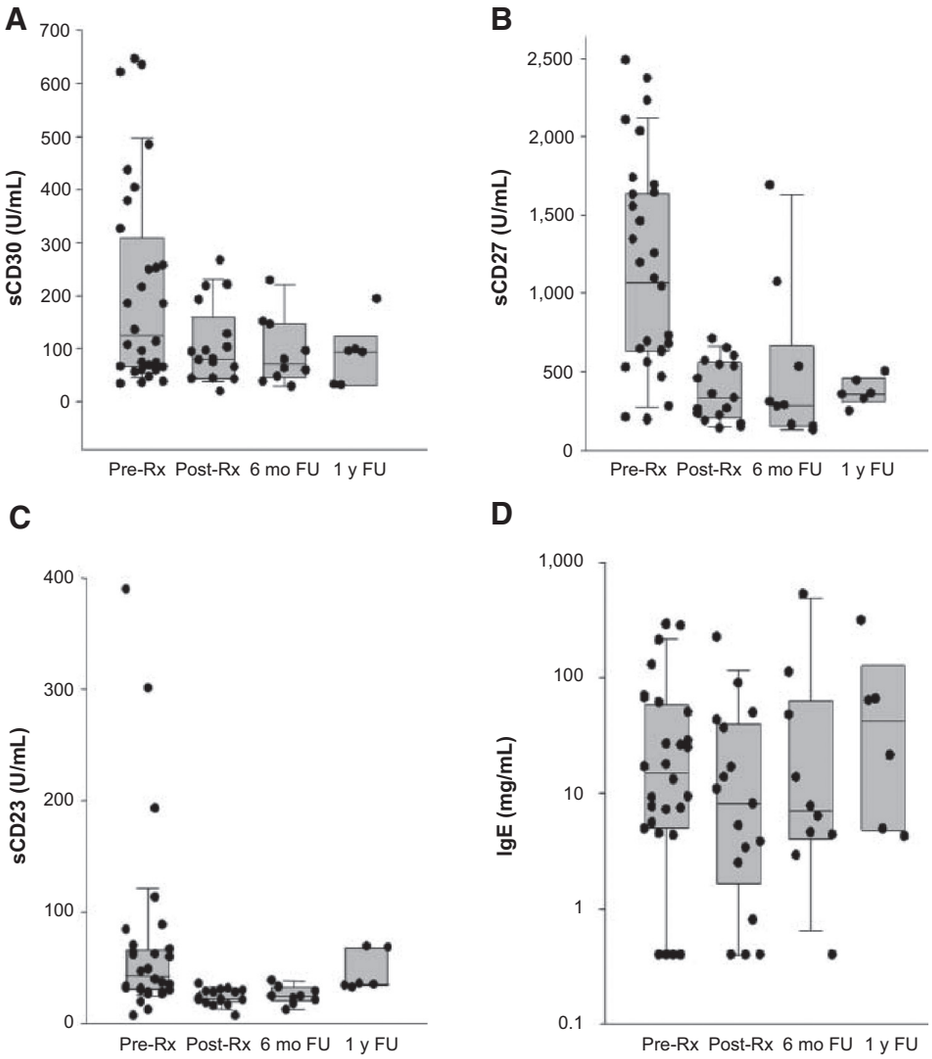

Figure 2.
Levels of immune activation biomarkers pre- and post-NHL treatment. Plasma sCD30 (A), sCD27 (B), sCD23 (C), and IgE (D) levels were measured in samples from persons who had been diagnosed with NHL, in samples from the same patients collected after lymphoma treatment (at the end of the first cycle of treatment, within a week after treatment initiation), 6 months after treatment, and 1 year after treatment. Each filled circle represents a sample from a single patient.

test; Fig. 2), and appeared to remain at lower levels at 6 months and 1 year after treatment completion, although this was not statistically significant ($P = 0.297$ and 0.063, respectively).

Similarly, plasma sCD27 levels were significantly decreased following the initiation of treatment ($P < 0.001$; Fig. 2), and remained lower at 6 months ($P = 0.016$) and 1 year after the completion of lymphoma treatment ($P = 0.063$), although this decrease was not statistically significant at one year posttreatment.

Plasma levels of sCD23 were seen to decrease significantly ($P < 0.001$, Wilcoxon signed rank test) after the initiation of lymphoma treatment and at 6 months posttreatment ($P = 0.16$), but not at 1 year after the completion of treatment ($P = 0.219$; Fig. 2).

Plasma levels of IgE appeared unchanged after lymphoma treatment (Fig. 2).

Association of biomarker levels with type of treatment

In this study, patients were treated with EPOCH with concurrent rituximab, or administration of rituximab after EPOCH treatment was complete. There were no significant differences seen between patients receiving concurrent or sequential rituximab, in terms of plasma levels of these biomarkers (not shown).

Association of biomarker levels with clinical response to lymphoma therapy, and survival

Lower levels of CXCL13, IL10, IL6, and LDH, as well as IPI scores, were significantly associated with complete response to therapy, in univariate analyses (Table 1). Similarly, when we assessed the association of these biomarkers with any clinical response (complete and partial responders vs. nonresponders), CXCL13, LDH, and IPI remained significant predictors of a clinical response and detectable IL10 was associated with nonresponders (not significant; Table 2). In addition, when a stepwise logistic regression was used to assess the relative contribution of these factors to complete response, only CXCL13 was seen to be associated with complete response ($P = 0.003$; OR = 5.5, 1.81–16.68).

We also looked at the association of these biomarkers with overall survival or PFS. In univariate analyses, the following

Table 1. Mean (IQR) of cytokines and stimulatory molecules and *P* values, when comparing complete responders with nonresponders or partial responders before treatment

	Complete responders [median (IQR)], *n* = 37	Nonresponders or partial responders [median (IQR)], *n* = 30–32	*P*
IL6 (pg/mL)	6.3 (4.0–32.2)	44.4 (8.2–96.1)	0.043
IL10 (% detectable)	41%	72%	0.0148
sCD23 (U/mL)	69.5 (29.8–94.0)	39.4 (26.0–68.7)	0.228
sCD30 (U/mL)	102 (76.1–227)	157 (62.4–376)	0.586
CXCL13 (pg/mL)	238 (155–395)	617 (252–1,030)	0.001
IgE (ng/mL)	21.8 (8.2–29.2)	40.2 (7.7–146)	0.190
sCD27 (U/mL)	800 (559–1,250)	758 (526–1,480)	0.966
LDH	305 (164–619)	555 (240–1,370)	0.0442
IPI (% who had IPI score of 0–1)	92%	66%	0.0198

factors were significantly associated with overall survival: IPI, CXCL13, IL6, IL10, and LDH (Table 3). However, when they were incorporated into a stepwise proportional hazard model for overall survival, only IL6 ($P = 0.010$, OR = 0.30, 0.12–0.75) was associated with overall survival.

The following factors were significantly associated with PFS in univariate analyses: IPI, CXCL13, IL6, IL10, and LDH (Table 3). However, when these markers were incorporated into a stepwise proportional hazard model, only IL10 ($P = 0.024$; OR = 2.86, 1.15–7.15) was significantly associated with PFS. Furthermore, patients with CXCL13, IL6, or IL10 levels higher than the median levels for the NHL group as a whole had lower overall survival and PFS, in Kaplan–Meier analyses (Fig. 3).

We also assessed the association of cytokine/activation markers with response to treatment by NHL pathologic types (DLBCL and Burkitt lymphoma). With respect to OS and PFS, the direction of the HRs was the same for Burkitt lymphoma and DBLCL, but frequently achieved statistical significance only when all patients are included (not shown). Therefore, while there was insufficient statistical power to definitively determine whether there was a difference in the results observed for these two NHL subgroups, it does not appear that there were marked differences seen between DLBCL and Burkitt lymphoma.

Discussion

In this study, we found that plasma levels of several molecules that are associated with immune activation and inflammation are detectable in those who have untreated AIDS-NHL, and that plasma levels of some of these molecules (sCD23, sCD27, sCD30, CXCL13) showed marked reductions after EPOCH and rituximab treatment. We did not see any differences in the levels of these biomarkers between the concurrent or sequential EPOCH and Rituximab treatment groups.

Table 2. Median (IQR) of cytokines and stimulatory molecules and *P* values, when comparing all responders (complete plus partial) versus nonresponders before treatment

	Complete and partial responders [median (IQR)], *n* = 44–52	Nonresponders [median (IQR)], *n* = 16–18	*P*
IL6 (pg/mL)	8.1 (4.2–44.4)	45.7 (4.2–84.1)	0.469
IL10 (% detectable)	50%	71%	0.168
sCD23 (U/mL)	47.1 (23.1–79.8)	43.4 (30.9–84.2)	0.485
sCD30 (U/mL)	96.6 (68.0–227)	171 (70.4–362)	0.273
CXCL13 (pg/mL)	247 (166–481)	663 (389–1,490)	0.003
IgE (ng/mL)	26.4 (9.4–84.5)	14.6 (7.1–88.6)	0.466
sCD27 (U/mL)	794 (523–1,250)	729 (525–1,270)	0.903
LDH	320 (190–691)	980 (260–1,660)	0.044
IPI (% who had IPI score of 0–1)	44%	12%	0.020

Most notably, we saw that pretreatment levels of CXCL13, IL6, IL10, and LDH, as well as IPI, were significantly lower in those patients who went on to have complete responses to treatment. After conducting a stepwise logistic regression analysis, CXCL13 was the only factor that significantly correlated with subsequent treatment response. Similarly, multivariate analyses showed that only pretreatment IL6 levels were associated with overall survival, and IL10 levels with PFS.

We also assessed the association of cytokine/activation markers with response to treatment by the major NHL pathologic types (DLBCL and Burkitt lymphoma) included in the AMC-034 study. Although there was insufficient statistical power to definitively determine whether there was a difference in the results observed for these two NHL subgroups, it did not appear that there were marked differences seen between DLBCL and Burkitt lymphoma.

Overall, these results suggest that plasma levels of CXCL13, IL6, and IL10 have significant potential as prognostic biomarkers for AIDS-NHL, and may add additional information over LDH or IPI, which are commonly used to assess prognosis (39). Certainly, the prognostic value of these cytokines needs to be confirmed in larger studies. In addition, it is important to determine whether the levels of these cytokines reflect tumor burden, or alternatively, if they are markers for the inability of patients to respond to therapy for other reasons relating to their poor health. Also, further studies are needed to define the prognostic value of measuring these cytokines when measured after the initiation of treatment for AIDS-NHL. However, these molecules show promise as new tools for the assessment of prognosis, and potentially, for the selection of treatment regimens for AIDS-NHL.

CXCL13 is a chemokine that directs the normal trafficking of B cells (40). It is expressed by T-follicular helper cells, dendritic cells, and stromal cells in secondary lymphoid tissue (41). Plasma levels of the chemokine, CXCL13, are elevated during HIV infection (42), and decrease with antiretroviral drug treatment (43). Other reports indicate that there are abnormalities in the CXCR5/CXCL13 system during HIV infection, including loss of expression of CXCR5 on mature B cells (44), and expression of CXCL13 by recirculating B cells (45). Together, these observations raise the possibility that the CXCR5/CXCL13 system may contribute to the abnormalities that are seen in the B-cell compartment during HIV infection, and thus could be involved in the genesis of AIDS-NHL. CXCL13 has been shown to be associated with Sjögren disease, in which CXCL13 contributes to the organization of ectopic reactive lymphoid tissue (46). In addition, elevated serum levels of CXCL13 have been seen before the diagnosis of non-AIDS NHL (47, 48), and CXCL13 and/or CXCR5 have been shown to be associated with several subtypes of non-AIDS–related B-cell lymphomas (49–50).

IL6 and IL10 are inflammation-associated cytokines that are secreted by monocytes, lymphocytes, and other cell types, and can enhance B-cell proliferation, survival, and antibody production. IL6 and IL10 are known to be elevated before lymphoma diagnosis, and their elevated levels are associated with

Table 3. Relationship between biomarkers and outcome measures

Factor	*N*	Complete response rate (%)	1-Year OS (%)(95% CI)	1-Year PFS (%)(95% CI)
IPI score				
0–1	25	72	95.8 (73.9–99.4)	95.8 (73.9–99.4)
2–4	44	43	62.8 (46.6–75.3)	54.0 (38.1–67.4)
OR/HR[c]		3.38 (1.06–11.47)	0.40 (0.15–1.09)	0.39 (0.16–0.97)
P		0.026[a]	0.028[b]	0.015[b]
CXCL13				
<Median	35	74	88.0 (71.2–95.3)	85.0 (67.6–93.5)
>Median	34	32	61.3 (42.8–75.4)	52.9 (35.1–67.9)
OR/HR[c]		6.04 (1.90–19.68)	0.31 (0.13–0.74)	0.41 (0.19–0.89)
P		<0.001[a]	0.008[b]	0.024[b]
sCD27				
<Median	34	53	70.1 (51.5–82.6)	61.1 (42.5–75.3)
>Median	34	56	81.9 (64.1–91.4)	79.1 (61.1–89.5)
OR/HR[c]		0.89 (0.31–2.56)	1.78 (0.78–4.08)	0.77 (0.83–3.78)
P		1.000[a]	0.171[b]	0.141[b]
sCD23				
<Median	34	47	82.1 (64.5–91.6)	73.1 (54.7–85.0)
>Median	35	60	68.0 (49.6–80.8)	65.2 (46.9–78.5)
OR/HR[c]		0.59 (0.20–1.71)	0.43 (0.18–1.01)	0.64 (0.31–1.35)
P		0.338[a]	0.051[b]	0.245[b]
sCD30				
<Median	35	60	69.5 (50.7–82.3)	58.0 (39.6–72.7)
>Median	34	47	79.8 (62.2–89.8)	79.8 (62.2–89.8)
OR/HR[c]		1.69 (0.59–4.88)	1.59 (0.71–3.56)	1.62 (0.77–3.41)
P		0.338[a]	0.261[b]	0.207[b]
IL6				
<Median	34	68	90.8 (74.1–96.9)	85.0 (67.7–93.5)
>Median	34	41	61.2 (42.6–75.3)	55.1 (36.9–70.1)
OR/HR[c]		2.99 (1.00–9.08)	0.26 (0.10–0.64)	0.34 (0.15–0.76)
P		0.051[a]	0.004[b]	0.008[b]
IL10				
Undetectable	31	71	93.3 (75.8–98.3)	90.1 (72.3–96.7)
Detectable	38	39	60.0 (42.6–73.6)	52.6 (35.8–67.0)
OR/HR[c]		3.75 (1.23–11.77)	0.25 (0.09–0.67)	0.31 (0.13–0.74)
P		0.015[a]	0.006[b]	0.008[b]
LDH				
<Median	30	60	85.9 (66.7–94.5)	79.6 (60.1–90.3)
>Median	30	40	60.0 (40.5–75.0)	53.3 (34.3–69.1)
OR/HR[c]		2.25 (0.71–7.18)	0.41 (0.17–0.98)	0.45 (0.20–0.98)
P		0.196[a]	0.044[b]	0.046[b]

[a]Fisher exact test.
[b]Log-rank test.
[c]OR and 95% confidence interval for complete responses; HR and 95% confidence interval for OS and PFS (unadjusted).

risk for the development of NHL in HIV+ persons (19–29). It is unclear how IL6 and IL10 are contributing to the development of lymphoma. They may be directly promoting the growth and/or viability of cancer cells and/or they may affect other immune cells, presumably creating a favorable environment for the development or growth of lymphoma cells. In addition, they may be secreted by the tumor cells. Thus, the reduction of these cytokines seen following treatment may be due to loss of tumor cells, as well as to the loss of tumor-reactive cells. Alternatively, the loss of cytokines maybe due to survival time bias, as partial responders and nonresponders are more likely not to survive.

In prior work, we reported that serum/plasma levels of sCD23 were significantly elevated some years before the development of AIDS-NHL, but sCD23 levels did not differ between AIDS-NHL cases and controls when measurements were made closer (<1 year) to the time of lymphoma diagnosis (19). Therefore, this molecule seems to be elevated several years before lymphoma diagnosis, but then drops as NHL diagnosis is approached. In this sense, the observed levels of sCD23 seen in NHL patients in this study are consistent with a progressive decrease in plasma sCD23, going from elevated several years before NHL diagnosis to decreased postdiagnosis. It is possible that sCD23 plays an etiologic role in early events in lymphomagenesis, but not in supporting the progressive growth of these cancers. These results are consistent with the known role of sCD23 in promoting *IgH* class switch recombination (CSR), a molecular event thought to contribute to the genesis of lymphomagenic chromosomal translocations that lead to Burkitt lymphoma (17).

As mentioned above, we and others have reported that these cytokines and molecules are elevated preceding AIDS-NHL (19–29). The results presented here extend, and are generally in agreement with, those prior studies, and support the notion that a B-cell stimulatory environment is associated with the development and progression of AIDS-NHL. In addition to this, some of these AIDS-NHL–associated plasma molecules, especially CXCL13, IL6, and IL-10, appear to have potential value as indicators of subsequent response to NHL treatment and survival.

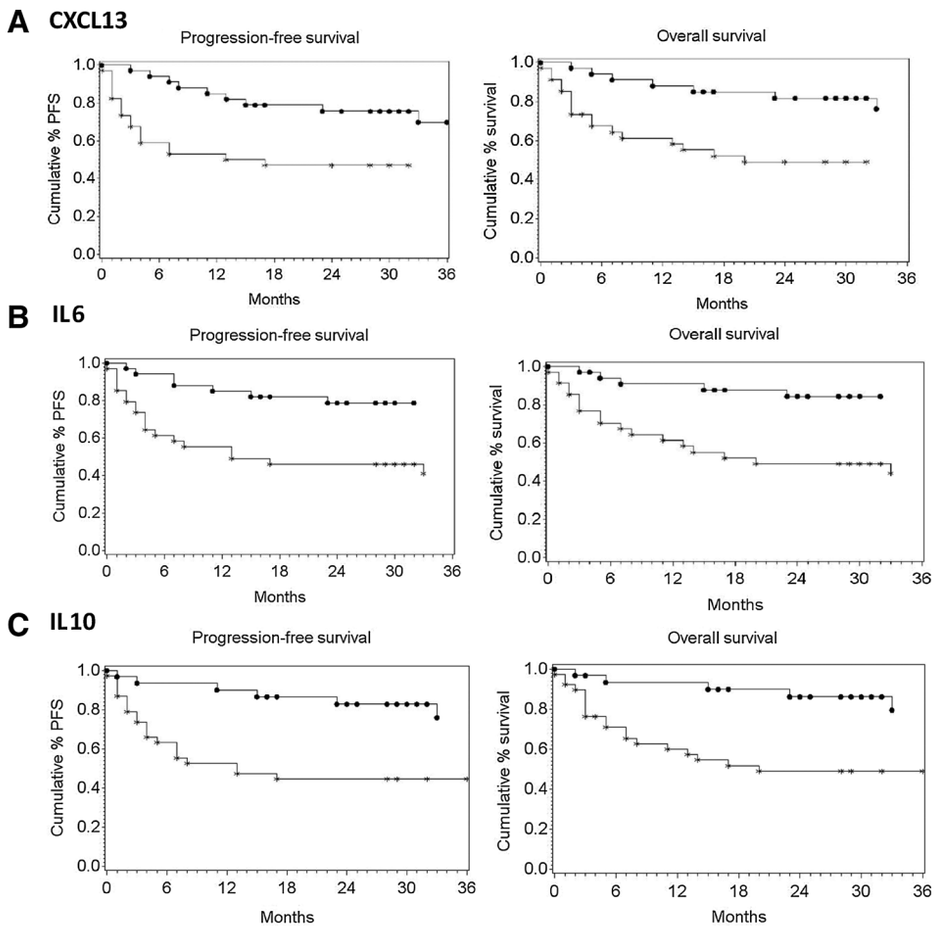

Figure 3.
Association of CXCL13, IL6, or IL10 plasma levels and PFS and overall survival. PFS and overall survival of subjects with pretreatment CXCL13 values higher than median (353 pg/mL; A), pretreatment IL6 values higher than median (18.03 pg/mL; B), or detectable pretreatment IL10 values (C). Filled circles, AIDS-NHL cases with CXCL13 or IL6 levels lower than median, or with no detectable plasma IL10; asterisks, cases with CXCL13 or IL6 levels higher than median, or with detectable IL10.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Beral V, Peterman T, Berkelman R, Jaffe H. AIDS-associated non-Hodgkin lymphoma. Lancet 1991;337:805–9.
2. Ziegler JL, Bragg K, Abrams D, Beckstead J, Cogan M, Volberding P, et al. High-grade non-Hodgkin's lymphoma in patients with AIDS. Ann N Y Acad Sci 1984;437:412–9.
3. Armenian HK, Hoover DR, Rubb S, Metz S, Martinez-Maza O, Chmiel J, et al. Risk factors for non-Hodgkin's lymphomas in acquired immunodeficiency syndrome (AIDS). Am J Epidemiol 1996;143:374–9.
4. Biggar RJ, Rosenberg PS, Cote T. Kaposi's sarcoma and non-Hodgkin's lymphoma following the diagnosis of AIDS. Multistate AIDS/Cancer Match Study Group. Int J Cancer 1996;68:754–8.
5. Goedert JJ, Cote TR, Virgo P, Scoppa SM, Kingma DW, Gail MH, et al. Spectrum of AIDS-associated malignant disorders. Lancet 1998;351:1833–9.
6. Hessol NA, Seaberg EC, Preston-Martin S, Massad LS, Sacks HS, Silver S, et al. Cancer risk among participants in the women's interagency HIV study. J Acquir Immune Defic Syndr 2004;36:978–85.

7. Biggar RJ. AIDS-related cancers in the era of highly active antiretroviral therapy. Oncology 2001;15:439–48.
8. Engels EA, Pfeiffer RM, Goedert JJ, Virgo P, McNeel TS, Scoppa SM, et al. Trends in cancer risk among people with AIDS in the United States 1980–2002. AIDS 2006;20:1645–54.
9. Seaberg EC, Hessol N, Jacobson L, Martínez-Maza O, Sutcliffe C, Levine A. Cancer incidence before and during the era of HAART. 9th International Workshop on HIV Observational Databases Budapest, Hungary; 2005.
10. Seaberg EC, Wiley D, Martinez-Maza O, Chmiel JS, Kingsley L, Tang Y, et al. Cancer incidence in the multicenter aids cohort study before and during the HAART era: 1984 to 2007. Cancer 2010;116:5507.
11. Biggar RJ, Jaffe ES, Goedert JJ, Chaturvedi A, Pfeiffer R, Engels EA. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. Blood 2006;108:3786–91.
12. Bonnet F, Balestre E, Thiebaut R, Morlat P, Pellegrin JL, Neau D, et al. Factors associated with the occurrence of AIDS-related non-Hodgkin lymphoma in the era of highly active antiretroviral therapy: Aquitaine Cohort, France. Clin Infect Dis 2006;42:411–7.
13. Bonnet F, Lewden C, May T, Heripret L, Jougla E, Bevilacqua S, et al. Malignancy-related causes of death in human immunodeficiency virus-infected patients in the era of highly active antiretroviral therapy. Cancer 2004;101:317–24.
14. van Baarle D, Hovenkamp E, Callan MF, Wolthers KC, Kostense S, Tan LC, et al. Dysfunctional Epstein-Barr virus (EBV)-specific CD8(+) T lymphocytes and increased EBV load in HIV-1 infected individuals progressing to AIDS-related non-Hodgkin lymphoma. Blood 2001;98: 146–55.
15. Epeldegui M, Widney DP, Martinez-Maza O. Pathogenesis of AIDS lymphoma: role of oncogenic viruses and B cell activation-associated molecular lesions. Curr Opin Oncol 2006;18:444–8.
16. Martinez-Maza O, Breen EC. B-cell activation and lymphoma in patients with HIV. Curr Opin Oncol 2002;14:528–32.
17. Epeldegui M, Vendrame E, Martinez-Maza O. HIV-associated immune dysfunction and viral infection - role in the pathogenesis of AIDS-related lymphoma. Immunol Res 2010;48:72–83.
18. Ambinder RF, Bhatia K, Martinez-Maza O, Mitsuyasu R. Cancer biomarkers in HIV patients. Curr Opin HIV AIDS 2010;5:531–7.
19. Breen EC, Hussain SK, Magpantay L, Jacobson LP, Detels R, Rabkin CS, et al. B-cell stimulatory cytokines and markers of immune activation are elevated several years prior to the diagnosis of systemic AIDS-associated non-hodgkin B-cell lymphoma. Cancer Epidemiol Biomarkers Prev 2011;20: 1303–14.
20. Pluda JM, Venzon DJ, Tosato G, Lietzau J, Wyvill K, Nelson DL, et al. Parameters affecting the development of non-Hodgkin's lymphoma in patients with severe human immunodeficiency virus infection receiving antiretroviral therapy. J Clin Oncol 1993;11:1099–107.
21. Breen EC, Boscardin WJ, Detels R, Jacobson LP, Smith MW, Chmiel JS, et al. Non-Hodgkin's B cell lymphoma in persons with acquired immunodeficiency syndrome is associated with increased serum levels of IL10, or the IL10 promoter -592 C/C genotype. Clin Immunol 2003;109:119–29.
22. Breen EC, van der Meijden M, Cumberland W, Kishimoto T, Detels R, Martinez-Maza O. The development of AIDS-associated Burkitt's/small noncleaved cell lymphoma is preceded by elevated serum levels of interleukin 6. Clin Immunol 1999;92:293–9.
23. Ouedraogo DE, Makinson A, Kuster N, Nagot N, Rubbo PA, Bollore K, et al. Increased T-cell activation and Th1 cytokine concentrations prior to the diagnosis of B-cell lymphoma in HIV infected patients. J Clin Immunol 2013;33:22–9.
24. Widney DP, Gui D, Popoviciu LM, Said JW, Breen EC, Boscardin WJ, et al. Expression and function of the chemokine, CXCL13, and its receptor, CXCR5, in AIDS-associated non-Hodgkin's lymphoma. AIDS Res Treat 2010;2010:164586.
25. Hussain SK, Zhu W, Chang SC, Breen EC, Vendrame E, Magpantay L, et al. Serum levels of the chemokine CXCL13, genetic variation in CXCL13 and its receptor CXCR5, and HIV-associated non-hodgkin B-cell lymphoma risk. Cancer Epidemiol Biomarkers Prev 2013;22: 295–307.
26. Hussain SK, Hessol NA, Levine AM, Crabb Breen E, Anastos K, Cohen M, et al. Serum biomarkers of immune activation and subsequent risk of non-Hodgkin B-cell lymphoma among HIV-infected women. Cancer Epidemiol Biomarkers Prev 2013;22:2084–93.
27. Vendrame E, Hussain SK, Crabb Breen E, Magpantay LI, Widney DP, Jacobson LP, et al. Serum levels of cytokines, and biomarkers for inflammation and immune activation, and HIV-associated non-Hodgkin B cell lymphoma risk. Cancer Epidemiol Biomarkers Prev 2014; 23:343–9.
28. Yawetz S, Cumberland WG, van der Meyden M, Martinez-Maza O. Elevated serum levels of soluble CD23 (sCD23) precede the appearance of acquired immunodeficiency syndrome–associated non-Hodgkin's lymphoma. Blood 1995;85:1843–9.
29. Widney D, Gundapp G, Said JW, van der Meijden M, Bonavida B, Demidem A, et al. Aberrant expression of CD27 and soluble CD27 (sCD27) in HIV infection and in AIDS-associated lymphoma. Clin Immunol 1999; 93:114–23.
30. Levine AM, Scadden DT, Zaia JA, Krishnan A. Hematologic Aspects of HIV/AIDS. Hematology 2001:463–78.
31. Edelman L, Deveau C, Raphael M, Monchatre E, Gabarre J, Deville-Chabrol A, et al. Serum interleukin-10 in acquired immunodeficiency syndrome lymphoma patients. Seroco-Hemoco Study Group. Eur Cytokine Netw 1996;7:785–91.
32. Pastore C, Gaidano G, Ghia P, Fassone L, Cilia AM, Gloghini A, et al. Patterns of cytokine expression in AIDS-related non-Hodgkin's lymphoma. Br J Haematol 1998;103:143–9.
33. Epeldegui M, Breen EC, Hung YP, Boscardin WJ, Detels R, Martinez-Maza O. Elevated expression of activation induced cytidine deaminase in peripheral blood mononuclear cells precedes AIDS-NHL diagnosis. AIDS 2007;21:2265–70.
34. Machida K, Cheng KT, Sung VM, Shimodaira S, Lindsay KL, Levine AM, et al. Hepatitis C virus induces a mutator phenotype: enhanced mutations of immunoglobulin and protooncogenes. Proc Natl Acad Sci U S A 2004;101:4262–7.
35. Imbeault M, Ouellet M, Giguere K, Bertin J, Bélanger D, Martin G, Tremblay MJ. Acquisition of host-derived CD40L by HIV-1 *in vivo* and its functional consequences in the B-cell compartment. J Virol 2011; 85:2189–200.
36. Sparano JA, Lee JY, Kaplan LD, Levine AM, Ramos JC, Ambinder RF, et al. Rituximab plus concurrent infusional EPOCH chemotherapy is highly effective in HIV-associated B-cell non-Hodgkin lymphoma. Blood 2010;115:3008–16.
37. Kanowith-Klein S, Saxon A. Fc epsilon receptors on human cell lines and peripheral blood lymphocytes detected by binding of IgE immune complexes. J Clin Immunol 1985;5:38–45.
38. Zhang K, Clark EA, Saxon A. CD40 stimulation provides an IFN-gamma-independent and IL-4-dependent differentiation signal directly to human B cells for IgE production. J Immunol 1991;146:1836–42.
39. Barta SK, Lee JY, Kaplan LD, Noy A, Sparano JA. Pooled analysis of AIDS malignancy consortium trials evaluating rituximab plus CHOP or infusional EPOCH chemotherapy in HIV-associated non-Hodgkin lymphoma. Cancer 2012;118:3977–83.
40. Muller G, Hopken UE, Stein H, Lipp M. Systemic immunoregulatory and pathogenic functions of homeostatic chemokine receptors. J Leukoc Biol 2002;72:1–8.
41. Crotty S, Johnston RJ, Schoenberger SP. Effectors and memories: Bcl-6 and Blimp-1 in T and B lymphocyte differentiation. Nat Immunol 2010;11: 114–20.
42. Widney DP, Breen EC, Boscardin WJ, Kitchen SG, Alcantar JM, Smith JB, et al. Serum levels of the homeostatic B cell chemokine, CXCL13, are elevated during HIV infection. J Interferon Cytokine Res 2005; 25:702–6.
43. Regidor DL, Detels R, Breen EC, Widney DP, Jacobson L, Palella F, et al. Effect of highly active antiretroviral therapy on biomarkers of B-lymphocyte activation and inflammation. AIDS 2011;25:303–14.
44. Förster R, Schweigard G, Johann S, Emrich T, Kremmer E, Nerl C, Lipp M. Abnormal expression of the B-cell homing chemokine receptor BLR1 during the progression of acquired immunodeficiency syndrome. Blood 1997;90:520–5.
45. Cagigi A, Mowafi F, Phuong Dang LV, Tenner-Racz K, Atlas A, Grutzmeier S, et al. Altered expression of the receptor-ligand pair CXCR5/CXCL13 in B cells during chronic HIV-1 infection. Blood 2008;112: 4401–10.

46. Barone F, Bombardieri M, Rosado MM, Morgan PR, Challacombe SJ, De Vita S, et al. CXCL13, CCL21, and CXCL12 expression in salivary glands of patients with Sjogren's syndrome and MALT lymphoma: association with reactive and malignant areas of lymphoid organization. J Immunol 2008;180:5130–40.
47. De Roos AJ, Mirick D, Edlefsen K, LaCroix AZ, Kopecky K, Madeleine M, et al. Markers of B-cell activation in relation to risk of non-Hodgkin lymphoma. Cancer Res 2012;72:4733–43.
48. Purdue MP, Hofmann JN, Kemp TJ, Chaturvedi AK, Lan Q, Park JH, et al. A prospective study of 67 serum immune and inflammation markers and risk of non-Hodgkin lymphoma. Blood 2013; 122:951–7.
49. Husson H, Freedman AS, Cardoso AA, Schultze J, Munoz O, Strola G, et al. CXCL13 (BCA-1) is produced by follicular lymphoma cells: role in the accumulation of malignant B cells. Br J Haematol 2002;119: 492–5.
50. Trentin L, Cabrelle A, Facco M, Carollo D, Miorin M, Tosoni A, et al. Homeostatic chemokines drive migration of malignant B cells in patients with non-Hodgkin lymphomas. Blood 2004;104: 502–8.