Immune Activation and Microbial Translocation as Prognostic Biomarkers for AIDS-Related Non-Hodgkin Lymphoma in the AMC-034 Study

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ABSTRACT

Purpose: AIDS-related non-Hodgkin lymphoma (ARL) is the most common cancer in HIV-infected individuals in the United States and other countries in which HIV-positive persons have access to effective combination antiretroviral therapy (cART). Our prior work showed that pretreatment/postdiagnosis plasma levels of some cytokines, such as IL6, IL10, and CXCL13, have the potential to serve as indicators of clinical response to treatment and survival in ARL. The aims of this study were to identify novel prognostic biomarkers for response to treatment and/or survival in persons with ARL, including biomarkers of microbial translocation and inflammation.

Experimental Design: We quantified plasma levels of several biomarkers (sCD14, LBP, FABP2, EndoCab IgM, IL18, CCL2/MCP-1, sCD163, IP-10/CXCL10, TARC/CCL17, TNFα, BAFF/BLyS, sTNFRII, sCD44, and sIL2Rα/sCD25) by multiplexed immunometric assays (Luminex) or ELISA in plasma specimens obtained from ARL patients enrolled in the AMC-034 trial, which compared infusional combination chemotherapy (EPOCH: etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) with concurrent or sequential rituximab. Plasma was collected prior to the initiation of therapy ($n = 57$) and after treatment initiation ($n = 55$).

Results: We found that several biomarkers decreased significantly after treatment, including TNFα, sCD25, LBP, and TARC (CCL17). Moreover, pretreatment plasma levels of BAFF, sCD14, sTNFRII, and CCL2/MCP-1 were univariately associated with overall survival, and pretreatment levels of BAFF, sTNFRII, and CCL2/MCP-1 were also associated with progression-free survival.

Conclusions: Our results suggest that patients with ARL who responded to therapy had lower pretreatment levels of inflammation and microbial translocation as compared with those who did not respond optimally.

Introduction

AIDS-related non-Hodgkin lymphoma (ARL) is the most common AIDS-defining cancer in HIV-infected individuals in the United States and other countries in which HIV-positive persons have access to effective combination antiretroviral therapy (cART; ref. 1). The introduction of cART has been associated with declines in the rates of ARL in developed countries (2–6). As previously described, the availability of cART appears to have had differential effects on the incidence of different ARL subtypes; on one hand, the incidence of primary central nervous system lymphoma (PCNSL) has decreased significantly, but the incidence of other ARL subtypes, such as Burkitt lymphoma or diffuse large B-cell lymphoma (DLBCL) has either not decreased or remained unchanged (7–9). This probably reflects the etiology of these cancers; PCNSLs develop due to the loss of immune control of Epstein–Barr virus (EBV)–infected B-cell clones (10), while the development of Burkitt lymphoma and DLBCL is more likely due to HIV infection–associated chronic B-cell activation (11, 12).

Chronic B-cell activation associated with HIV infection is believed to contribute to the development of NHL (11, 13, 14) and plays an important role in lymphomagenesis, even in the cART era. Epidemiologic studies have revealed that pre-ARL diagnosis serum levels of inflammatory cytokines, such as IL6, IL10, IP-10/CXCL10, CXCL13, TNFα, and sCD23, are associated with increased risk for ARL (15–19). Another factor that leads to immune activation and/or inflammation is microbial translocation. Microbial translocation is the leakage of bacterial products from the gut lumen into the peripheral circulation, which results in high levels of lipopolysaccharide (LPS) in the circulation of persons living with HIV infection, further leading to chronic immune activation and inflammation (20, 21). Importantly, several studies have demonstrated the limited effects of cART in antagonizing microbial translocation and the mechanisms by which it arises, indicating that it remains a problem in those who are receiving cART (22, 23). Moreover, serum levels of markers of microbial translocation, including FABP2, LPS-binding protein (LBP), haptoglobin, sCD14, and endotoxin core antibody (EndoCab) IgM, and markers of macrophage activation, such as sCD163, are all associated with ARL risk (24). Hence, it is clear that B-cell stimulatory cytokines, inflammation, macrophage activation, and microbial translocation may contribute to the development of non-Hodgkin lymphoma (NHL). However, the prognostic value of these molecules have not been defined.

Serum lactate dehydrogenase (LDH) is commonly elevated in lympho-proliferative disorders and currently, serves as the only

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Clin Cancer Res 2021;27:4642–51

doi: 10.1158/1078-0432.CCR-20-4167

Translational Relevance

Chronic HIV infection increases the risk for the development of non-Hodgkin lymphoma (NHL), the most common AIDS-defining cancer among HIV-infected individuals who have access to effective combination antiretroviral drug treatment (cART) regimens. Microbial translocation has been implicated as a possible cause of systemic immune activation and disease progression among HIV-infected individuals. Serum levels of microbial translocation biomarkers are associated with AIDS-related NHL (ARL) risk; however, their prognostic value remains unknown. The identification of new biomarkers with prognostic significance is warranted. Currently, imaging techniques used for the diagnosis of lymphomas (i.e., PET) remain increasingly challenging by the different clinical presentations of AIDS-related malignancies. Our data show that pretreatment plasma levels of biomarkers of inflammation/immune activation (sTNFRII, sCD25) and microbial translocation/macrophage activation (BAFF/BLyS, sCD14, CCL2/MCP-1) are associated with overall survival and progression-free survival in patients with ARL, and they may serve as potential prognostic biomarkers.

prognostic factor in HIV-related NHLs (25). The International Prognostic Index (IPI) scoring system is used routinely to assess NHL prognosis and predict the survival of patients with aggressive NHLs; the IPI score increases by one for stage III or IV disease, elevated serum lactate dehydrogenase above normal, and Eastern Cooperative Oncology Group (ECOG) performance status (26). However, the development of prognostic biomarkers is of great clinical importance, as common techniques for assessing NHL prognosis (i.e., PET) are costly, and have significant limitations when used in HIV-infected patients. Therefore, identifying a group of molecules that efficiently provide prognostic information for ARL is of great relevance and would be a useful tool for clinicians.

We have recently shown that pretreatment, postdiagnosis plasma levels of some cytokines, including IL6, IL10, and CXCL13, have the potential to serve as indicators of response to treatment and survival in ARL (27). However, other molecules seen to be elevated pre-ARL in epidemiologic studies, including markers of microbial translocation, have not been tested for their prognostic value. Therefore, identifying a group of molecules that efficiently provide prognostic information for ARL is of great relevance and may provide useful tools for clinicians.

Materials and Methods

Study population

Of the 106 patients with AIDS-NHL 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, plasma specimens were available from 57 patients with intermediate- or high-grade HIV-associated B-cell NHL (46 patients had DLBCL, 7 patients had Burkitt lymphoma, and 4 were classified as other lymphoma). The median age (interquartile range, IQR) of patients with lymphoma was 44 (38–48) years. Lymphoma patients had a median (IQR) HIV plasma level of 9,667 (undetectable–87,926), and a median (IQR) $CD4^+$ number of 231 (82–337) cells/mm^3. Plasma samples available for this investigation were collected before the initiation of therapy, after the initiation of treatment (i.e., at the end of the first cycle - within a week or less of treatment), or at 6 months or one year following the completion of treatment. Clinical responses were defined as described in the report detailing the AMC-034 trial results (28).

Rituximab, EPOCH, supportive care, and clinical evaluation

Details regarding the treatment protocol have been reported by Sparano and colleagues (28). Clinical responses were defined by the International Response Criteria for NHL (which uses anatomic, but not functional imaging). 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 complete response (CR; for a minimum of four and a 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. 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.

Biomarker determination

As previously described (24), plasma levels of anti-endotoxin core protein IgM (EndoCab IgM; Hycult Biotech) were determined by ELISA, according to the manufacturers' instructions. Plasma levels of all other biomarkers (sCD14, LBP, FABP2, IL18, CCL2/MCP-1, sCD163, IP-10/CXCL10, TARC/CCL17, TNFα, BAFF/BLyS, sTNFRII, sCD44, and sIL2Rα/sCD25) were determined using the Luminex multiplex assay platform with custom-made panels produced by R&D Systems. Briefly, Luminex microparticles precoated with analyte-specific antibodies were incubated with diluted plasma samples, followed by a biotin antibody and by a streptavidin–phycoerythrin conjugate. The fluorescence intensity of each analyte's microparticles was quantified using a Bioplex 200 (Luminex) System Analyzer (Bio-Rad), and the data analyzed using BioPlex Manager (v 4.1.1) software. The lower limit of detection (LLD) for each biomarker was set either as the lowest value that the BioPlex Manager software could calculate using the standard curve or as the lowest value of the standard curve, whichever was smaller. For quality control, case and control samples were equally distributed across reaction plates, and replicates were included across the reaction plates to calculate coefficients of variation. All laboratory personnel were blinded to the case–control status of samples. For this study, samples were available for a subset of the participants in the AMC-034 study.

Statistical analysis

Changes from pre- to posttreatment in biomarkers were evaluated for significance using paired nonparametric Wilcoxon signed rank tests. Results were averaged for one participant with two posttreatment samples. In addition, pretreatment biomarkers were compared according to subtype (DLBCL vs. Burkett), IPI score (0–1 vs. 2–3), and response using nonparametric two-sample Wilcoxon rank-sum tests. Kaplan–Meier estimates of overall survival (OS), the time from enrollment to death, progression-free survival (PFS), the time from enrollment to progression or death, and time-to-progression (TTP), the time from enrollment to progression, were computed and compared according to low and high pretreatment biomarker levels ($<$ median vs. $\geq$ median) using log-rank tests. HRs and their 95% confidence intervals were obtained from single-variable Cox proportional hazards regression models. Multivariable Cox regression models

were fit using stepwise variable selection procedures that entered and maintained biomarkers that were significant at alpha = 0.10; age-adjusted IPI (29) was also investigated in the model as it has been validated in patients with HIV-associated NHL (30–33). Complete response rates were also compared according to low and high pretreatment biomarker levels using Fisher exact test. As there are no normal ranges for these biomarkers, median cut-off points were used to facilitate interpretation; a sensitivity analysis was also conducted to investigate qualitative differences in terms of statistical significance between results based on the above analyses versus those investigating relationships between continuous biomarker values and response and survival outcomes. *P* values were not adjusted for multiple comparisons.

Ethics statement

This study involved the use of samples obtained from human subjects. The AIDS Cancer Specimen Repository (ACSR) acts as an honest broker by providing specimens and data obtained from human subjects, with personal identifying information removed. The ACSR is the biorepository of record for AIDS Malignancy Trials Consortium (AMC) and as such provides samples and associated annotation collected from participants who consent to donate and/or have leftover clinical trial materials use for future research. Materials are distributed through a letter intent process. AMC-034 clinical trial was conducted in accordance with the Declaration of Helsinki and all participants provided written informed consent to have their leftover samples and data used for future research. The current study was determined by the UCLA IRB to be exempt from IRB review, as the information was provided in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.

Results

Biomarkers of microbial translocation and inflammation decrease after cancer treatment

In this study, we examined plasma levels of pre- and posttreatment initiation specimens collected from persons enrolled in the AMC-034 trial, comparing infusional combination chemotherapy (EPOCH) with concurrent or sequential rituximab (27). Of the 57 participants, 55 participants had both pretreatment and posttreatment biomarker data (posttreatment samples were not available for two participants). Plasma levels of EndoCab IgM were measured by ELISA, and plasma levels of biomarkers of macrophage activation (BAFF/BLyS, IL18, CCL2/MCP-1, TNFα, TARC/CCL17, sCD163), B-cell activation and inflammation-associated molecules (sCD25, sTNFRII, sCD44, IP-10/CXCL10), and microbial translocation (sCD14, LBP, FABP2) were measured by multiplexed Luminex assay. We found that plasma levels of BAFF/BLyS increased after cancer treatment ($P < 0.001$; **Fig. 1A**). Plasma levels of TNFα significantly decreased posttreatment, compared with pretreatment levels ($P = 0.005$, Wilcoxon rank-sum test, **Fig. 1B**). Similarly, significant decreases were seen in sCD25 ($P < 0.001$), LBP ($P < 0.001$), and TARC/CCL17 ($P < 0.001$; **Fig. 1C–E**). No other biomarkers differed significantly when comparing levels seen posttreatment with levels seen prior to treatment.

Associations of pretreatment biomarkers of microbial translocation and inflammation and ARL subtype and IPI score

Pretreatment biomarkers were not significantly related to ARL subtype [DLBCL ($n = 46$) vs. Burkitt ($n = 7$)], but this comparison is limited by the study population with biomarker samples being predominantly DLBCL subtype. For the subset with DLBCL, the group with higher IPI scores (2–3 vs. 0–1) had significantly higher levels of BAFF/BLyS ($P = 0.029$), sCD163 ($P = 0.005$), sCD14 ($P = 0.003$), sCD25 ($P < 0.001$), sTNF-RII ($P < 0.001$), and IL18 ($P = 0.006$; **Fig. 2**).

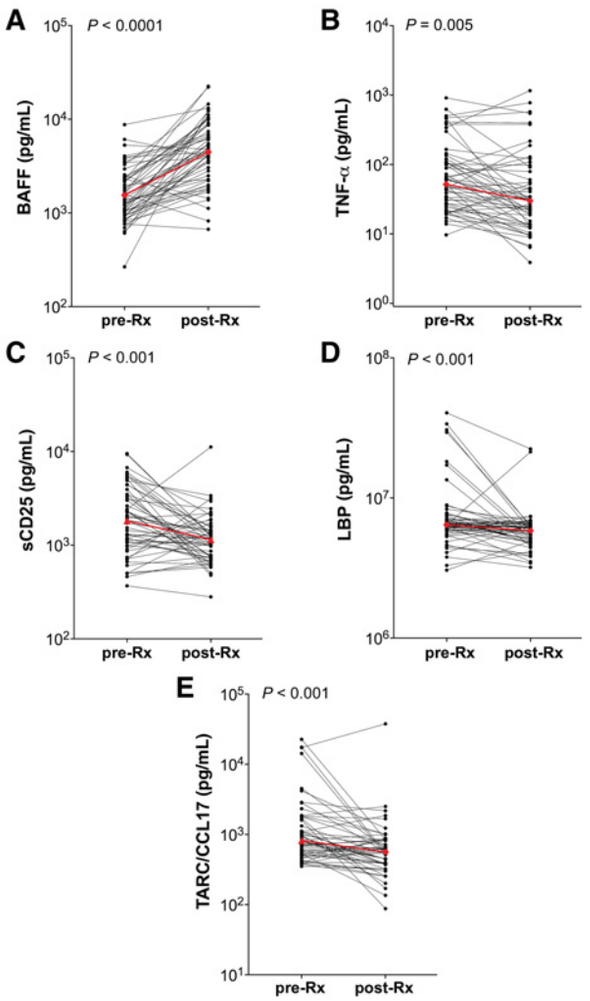

Figure 1.

Biomarkers of microbial translocation and inflammation significantly decrease after cancer treatment. Plasma levels of BAFF (**A**), TNFα (**B**), sCD25 (**C**), LBP (**D**), and TARC/CCL17 (**E**) were measured in samples from persons diagnosed with NHL. Shown are results for samples prior to the first cycle of lymphoma treatment (pre-Rx) and posttreatment initiation (post-Rx). Results are shown for 55 patients (pre-Rx and post-Rx), and each filled circle represents a sample from a single patient. Median values of pre-Rx and post-Rx are shown as red circles and lines [BAFF, median pre-Rx (1,547 pg/mL) and post-Rx (4,462 pg/mL); TNFα, median pre-Rx (52 pg/mL) and post-Rx (30 pg/mL); sCD25 median pre-Rx (1,821 pg/mL) and post-Rx (1,147 pg/mL); LBP, median pre-Rx (6,429,700 pg/mL) and post-Rx (5,838,000 pg/mL); and TARC/CCL17, median pre-Rx (818 pg/mL) and post-Rx (562 pg/mL)]. Statistical comparisons were made using paired nonparametric Wilcoxon signed-rank tests.

Association of pretreatment biomarkers of microbial translocation and inflammation and clinical response to treatment

We then investigated the association between pretreatment biomarker levels and treatment response. Fifty-two participants with

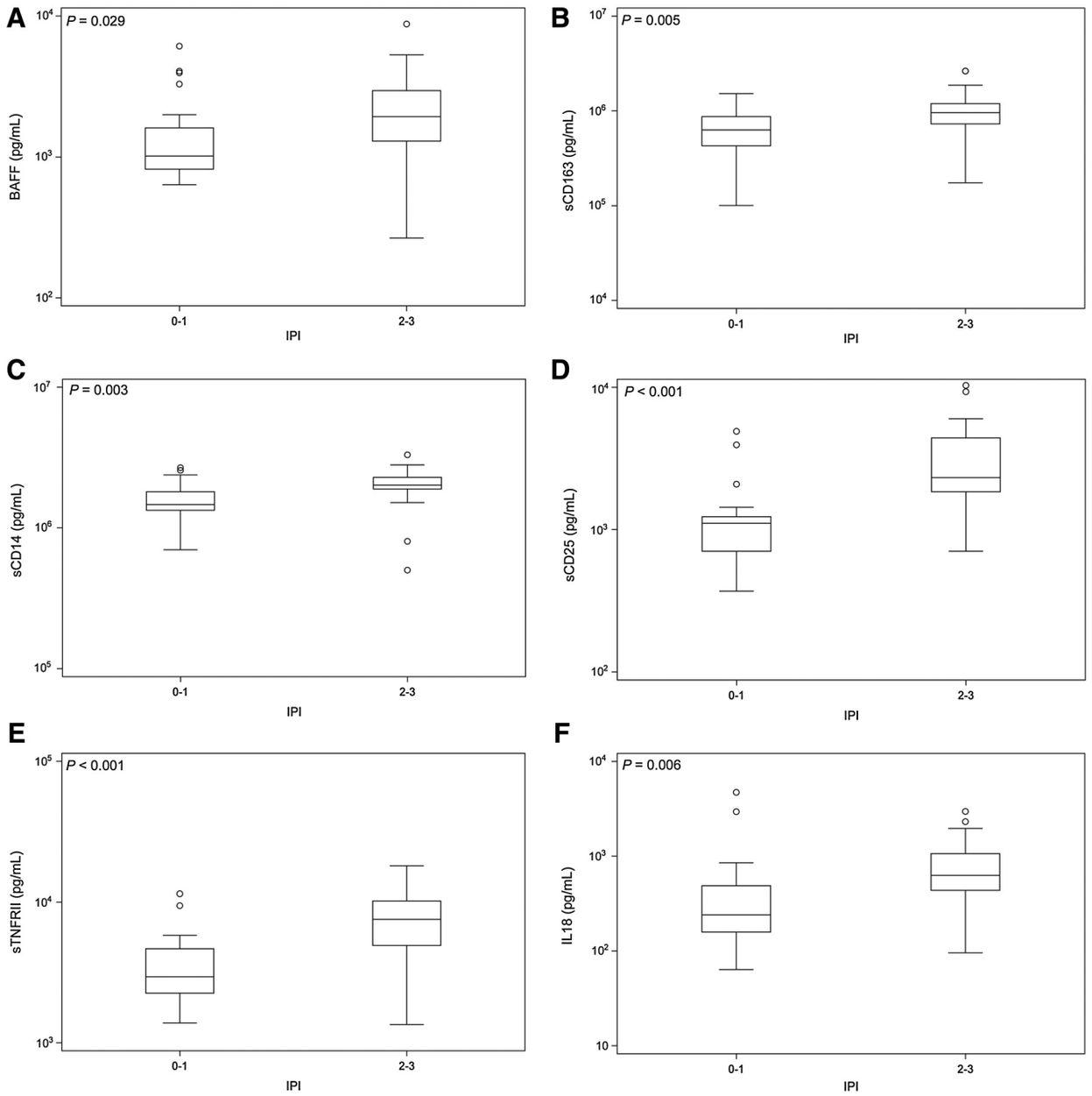

Figure 2.

Pretreatment plasma levels of biomarkers of microbial translocation and inflammation are significantly higher with worse (higher) IPI scores in DLBCL subtype. Pretreatment biomarker levels of BAFF/BLyS (**A**), sCD163 (**B**), sCD14 (**C**), sCD25 (**D**), sTNFRII (**E**), and IL18 (**F**) were measured in 46 participants with DLBCL with low (0–1, $n = 20$) and high (2–3, $n = 26$) IPI scores. Median biomarker levels are presented as line within the box (first and third quartiles) with whiskers extending to most extreme observation within 1.5 times the interquartile range. Statistical comparisons were made using two-sample nonparametric Wilcoxon rank sum tests.

pretreatment biomarker data were evaluable for response. We found that pretreatment levels of BAFF/BLyS trended lower in complete responders compared to partial/nonresponders (median, 1,207.3 vs. 1,586.2, $P = 0.065$), as did sCD14 (median, 1.8×10^7 vs. 2.0×10^7, $P = 0.064$), sCD44 (2,243.9 vs. 3,779.8, $P = 0.061$), and IP-10/CXCL10 (median, 398.3 vs. 629.1, $P = 0.051$). When biomarkers were dichotomized based on median values, biomarkers were not significantly related to CR rates, but there was a trend for higher complete response rates for those with low levels of sTNFRII compared with those with high levels (80% vs. 56% CR rates, $P = 0.080$; **Table 1**).

Associations of pretreatment biomarkers of microbial translocation and inflammation and survival outcomes

When survival outcomes were compared according to low or high levels of pretreatment biomarkers, OS was significantly lower for individuals who had higher BAFF/BLyS (HR = 3.43, $P = 0.024$),

Table 1. Relationship between baseline biomarker levels and outcome measures.

Factor	*N*	Complete response rate (%)	*N*	1-Year OS (%) (95% CI)	1-Year PFS (%) (95% CI)
IPI					
0–1	22	73	23	95.7 (72.9–99.4)	91.3 (69.5–97.8)
2–3	30	63	34	75.4 (56.7–86.9)	63.9 (45.2–77.7)
OR/HR (95% CI)[a]		1.54 (0.47–5.11)		1.87 (0.65–5.42)	1.85 (0.74–4.62)
P		0.558[b]		0.239[c]	0.181[c]
BAFF/BlyS					
<Median	28	75	28	92.9 (74.3–98.2)	89.3 (70.4–96.4)
≥Median	24	58	29	74.3 (53.4–86.9)	61.0 (40.7–76.2)
OR/HR (95% CI)[a]		2.14 (0.66–6.97)		3.43 (1.10–10.69)	3.73 (1.36–10.23)
P		0.245[b]		0.024[c]	0.006[c]
sCD163					
<Median	26	69	28	81.2 (60.6–91.8)	74.3 (53.5–86.8)
≥Median	26	65	29	85.9 (66.7–94.5)	75.9 (55.9–87.7)
OR/HR (95% CI)[a]		1.19 (0.37–3.80)		0.84 (0.31–2.23)	0.66 (0.28–1.58)
P		>0.999[b]		0.721[c]	0.350[c]
LBP					
<Median	25	72	28	85.0 (64.7–94.1)	74.2 (53.3–86.8)
≥Median	27	63	29	82.6 (63.1–92.4)	75.9 (55.9–87.7)
OR/HR (95% CI)[a]		1.51 (0.47–4.88)		0.86 (0.32–2.29)	0.65 (0.27–1.54)
P		0.562[b]		0.757[c]	0.340[c]
sCD14					
<Median	26	77	28	96.3 (76.5–99.5)	85.2 (65.2–94.2)
≥Median	26	58	29	72.4 (52.3–85.1)	65.5 (45.4–79.7)
OR/HR (95% CI)[a]		2.44 (0.74–8.11)		3.05 (0.98–9.48)	1.71 (0.71–4.15)
P		0.237[b]		0.043[c]	0.220[c]
sCD25					
<Median	26	73	28	92.6 (73.5–98.1)	85.2 (65.2–94.2)
≥Median	26	62	29	79.0 (59.1–90.0)	65.5 (45.4–79.7)
OR/HR (95% CI)[a]		1.70 (0.53–5.48)		2.28 (0.79–6.59)	2.18 (0.88–5.43)
P		0.555[b]		0.118[c]	0.087[c]
sTNFRII					
<Median	25	80	28	92.4 (73.0–98.1)	88.9 (69.4–96.3)
≥Median	27	56	29	75.4 (55.1–87.5)	62.1 (42.1–76.9)
OR/HR (95% CI)[a]		3.20 (0.93–11.05)		3.27 (1.05–10.18)	2.79 (1.08–7.22)
P		0.080[b]		0.031[c]	0.027[c]
EndoCab IgM					
<Median	25	64	28	89.3 (70.4–96.4)	75.0 (54.6–87.2)
≥Median	27	70	29	78.1 (57.6–89.5)	75.1 (54.7–87.3)
OR/HR (95% CI)[a]		0.75 (0.23–2.39)		1.05 (0.39–2.82)	0.75 (0.31–1.78)
P		0.769[b]		0.916[c]	0.518[c]
FABP2					
<Median	26	65	28	88.9 (69.3–96.3)	78.0 (57.4–89.5)
≥Median	26	69	29	78.9 (58.8–89.9)	72.4 (52.3–85.1)
OR/HR (95% CI)[a]		0.84 (0.26–2.68)		1.42 (0.52–3.84)	1.42 (0.59–3.41)
P		>0.999[b]		0.494[c]	0.416[c]
CCL2/MCP-1					
<Median	26	77	28	100.0 (100.0–100.0)	92.6 (73.5–98.1)
≥Median	26	58	29	68.1 (47.6–82.0)	58.6 (38.8–74.0)
OR/HR (95% CI)[a]		2.44 (0.74–8.11)		3.96 (1.27–12.33)	4.14 (1.51–11.35)
P		0.237[b]		0.011[c]	0.003[c]
sCD44					
<Median	28	75	28	85.6 (66.0–94.3)	82.1 (62.3–92.1)
≥Median	24	58	29	81.7 (61.4–92.0)	68.1 (47.7–82.0)
OR/HR (95% CI)[a]		2.14 (0.66–6.97)		1.46 (0.54–3.92)	1.33 (0.56–3.13)
P		0.245[b]		0.453[c]	0.529[c]
IL18					
<Median	26	69	28	85.6 (66.0–94.3)	82.1 (62.3–92.1)
≥Median	26	65	29	82.0 (62.0–92.1)	68.1 (47.7–82.0)
OR/HR (95% CI)[a]		1.19 (0.37–3.80)		1.46 (0.54–3.92)	1.30 (0.55–3.08)
P		>0.999[b]		0.457[c]	0.532[c]

(Continued on the following page)

Table 1. Relationship between baseline biomarker levels and outcome measures. (Cont'd)

Factor	*N*	Complete response rate (%)	*N*	1-Year OS (%) (95% CI)	1-Year PFS (%) (95% CI)
TARC/CCL17					
<Median	27	67	28	74.7 (54.1–87.1)	67.9 (47.3–81.8)
≥Median	25	68	29	93.0 (74.7–98.2)	82.4 (62.7–92.3)
OR/HR (95% CI)[a]		0.94 (0.30–3.00)		0.42 (0.15–1.21)	0.58 (0.24–1.41)
P		>0.999[b]		0.098[c]	0.218[c]
CXCL10					
<Median	27	78	28	92.7 (73.9–98.1)	89.3 (70.4–96.4)
≥Median	25	56	29	74.7 (54.0–87.1)	61.1 (40.8–76.2)
OR/HR (95% CI)[a]		2.75 (0.83–9.16)		1.98 (0.72–5.48)	1.94 (0.80–4.70)
P		0.140[b]		0.182[c]	0.134[c]
TNFα					
<Median	25	68	28	77.8 (57.1–89.4)	70.8 (50.0–84.2)
≥Median	27	67	29	89.1 (69.9–96.4)	79.3 (59.6–90.1)
OR/HR (95%CI)[a]		1.06 (0.33–3.39)		0.48 (0.18–1.34)	0.69 (0.29–1.64)
P		>0.999[b]		0.152[c]	0.398i[c]

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

sCD14 (HR = 3.05, $P = 0.043$), sTNFRII (HR = 3.27, $P = 0.031$), and CCL2/MCP-1 (HR = 3.96, $P = 0.011$), as compared with individuals with lower biomarker levels in univariate analyses (**Table 1**; **Fig. 3**). Although not significant as a dichotomized variable, in sensitivity analyses investigating biomarkers as continuous variables, higher levels of IP-10/CXCL10 were related to death [HR = 1.05 (per 100), $P = 0.013$]. Other biomarkers were not significant. The final multivariable model included BAFF/BLyS (HR = 3.74, $P = 0.049$), CCL2/MCP-1 (HR = 3.97, $P = 0.032$), TNFα (HR = 0.22, $P = 0.006$), and continuous IP-10/CXCL10 [HR = 1.05 (per 100), $P = 0.021$].

PFS also significantly differed for BAFF/BLyS, sTNFRII, and CCL2/MCP-1 (**Table 1**). However, the difference in PFS did not meet statistical significance for sCD14 based on a median split (HR = 1.71, $P = 0.220$). Individuals with higher BAFF/BLyS ($P = 0.006$), sTNFRII ($P = 0.027$), and CCL2/MCP-1 ($P = 0.003$) plasma levels had overall lower PFS outcomes (**Fig. 4**). Also, continuous IP-10/CXCL10 was significant [HR = 1.04 (per 100), $P = 0.044$]. No other PFS endpoint comparisons were statistically significant. The final multivariable model included sTNFRII (HR = 3.04, $P = 0.023$) and CCL2/MCP-1 (HR = 4.40, $P = 0.004$).

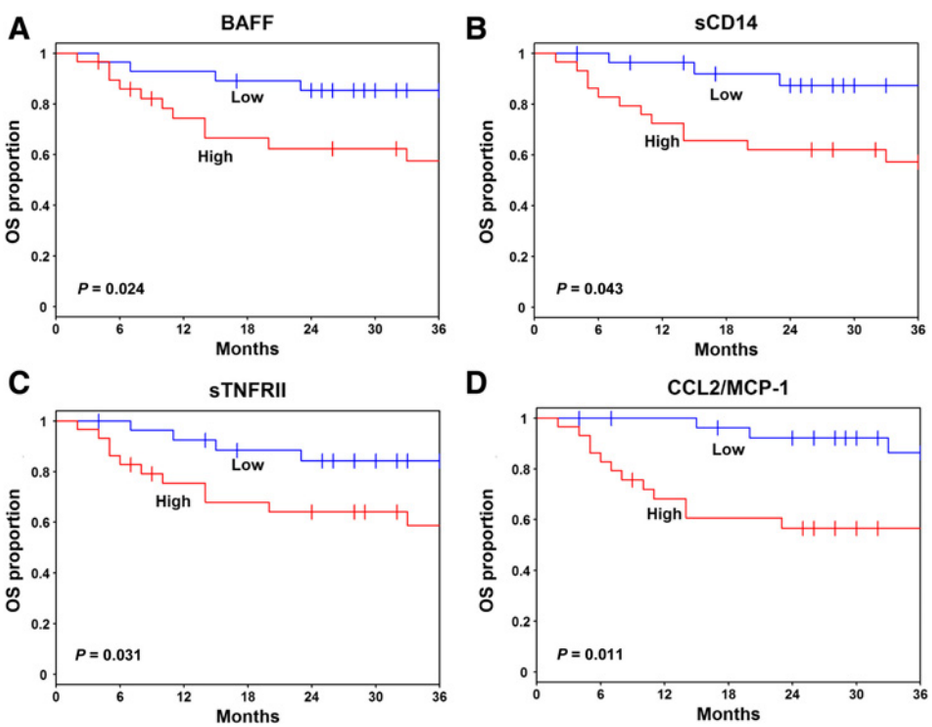

Figure 3.
Pretreatment plasma levels of biomarkers of microbial translocation and immune activation are significantly associated with overall survival. Kaplan–Meier estimates showing the relationship of low and high pretreatment biomarker levels of BAFF (**A**), sCD14 (**B**), sTNFRII (**C**), and CCL2/MCP-1 (**D**) and overall survival over time for 57 patients with intermediate- or high-grade HIV-associated B-cell NHL. Statistical comparisons were made between low and high pretreatment biomarker levels (< median vs. ≥ median) using log-rank tests.

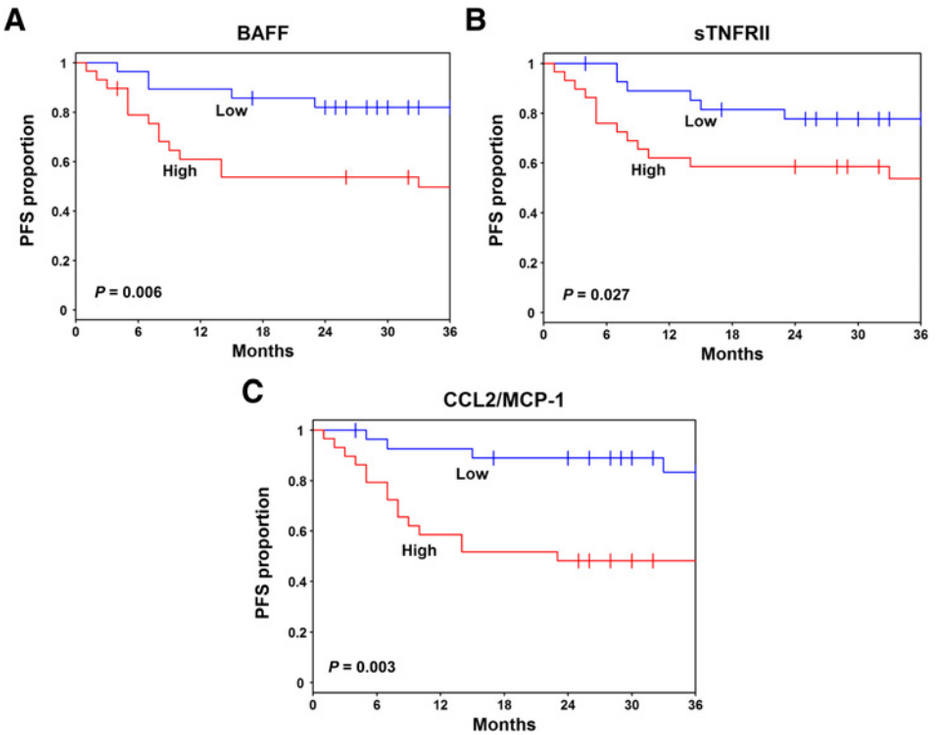

Figure 4.
Pretreatment plasma levels of BAFF, sTNFRII, and CCL2/MCP-1 are significantly associated with PFS. Kaplan–Meier estimates showing the relationship of low and high pretreatment biomarker levels of BAFF (**A**), sTNFRII (**B**), CCL2/MCP-1 (**C**), and PFS over time for the 57 patients with intermediate- or high-grade HIV-associated B-cell NHL. Statistical comparisons were made between low and high pretreatment biomarker levels (< median vs. ≥ median) using log-rank tests.

In terms of TTP, individuals with high BAFF/BLyS, sTNFRII, and CCL2/MCP-1 plasma levels exhibited lower proportions without progression (Supplementary Fig. S1). Continuous IP-10/CXCL10 was related to progression [HR = 1.04 (per 100), $P = 0.011$].

Correlations of biomarkers of microbial translocation, inflammation, HIV viral load, and CD4$^+$ T-cell count

To determine whether plasma biomarkers of immune activation, inflammation, and microbial translocation correlated with HIV viral load, we examined pretreatment biomarker levels. We found that pretreatment HIV viral load correlated with pretreatment plasma biomarker levels of BAFF/BLyS (Spearman $\rho = 0.28$, $P = 0.043$), sCD14 (Spearman $\rho = 0.35$, $P = 0.012$), sCD25 (Spearman $\rho =$ s0.47, $P < 0.001$), sTNFRII (Spearman $\rho = 0.59$, $P < 0.001$), IL18 (Spearman $\rho = 0.39$, $P = 0.005$), and IP-10/CXCL10 (Spearman $\rho = 0.35$, $P = 0.012$). HIV viral loads during cycles 1 to 6 were correlated with posttreatment initiation biomarkers of sCD25 (Spearman $\rho = 0.32$, $P = 0.027$), IL18 (Spearman $\rho = 0.30$, $P = 0.036$), and TARC/CCL17 (Spearman $\rho = -0.39$, $P = 0.006$). HIV viral loads posttreatment completion were positively associated with posttreatment initiation biomarkers of sCD25 (Spearman $\rho = 0.42$, $P = 0.013$).

Pretreatment biomarkers correlations with CD4$^+$ T-cell count were not as strong as those for HIV viral load. Of the biomarkers provided in **Fig. 1**, correlations of plasma biomarkers with CD4$^+$ T-cell counts at pretreatment were not significant; we found the stongest correlation for pretreatment sCD14 (Spearman $\rho = -0.22$, $P = 0.108$) and CCL2/MCP-1 (Spearman $\rho = -0.26$, $P = 0.056$) levels. CD4$^+$ T-cell counts during cycles 1 to 6 were not correlated with posttreatment initiation biomarkers, but CD4$^+$ T-cell counts posttreatment completion were negatively associated with CCL2/MCP-1 (Spearman $\rho = -0.34$, $P = 0.038$).

Discussion

We previously showed, in a nested case–control study done in a large prospective cohort study (Multicenter AIDS Cohort Study), that microbial translocation markers (sCD14, LPB, and EndoCab IgM) were predictive of risk for a subsequent ARL diagnosis in HIV-infected individuals, and these associations were observed after adjustment of HIV disease status, immune suppression, and antiretroviral drug therapy (24). In this study, we found that plasma levels of molecules associated with microbial translocation and inflammation (TNFα, sCD25, LBP, and TARC/CC17) were significantly reduced after treatment with EPOCH and rituximab, in a study done in the AIDS Malignancies Consortium (AMC-034). Moreover, we demonstrate that higher pretreatment plasma levels of BAFF/BLyS, sCD14, sTNFRII, and CCL2/MCP-1 were associated with lower overall survival, and pretreatment levels of BAFF/BLyS, sTNFRII, and CCL2/MCP-1 were also associated with PFS.

These biomarkers have different functions. For instance, TNFα is an inflammatory cytokine associated with early disease progression in patients with HIV (34). Its soluble form, TNFα receptor type II (sTNFRII), which binds TNFα with high affinity, results in downstream signaling, and mediates the biological effects of TNFα. Reduced levels of both TNFα and sTNFRII suggest that cancer treatment reduced HIV-associated immune activation and inflammation.

The B-cell activation factor, BAFF, is a member of the TNF ligand superfamily. BAFF promotes the survival of B lymphocytes and is essential for B-cell maturation. BAFF may also activate B cells and promote their proliferation, and thus play a role in the pathogenesis of NHL (35). Moreover, others have shown an inverse, rather than positive, association between levels of BAFF and risk of NHL and chronic lymphocytic leukemia/small lymphocytic (CLL/SLL; ref. 36). Thus, it was hypothesized that BAFF is sequestered by receptors found on expanding clones of B cells, which may subsequently activate

NF-κB. Moreover, BAFF levels remained siginifcantly inversely correlated for CLL/SLL risk over 10+ years of blood draw. In this study, we found significantly increased levels of BAFF/BLyS after cancer treatment. Treatment may have resulted in increased serum levels of BAFF by decreasing the amount of this cytokine bound by tumor B cells, due to a decrease in tumor load. Alternatively, treatment may have resulted in enhanced production of BAFF.

The chemokine CCL2/MCP-1 has been shown to be a potent chemoattractant. Tumor cells produce chemokines, such as CCL2, to drive the generation different types of regulatory immune cells, including B cells (37). The CCL2/MCP-1 and CC chemokine receptor 2 (CCR2) axis has been shown to facilitate tumor survival and invasion. Others have shown that high CCL2/MCP-1 levels or high CCR2 expression levels serve as a progonostic factor for overall survival and progression-free survival of patients with DLBCL (38). Our results indicate that pretreatment levels of CCL2/MCP-1 are univaritely associated with OS and PFS, suggesting that CCL2/MCP-1 may serve as a prognostic biomarker for ARL.

Soluble CD14 is a coreceptor for LPS that is released from monocytes upon activation and is considered to be a marker of microbial translocation; elevated plasma levels of sCD14 have been associated with a poor prognosis of HIV-infected individuals (39–41), and increased morbidity and mortality in the course HIV disease (42). In this study, we observed that plasma levels of sCD14 decrease after cancer treatment, suggesting that overall immune activation and microbial translocation diminishes after cancer therapy. CD25, the IL2 receptor, is widely expressed in many leukocytes. Its soluble form, sCD25, has been characterized as a biomarker in inflammatory disorders, such as sarcoidosis (43–45) and autoimmune diseases (46–48). The decrease noted for sCD25 posttreatment suggests that these therapies inhibit immune activation.

In prior work, we showed that biomarkers of bacterial translocation, LPB, FABP2, and sCD14 were significantly increased prior to ARL diagnosis (24). In this study, we studied whether these biomarkers, and other biomarkers associated with significant ARL risk, also have prognostic value, and investigated their associations with disease progression posttreatment. Multivariate analyses of OS and PFS demonstrated that patients with ARL who responded to therapy had overall lower pretreatment levels of BAFF/BLyS, sCD14, and sTNFRII, and higher pretreatment levels of CCL2/MCP-1. Together, our data suggest that biomarkers of microbial translocation and inflammation are good prognostic biomarkers and may serve as important tools to assess therapies for ARL. In addition, our results suggest that the state of the immune system prior to cancer treatment, including systemic inflammation associated with HIV infection and microbial translocation, may be an important determinant for clinical outcome.

Moreover, we found that pretreatment HIV viral load correlated with pretreatment plasma biomarker levels of BAFF, sCD14, CD25, sTNFRII, IL18, and CXCL10. HIV viral load has been associated previously with increased risk of NHL (13, 49) as some of these biomarkers are. Moreover, HIV viral load may be contributing directly to the induction of these biomarkers by activating directly B cells and macrophages. HIV carries CD40L in their envelope and by binding with CD40 on B cells can activate B cells directly (50). Therefore, HIV viral load may be contributing directly to inflammation/macrophage activation biomarkers associated with ARL risk.

One limitation to our study was that our study did not include results from HIV+ individuals and healthy control participants without cancer. Certainly, inclusion of such results would be valuable for interpreting the magnitude of change in plasma biomarker levels we observed. However, specimens from relevant control subjects were not collected in AMC-034. Because collection procedures and storage conditions can affect the measurement of biomarker levels, we did not utilize specimens collected from other studies or cohorts for this purpose.

Additional research is required to identify driver pathways of inflammation and microbial translocation to improve the outcome of HIV-associated malignancies. Altered microbial regulation in HIV infection can lead to microbiome dysbiosis (51), which can subsequently exacerbate microbial translocation, disruption of the epithelial cell barrier, and contribute to immune cell activation and inflammation. Moreover, changes in gut microbiota composition may affect mucosal T-cell responses and immune recovery in HIV-1–infected subjects (52–54). Thus, understanding the mechanisms underlying microbial translocation and microbiome dysbiosis, and mucosal immune responses in the pathogenesis of AIDS and the development of ARL, can identify new biomarkers and inform the development of therapeutic, cancer inteventions.

Authors' Disclosures

L.E. Martínez reports grants from NIH during the conduct of the study. S. Lensing reports grants from NIH/NCI during the conduct of the study. D. Chang reports grants from NIH during the conduct of the study. L.I. Magpantay reports grants from NIH/NCI during the conduct of the study. R. Misuyasu reports grants from NIH/NCI during the conduct of the study. O. Martínez-Maza reports grants from NIH during the conduct of the study. M. Epeldegui reports grants from NIH/NCI during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

L.E. Martínez: Data curation, formal analysis, investigation, writing–original draft, writing–review and editing. **S. Lensing:** Formal analysis, methodology, writing–original draft, writing–review and editing. **D. Chang:** Formal analysis, methodology, writing–original draft, writing–review and editing. **L.I. Magpantay:** Data curation, formal analysis, validation, methodology. **R. Misuyasu:** Data curation, investigation, writing–original draft, writing–review and editing. **R.F. Ambinder:** Data curation, investigation, writing–original draft, writing–review and editing. **J.A. Sparano:** Data curation, investigation, writing–original draft, writing–review and editing. **O. Martínez-Maza:** Conceptualization, resources, supervision, investigation, writing–original draft, writing–review and editing. **M. Epeldegui:** Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, investigation, writing–original draft, writing–review and editing.

Acknowledgments

This study received financial support from the following sources: an AIDS Malignancy Consortium (AMC) Lab Translational Fellowship (UM1-CA121947) to M. Epeldegui, R21-CA220475, R01-CA228157, and a UCLA Tumor Immunology T32 Training Grant Fellowship to L.E. Martínez (T32-CA009120). Flow cytometry was performed in the UCLA JCCC and CFAR/AIDS Institute Flow Cytometry Core Facility that is supported by NIH awards P30-CA016042 and P30-AI028697 and by the David Geffen School of Medicine at UCLA and the UCLA Vice Chancellor's Office of Research. In addition, this work was supported by the Pendleton Charitable Trust and the McCarthy Family Foundation.

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Received October 23, 2020; revised April 30, 2021; accepted June 9, 2021; published first June 15, 2021.

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