Title

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Stromal immune infiltration in HIV-related diffuse large B-cell lymphoma is associated with HIV disease history and patient survival

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Abstract

Objective—Understanding tumor microenvironment and its impact on prognosis of HIV-related lymphomas may provide insight into novel therapeutic strategies.

Design—We characterized the relationship between infiltrating immune cells with tumor characteristics, HIV disease history and survival in 80 HIV-related diffuse large B-cell lymphoma (DLBCL) patients diagnosed in the era of combined antiretroviral therapy (1996–2007) at Kaiser

Permanente (KP) California. Eighty HIV-unrelated DLBCL patients were included for comparison.

Methods—Data on patients' clinical history were obtained from KP's electronic health records. The density of stromal CD4+, CD8+ and FOXP3+ T cells and CD68+ macrophages, as well as tumor molecular characteristics were examined using immunohistochemistry. The associations between stromal immune infiltration and patient's clinical history or tumor characteristics were examined using Kruskal Wallis tests or Peasrons' correlation coefficient. The effect of stromal immune infiltration on two-year mortality was evaluated in multivariable logistic regression.

Results—Compared to HIV-unrelated DLBCL, patients with HIV-related DLBCL had significantly reduced stromal CD4+ and FOXP3+ T cells, but increased density of macrophages. Increased density of stromal macrophages was correlated with lower circulating CD4 cell count at DLBCL diagnosis. Tumor molecular characteristics, including BCL6, p53 and cMYC expression, but not EBV infection status, were significantly correlated with stromal immune infiltration, particularly FOXP3+ T cells. A higher density of infiltrating CD8+ T cell was significantly associated with reduced mortality in HIV-related DLBCL patients [odds ratio=0.30 (0.09–0.97) for 25% vs. <10%].

Conclusion—These data provide evidence for the prognostic significance of cytotoxic T cells in determining outcomes of HIV-related lymphoma.

Introduction

The tumor microenvironment plays a vital role in cancer development[1, 2]. The interaction between tumor cells and stromal cells, such as infiltrating immune cells, fibroblasts and endothelial cells may create a local environment that supports or inhibits tumor growth. Anti-tumor immunity mediated by infiltrating T cells, macrophages and dendritic cells play an important role in determining disease progression and patient outcome in a variety of cancers, including diffuse large B-cell lymphomas[3–5]. To this end, several studies have demonstrated the prognostic significance of the stromal immune cells through gene expression profiling[6–8], immunohistochemistry[9–11] or functional/animal studies[12, 13], lending support to therapeutic strategies that aim to restore immune surveillance in the tumor microenvironment.

Despite knowledge advancements about anti-tumor immunity and its treatment implications in lymphomas in the general population, there is a lack of studies examining the microenvironment for HIV-related lymphomas. As such, little is known about the microenvironment and the role of anti-tumor immunity among immunodeficient populations, such as HIV-infected patients. Liapis and colleagues have for the first time attempted to characterize the microenvironment of HIV-related DLBCL[14]. They showed increased vessel density and altered infiltrating T cell populations compared to patients with sporadic DLBCL. These findings highlighted the need to better understand tumor microenvironment unique to HIV-related lymphomas and its therapeutic implications.

In this study we examined the immune component of the microenvironment, including stromal T-cell subpopulations and tumor-associated macrophages in HIV-related DLBCL. We sought to understand (1) if the composition of infiltrating immune cells differs by clinical and molecular characteristics of the tumor, (2) if the composition of infiltrating immune cells may be affected by HIV disease history such as severe immunosuppression, and (3) if the density of specific infiltrating immune cells can predict patient survival in HIV-related DLBCL. To warrant generalizability to current clinical management of DLBCL in HIV-infected patients, the present study is entirely based on contemporary patients diagnosed in the post-combination antiretroviral therapy (ART) era.

Methods

Study Population

All adult HIV-infected patients (≥18 years) diagnosed with incident DLBCL between 1996 and 2007 were identified from the Kaiser Permanente (KP) Southern and Northern California Health Plans, two large integrated health care delivery systems. DLBCL diagnoses were ascertained from KP's Surveillance, Epidemiology, and End Results (SEER)-affiliated cancer registries. HIV infection status was identified through record linkage with the health plans' HIV registries.

A group of HIV-unrelated DLBCL patients were also selected for comparison. Tumor biology can differ by age and DLBCL tend to be diagnosed at younger age in HIV-infected persons. Furthermore, the HIV-infected cohort in California was 95% male and the majority was of non-Hispanic white race. To ensure comparability of HIV-unrelated DLBCL patients to the HIV-related DLBCL patients, we matched subjects 1:1 by age groups (i.e., <30, 30–50 and >50 years), gender and race (white vs. non-white). Appropriate tumor specimens of matched HIV-unrelated DLBCL patients for 4 HIV-related DLBCL patients were unavailable. The comparisons for those 4 HIV-infected patients were thus replaced by additional matched HIV-unrelated DLBCL patients for other HIV-infected patients.

Pathology Review and Tissue Microarray Construction

Archived tumor specimens were retrieved and hematoxylin and eosin stained (H&E) slides were reviewed to confirm the DLBCL diagnosis as well as to identify representative tumor blocks for tissue microarray (TMA) construction (at the UCLA Core Microarray Facility). Whenever possible three 0.6-mm cores from different areas of the donor block were obtained from each case and inserted in a grid pattern into a recipient paraffin block using a tissue arrayer (Beecher Instruments, Silver Spring, MD).

Immunohistochemistry Staining

Immunohistochemistry staining was performed on TMA cores to analyze the stromal expression of CD4 (helper T-cells), CD8 (cytotoxic T-cells), FOXP3 (T_{reg} cells), and CD68 (macrophage). In addition, the expression of the following markers was assessed in the tumor cells: BCL2, BCL6, p53, Ki-67, cMYC. Expression of CD10, MUM1 and BCL6 were used to determine the germinal center (GC) phenotype using the Hans' algorithm[15]. The percent of stromal cells that expressed CD4, CD8, FOXP3 and CD68, and the percent of

tumor cells that expressed Ki-67 were digitally scored on a computerized automated platform. The percent of tumor cells that expressed BCL2, BCL6, p53 and cMYC was scored manually by one study pathologist and confirmed by another. Cases with discrepant scores (about 10%) were resolved by re-review with double headed microscope. Tumor EBV infection was determined by *in situ* hybridization of EBV encoded RNA and was considered positive if 75% of the DLBCL cells had detectable EBV. Among EBV-positive tumors, LMP1 expression was determined based on immunohistochemistry staining.

Normal tonsillar lymphoid tissue was included as a positive control. Negative controls for each case consisted of substituting the primary antibody with isotype specific non-cross reacting antibody matching the primary antibody. The detailed information on TMA construction, antibody, incubation method and signal detection for each marker were described elsewhere[16].

DLBCL Morphologic Variants

DLBCL morphologic variant subtyping was performed by the two study pathologists (Said J and Zha H), who independently reviewed pathology reports, H&E slides and stained tumor marker expression data. Minor classification discrepancies on two patients were resolved by the pathologists after applying the World Health Organization's 2008 classification system of tumors of the hematopoietic and lymphoid tissues.

Ascertainment of Patient Survival

Two-year mortality was chosen as the outcome because most deaths in HIV-infected patients (85% in our study) occurred within two years after DLBCL diagnosis. Overall mortality ascertainment was complete for all subjects (even if the person terminated KP membership) through record linkage with KP's membership and utilization files, California's state death file, and Social Security death records. As such, there was no loss-to-follow up.

Covariates

The International Prognostic Index (IPI) was calculated based on age, clinical stage, extranodal involvement, serum lactose dehydrogenase (LDH), and performance status[17, 18]. Age at DLBCL diagnosis, stage at diagnosis, extranodal involvement, and initial chemotherapy were collected from KP's cancer registries. Serum LDH level and circulating CD4 cell counts at DLBCL diagnosis (and their nadir) were obtained from the laboratory databases. Performance status and chemotherapy were ascertained from standardized medical record review[19]. We collected HIV disease factors from HIV registries, including prior AIDS diagnosis, use of ART, and duration of known HIV infection.

Statistical Analysis

The density of the five stromal immune cells were calculated and compared by HIV status using the t-test statistic. Next, among HIV-infected patients, we compared the density of the stromal immune cells by stage at diagnosis, DLBCL variant (centroblastic, immunoblastic and plasmablastic), GC phenotype, prior AIDS diagnosis, ART use prior to DLBCL diagnosis, and tumor EBV infection and LMP1 status, using the Kruskal Wallis tests. The

association between circulating CD4 cell count (both at DLBCL diagnosis and nadir) and infiltrating immune cells was examined using Pearson's correlation coefficient. Similarly, the association between infiltrating immune cells and tumor molecular characteristics, including the expression of BCL2, BCL6, p53, Ki-67 and cMYC, was examined using Pearson's correlation coefficient.

Kaplan-Meier survival curves were generated for CD8+ and FOXP3+ T cells and CD68+ macrophage for the following density categories: <10%, 10–24%, 25–49%, 50–74%, and 75%. However, because less than 5 subjects had stromal immune cell density at 50% or greater, the following combined categories were presented: <10%, 10–24%, and 25%. Because most HIV-related DLBCL patients had minimal CD4+ T cell infiltration, we dichotomized CD4+ T cells into two categories: <1% and 1%. The association between infiltrating immune cells and 2-year overall mortality was examined using bivariate and multivariable logistic regression adjusting for IPI, GC phenotype and DLBCL variant. Multivariable models restricted to those who received chemotherapy treatment were also performed. Missing data were handled using the multiple imputation method described by Rubin[20]. All analyses were performed with SAS Version 9.2; Cary, North Carolina, USA.

Results

The demographic and clinical characteristics of the 80 HIV-related and the 80 matched HIV-unrelated DLBCL patients are presented in Table 1. The mean age at DLBCL diagnosis was similar by HIV status (50 years old) due to matching. Compared with HIV-uninfected patients, HIV-infected patients were more likely to be diagnosed at advanced stage (48% and 29%; p=0.01), with extranodal involvement (43% vs. 11%, p<0.01), GC phenotype (39% vs. 26%; p=0.01), and immunoblastic (23% vs. 6%) or plasmablatic subtypes (8% vs. 1%, p<0.01). HIV-infected patients in this study had a mean circulating CD4 cell count at DLBCL diagnosis of 206 cells/mm^3, and a mean 5-years duration of known HIV infection prior to DLBCL diagnosis. In addition, 43% of the HIV-infected DLBCL patients had a prior AIDS diagnosis, and 65% patients had a history of ART use at time of DLBCL diagnosis (Table 1).

HIV infection status and stromal immune cells

Density of infiltrating immune cells by HIV infection status is shown in Table 2. The majority of HIV-related DLBCL patients had minimal CD4 T cell infiltration (i.e., 75% of the patients had <1% of stromal CD4+ T cells, data not shown). Compared to HIV-unrelated DLBCL, HIV-related DLBCL showed significantly reduced density of infiltrating CD4 T cells (mean = 1.8% vs. 14.8% for HIV-related vs. HIV-unrelated DLBCL, respectively. P<0.001) and FOXP3+ T_{reg} cells (7.3% vs. 22.5%, p<0.001), but significantly increased density of infiltrating CD8+ T cells (19.9% vs. 14.6%, p=0.017) and macrophages (15.0% vs. 8.7%, p=0.006).

Stromal immune cells and HIV disease history

There was a greater degree of stromal macrophage infiltration in those with lower CD4 cell count at DLBCL diagnosis (Pearson's correlation coefficient = −0.25, p-value= 0.05). A

similar but weaker association with stromal macrophages and the nadir circulating CD4 cell count was also observed (Pearson's correlation coefficient = −0.18, p-value= 0.14). No association was found between circulating CD4 T cells and stromal density of any of the T cells examined (data not shown). However, having an AIDS diagnosis prior to the DLBCL diagnosis was associated with a substantially reduced infiltrating CD4 T cells (mean (SD) =0.6% (6.91%) and 2.7% (0.9%), p-value=0.09). Although all our HIV-related DLBCL patients were diagnosed in the ART era, not all patients had initiated ART at the time of DLBCL diagnosis. To this end, we did not find an association between ART use prior to DLBCL diagnosis and any of the stromal immune cells examined (data not shown).

EBV infection status and stromal immune cells in HIV-related DLBCL

Seventy HIV-related DLBCL patients who had a valid EBV results were included in this analysis. One-third (22 of 70) of them were positive for tumor EBV infection. When we examined the stromal immune cells by EBV status, a greater density of CD4+ T cells was found in the EBV-negative compared to the EBV-positive DLBCLs (mean: 2.2% vs. 0.5%, respectively), although this difference was not statistically significant (p=0.19). EBV-negative HIV-related DLBCL also demonstrated slightly lower density of FOXP3+ T_{reg} cells compared to EBV-positive DLBCL (mean: 6.2% vs. 9.6%, respectively, p=0.07). The level of stromal CD8+ T cell (20.6% vs. 19.8%) and CD68+ macrophages (13.4% vs. 15.9%) appeared to be similar by tumor EBV status. When we explored the link between LMP1 expression and these stromal immune cells among EBV-positive HIV-related DLBCLs, no association was found between LMP1 expression and CD4+ or FOXP3+ T cells (data not shown).

Stromal immune cells and DLBCL characteristics

When we examined the stromal immune cells by DLBCL variant in HIV-related DLBCL, we observed a significantly elevated density of stromal FOXP3+ T_{reg} cells in plasmablastic subtype, compared to centroblastic or immunoblastic subtypes (mean (SD)=13.4% (9.1%), 6.5% (9.2%), and 7.7% (10.6%), respectively. P=0.04). Stromal macrophage density appeared to be elevated in immunoblastic subtype compared to the centroblastic subtype despite lack of statistical significance (mean (SD) = 24.2% (21.3%) and 11.8% (12.0%), respectively). No clear association was found between the stromal immune cells examined and clinical stage or cell-of-origin (data not shown).

When we examined the relationship between stromal immune cells and tumor molecular characteristics, stromal FOXP3+ T_{reg} cells were found to be positively associated with tumor expression of p53 and cMYC [Pearson's correlation coefficient =0.37 (p<0.01) and 0.32 (p<0.1), respectively], and inversely associated with BCL6 [Pearson's correlation coefficient = −0.31 (p=0.01)]Table 3).

Stromal immune cells and patient survival

Figure 1 shows the Kaplan-Meier survival curve in HIV-related DLBCL by stromal immune cell density. In multivariable logistic regression adjusting for IPI, germinal center phenotype and DLBCL variant, a higher density of stromal CD8+ T cells was significantly associated with reduced mortality [odds ratio (OR) for 25% vs. < 10% = 0.30, 95% confidence

interval 0.09–0.97]. Reduced mortality was also suggested for those with higher density of stromal CD4+ T cells [OR for >1% vs. 1% = 0.43 (0.14–1.31)], and those with lower density of stromal macrophages [OR for >25% vs. <10% = 2.14 (0.48–9.43)], despite lack of statistical significance (Table 4). Similar OR estimates were obtained when we restricted the analyses to only those who received chemotherapy (data not shown).

Discussion

Little is known about the tumor microenvironment in the setting of HIV-related lymphomas. In this study, we found significant differences in the density of important stromal immune cells between HIV-related and HIV-unrelated DLBCL, confirming findings previously reported by Liapis and colleagues based on a smaller sample[14]. In addition, we observed an association between stromal immune cells and HIV disease history, a novel finding that may have implication for HIV disease management. Most importantly, we are among the first to link the density of stromal immune cells to patient survival outcomes in HIV-related DLBCL and reported the prognostic significance of CD8+ T cells on survival. These findings suggest that novel therapeutic approaches targeting the microenvironment, e.g., by restoring T-cell mediated tumor surveillance may have particular benefits among HIV-infected patients who do not respond well to standard chemotherapy.

The reduced density of T_{reg} cells and elevated infiltration of macrophages seen in HIV-related DLBCL is consistent with a pattern generally associated with poorer prognosis. Intratumoral T cells are thought to have an important impact on antitumor immunity[21]. T_{reg} cells affects immune responses of a variety of immune cells, including T cells, B cells and natural killer cells[22]. Although T_{reg} cells are thought to induce immunosuppressive phenotype, several studies of DLBCL and follicular lymphoma have linked high number of T_{reg} cells to superior survival[23–25]. Tumor-associated macrophages, on the other hand, have generally been shown to correlate with disease progression in a variety of cancers, including DLBCL and follicular lymphomas[26, 27]. As shown in *in vitro* and *in vivo* studies, macrophages may promote angiogenesis by secreting cytokines and other angiogenic factors such as the VEGFA and MMP9, which also support the growth of lymphomas[28]. We also observed very limited infiltrating CD4+ T cell population in HIV-related DLBCL. The prognostic significance of infiltrating CD4+ T cells is less clear and may depend on the distribution of subpopulations of CD4+ T cells. However, some studies suggest favorable survival profiles for patients with higher number of overall stromal CD4+ T cells[29, 30].

Interestingly, HIV-related DLBCL patients did not have reduced level of stromal CD8+ T cells in our study. Studies suggest that CD8+ T cells may directly target lymphoma cells[31]. Higher stromal density of CD8+ T cells significantly predicted improved patient survival in our cohort of HIV-related DLBCL, independent of the IPI score. Our finding is consistent with those in lymphomas in the general population[32–34]. The relationship between stromal CD8+ T cells and patient survival outcomes may reflect compromised major histocompatibility complex (MHC) restricted immune function, resulting in a loss of effective tumor immunosurveillance[8]. Loss of cell adhesion molecules in the microenvironment has also been linked to loss of stromal CD8+T cell function, when loss of

stromal CD8+ T cell function has been linked to poor patient outcomes[35]. Our findings suggest that CD8+ T cells may be an important component of lymphoma-specific immune response in HIV-related lymphomas. These stromal immune cells may also serve as useful markers for patient risk stratification beyond the traditional clinical IPI algorithm.

An additional novel contribution of this study is the preliminary evidence that host immune history may play a role in shaping the tumor microenvironment. We showed that patients with prior AIDS-defining conditions, primarily opportunistic infections, and low circulating CD4 cell count at DLBCL diagnosis tended to have reduced density of stromal CD4+ T cells and increased macrophage infiltration, respectively. Several previous studies, including our own work, have shown that circulating CD4 cell count and/or prior AIDS are independent prognostic predictors in patients with HIV-related lymphoma[36, 37]. Our data suggest that one of the potential mechanisms of the prognostic impact of these HIV disease factors may be though modifying the tumor microenvironment. If this is confirmed, then the importance of maintaining circulating CD4 cell count would not only be relevant to preventing the development of HIV-related lymphomas, but also would promote a better prognosis in those who develop such malignancies.

EBV infection of tumor cells is seen in about one-third of the contemporary HIV-related DLBCL cases. Recent data suggest that EBV-mediated pathogenesis involved interaction with the tumor microenvironment[38]. Thus, we hypothesized that EBV-positive DLBCLs recruited a particular subset of immune cells. However, contrary to our hypothesis, no clear pattern of stromal immune cells was seen in DLBCLs positive for EBV infection. Although a statistically significant difference was found for T_{reg} cells, the degree of the difference appears unlikely to be clinically meaningful. LMP1 expression was not associated with stromal CD4+ T cells or T_{reg} cells. However, only 22 EBV-positive DLBCL patients were included in this comparison; thus the LMP1 analysis should be considered exploratory. A previous study that examined the presence of EBV infection in pediatric DLBCL patients also found no alteration of the T-cell subsets in EBV-positive tumors[39]. However, these results do not exclude the possibility that the presence of EBV may adversely affect the function of these T cells. In fact, studies suggest that EBV may impair the function of cytotoxic T cells and contribute to decreased immune surveillance[40].

Our results provide some evidence for the bi-directional interactions between the tumor and the microenvironment, as we observed significant correlations between certain molecular tumor characteristics and stromal immune cells. We examined the expression of five oncogenic proteins that are known to play a central role in lymphoma development and progression; three of these are correlated with the stromal density of T_{reg} cells. We also observed a positive association between p53 expression and infiltrating CD4+ T cells population. It has been proposed that functional p53 protein is involved in the induction of anti-tumor CD4+ T-cell response via expression of major-histocompatibility-complex (MHC) class-II antigens, and the absence of p53 may reduce the induction of CD4+ T cell activity[41]. Another potential explanation to the association we observed might be that the p53 peptides presented to immune cells may serve as a potential mechanism for triggering anti-tumor immune response[42]. It is also possible that some of the correlations we observed simply reflected certain microenvironment characteristics that simultaneously

promote/inhibit biologic processes in both the tumor and the stromal. For example, pro-inflammatory cytokines such as IL6 and TNF-α have been shown to trigger the expression of BCL6 in multiple myeloma cells[43]. IL-6 is known to be a negative regulator for T_{reg} cells[44]. It is thus possible that the inverse association observed between tumor BCL6 expression and infiltrating T_{reg} cells may be due to a pro-inflammatory cytokine environment. Our study, however, was not designed to address if it is the specific tumor characteristics that recruit (or through de novo generation) the T_{reg} cells, or whether the specific stromal immunity that induces or promotes the expression of certain oncogenic proteins, or both. To date, knowledge on the biologic processes that underlie the interplay between tumor and its microenvironment remains limited. Additional laboratory and clinical studies are needed to further elucidate the interaction between tumor, tissue and system level factors.

A potential limitation of this study was the limited sample size for certain subgroup comparisons. This limitation in sample size may also explain the lack of statistical significance between the density of stromal T_{reg} cells and macrophages with survival outcomes. However, our study is based on a well-defined cohort of HIV-related DLBCL patients from the ART era that is among the largest reported in the literature. Another limitation is that we did not distinguish the sub-populations of stromal CD4+ T cells and tumor associated macrophages, and we did not measure cytokine production or other factors that may affect immune cell functions.

In conclusion, we found that the composition of stromal immune cells in HIV-related DLBCL was significantly different from that found in HIV-unrelated DLBCL, and were consistent with a pattern associated with poorer prognosis. We also determined the prognostic significance of stromal CD8+ T cells in HIV-related lymphomas in a clinical population. We found that host HIV disease history, including low circulating CD4 cell count and prior AIDS diagnosis were associated with the composition of tumor microenvironment. As such, early initiation of ART and maintenance of immune function may have an impact not only on the incidence of lymphomas but also on disease prognosis. These data suggest that antitumor immunity plays an important role in disease progression and treatment outcomes in HIV-infected patients, but it can be compromised. Patient management strategies directed at restoring immunosurveillance at the tumor microenvironment level may thus hold promise for treating HIV-related lymphomas.

Acknowledgments

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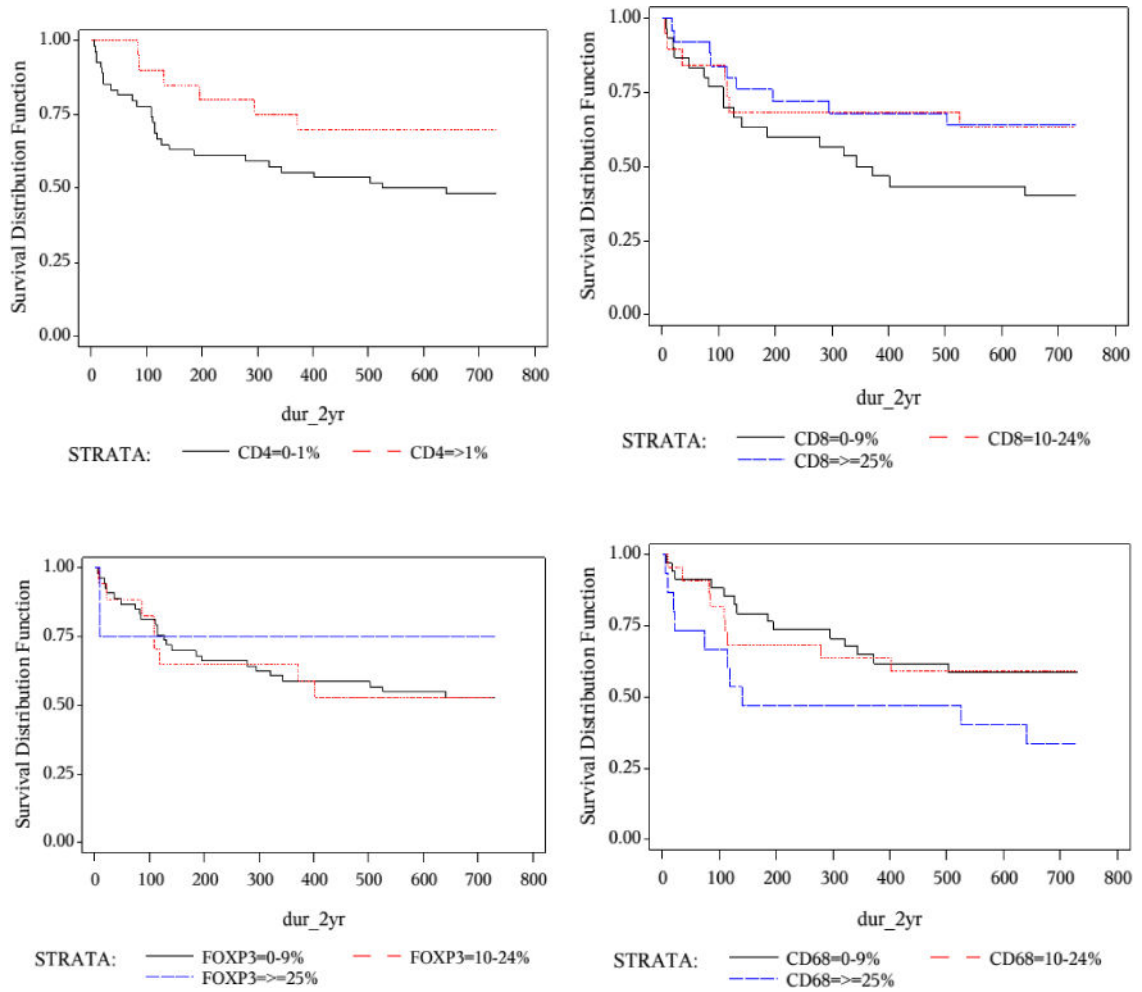
Figure 1. Kaplan-Meier survival curve for HIV-related DLBCL by density of infiltrating immune cells

Log-rank test *P*-value:

p=0.08 for CD4+ T cells; p= 0.17 for CD8+ T cells;

p=0.81 for FOXP3+ T cells; p=0.14 for CD68+ macrophage.

Table 1

Diffuse large B-cell lymphoma patient characteristics and two-year mortality by HIV infection status.

	HIV-positive (N=80)	HIV-negative (N=80)	*P* value
Age, year, mean (SD)	47.9 (9.2)	50.6 (15.9)	0.97
Male gender[a]	74 (92.5%)	70 (87.5%)	0.29
Race/Ethnicity			
White	47 (58.8%)	47 (58.8%)	1.00
Non-White	33 (41.2%)	33 (41.2%)	
Known duration of HIV infection, year, mean (SD)	5.2 (5.80)	–	–
Prior AIDS diagnosis	34 (42.5%)	–	–
Prior use of ART	52 (65.0%)	–	–
CD4 cell count at DLBCL diagnosis, cells/mm³, mean (SD)	206.2 (166.9)	–	–
Lowest CD4 cell count recorded in KP prior to DLBCL diagnosis, cells/mm³, mean (SD)	71.2 (66.7)	–	–
SEER summary stage			
I(Localized)	20 (25%)	38 (47.5%)	0.01
II(Regional)	14 (17.5%)	15 (18.8%)	
III(Distant)	38 (47.5%)	23 (28.8%)	
Unknown	8 (10%)	4 (5%)	
Extranodal involvement			
Yes	33 (41.3%)	9 (11.3%)	<0.01
Unknown	0 (0%)	19 (23.8%)	
Germinal Center (GC) Phenotype			
GC	31 (38.8%)	21 (26.3%)	
Non GC	41 (51.3%)	58 (72.5%)	0.01
Unknown	8 (10.0%)	1 (1.3%)	
DLBCL subtype			
Centroblastic	56 (70.0%)	72 (90%)	<0.01
Immunoblastic	18 (22.5%)	5 (6.3%)	
Plasmablastic	6 (7.5%)	1 (1.3%)	
Unknown	0 (0%)	2 (2.5%)	
Received chemotherapy	60 (75.0%)	67 (83.8%)	0.17
Two-year mortality	37 (46.3%)	13 (16.3%)	<0.01

Table 2

Density of infiltrating immune cells in diffuse large B-cell lymphoma by HIV infection status.

Stromal marker		HIV-infected N=80	HIV-uninfected N=80	*P*-value
		% among all stromal cells		
CD4+	Mean (SD)	1.8% (5.3%)	14.8% (21.7%)	<0.001
	Range	(0.0%–41.6%)	(0.0%–94.6%)	
CD8+	Mean (SD)	19.9% (16.1%)	14.6% (15.6%)	0.017
	Range	(0.4%–67.5%)	(0.0%–73.8%)	
FOXP3+	Mean (SD)	7.3% (9.5%)	22.5% (20.3%)	<0.001
	Range	(0.2%–62.5%)	(0.5%–82.8%)	
CD68+	Mean (SD)	15.0% (15.4%)	8.7% (9.7%)	0.006
	Range	(0.6%–68.0%)	(0.1%–49.6%)	

Table 3

Correlation between selected tumor marker expression and infiltrating immune cells in HIV-related DLBCL.

Stromal immune cells	CD4	CD8	FOXP3	CD68
Selected tumor markers	Pearson's Correlation Coefficient[a] (*P*-value)			
BCL2	−0.01 (p=0.93)	0.08 (p=0.52)	−0.08 (p=0.53)	−0.07 (p=0.57)
BCL6	−0.17 (p=0.17)	−0.00 (p=0.98)	**−0.31 (p=0.01)**	−0.14 (p=0.26)
P53	**0.25 (p=0.04)**	−0.1 (p=0.40)	**0.37 (p<0.01)**	0.13 (p=0.30)
cMYC	0.16 (p=0.18)	−0.01 (p=0.95)	**0.32 (p<0.01)**	0.01 (p=0.91)
Ki-67	0.04 (p=0.73)	0.03 (p=0.83)	0.09 (p=0.50)	**−0.22 (p=0.07)**

Numbers in bold are correlations that are statistically significant at *P*-value <0.10 level.

[a]Pearson's correlation coefficient ranges from 0 to 1, with 0 being independent and 1 being perfect correlation. A negative value indicates an inverse association.

Table 4

Association between infiltrating immune cells and two-year overall mortality in HIV-related DLBCL patients, crude and adjusted for International Prognostic Index (IPI).

HIV-infected patients (n=80) Stromal immune cells	Crude	Adjusted for IPI, germinal center phenotype and DLBCL variant	
	Odds ratio (95% CI)	Odds ratio (95% CI)	*P*-value
CD4 (reference: <1%)			
1%	0.49 (0.18–1.31)	0.43 (0.14–1.31)	0.14
CD8 (reference: <10%)			
10–24%	0.41 (0.12–1.37)	0.37 (0.10–1.31)	0.12
25%	0.37 (0.13–1.06)	**0.30 (0.09–0.97)**	**0.05**
10% vs. <10%	0.38 (0.15–0.99)	**0.33 (0.12–0.92)**	**0.04**
FOXP3 (reference: <10%)			
10–24%	0.97 (0.32–2.95)	0.73 (0.21–2.51)	0.62
25%	0.36 (0.04–3.33)	0.14 (0.01–1.68)	0.12
CD68 (reference: <10%)			
10–24%	0.86 (0.30–2.48)	1.04 (0.33–3.21)	0.95
25%	2.49 (0.74–8.34)	2.14 (0.48–9.43)	0.32
25% vs. <25%	2.65 (0.86–8.20)	2.11 (0.54–8.14)	0.28