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Primary Central Nervous System Diffuse Large B-cell Lymphoma Has Poorer Immune Cell Infiltration and Prognosis than Peripheral Counterpart

(Short title: Immune cells in brain large B-cell lymphoma)

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Abstract

***Aims*:** Primary central nervous system (CNS) diffuse large B-cell lymphoma (PCNSL) is an ominous disease with poor prognosis. The brain is an immune-privileged sanctuary which may contribute to an ineffective host anti-immune response and thus poorer outcome. We, thus, attempted to study the difference in the immune composition in PCNSL and non-CNS DLBCL, and the role of the immune response in PCNSL prognosis.

***Methods and results*:** Thirty-two biopsy specimens of PCNSL and 30 specimens of low-stage non-CNS DLBCL from immunocompetent patients formed the study group. The density and distribution of immune cells, including dendritic cells (DC-LAMP$^+$ and S100$^+$), effector/memory T-cells (CD45RO$^+$) and cytotoxic T-cells (granzyme B$^+$), and expression of HLA-DR by lymphoma cells were evaluated immunohistochemically. PCNSL patients showed poorer overall survival (P=0.032). Comparing the PCNSL and DLBCL biopsy specimens, the PCNSL cells showed less HLA-DR expression (P=0.003) and there were fewer S100$^+$ cells (P<0.01), and effector T cells (P=0.026) infiltrating PCNSL versus DLBCL. For PCNSL patients, fewer cytotoxic T cells in the background were a poor prognostic factor (P=0.004). Intratumoral S100$^+$-cell infiltration was positively correlated with T-cell infiltration, and a T-cell rimming pattern.

***Conclusions*:** In PCNSL, the baseline anti-tumor immune response is inadequate compared with non-CNS DLBCL and this response may play a role in poorer prognosis. Adjuvant dendritic-cell and T-cell immunotherapy may further boost treatment responses in PCNSL patients.

Key Words: Primary central nervous system; diffuse large B-cell lymphoma; antitumor immunity; dendritic cells; T cells; prognosis

Introduction

Patients with primary central nervous system (CNS) diffuse large B-cell lymphoma (PCNSL) have a poor prognosis with only a 1- to 2-year median survival.[1] The incidence rate of PCNSL correlates with host immunity. For immunocompromised patients, CNS involvement occurs in about 22% of patients with post-transplantation lymphomas, about 55% of which are confined to the CNS.[2] In immunocompetent hosts, PCNSL is rare, representing approximately 2% of all primary brain tumors,[3] and affects predominantly patients older than 65 years with a slight male predominance.[4] The overall incidence of PCNSL increased from the 1970s (0.2 per 100,000 people) to the 1990s (0.9 per 100,000 people) and declined in the 2000s (0.7 per 100,000 people). The reason is believed to be a consequence of the AIDS epidemic and the subsequent introduction of highly active antiretroviral therapy (HAART).[4] PCNSL is a highly aggressive lymphoma associated with multiple relapses. Surgical excision or radiotherapy alone is insufficient for long-term survival. Methotrexate-based chemotherapy with or without whole brain irradiation achieves more promising survival, but increases the patient's risk of subsequent debilitating neurotoxicity.[5]

Anti-tumor immunity plays an important role in eradicating tumor cells.[6] Gene expression profiling has identified genes representing immune escape or tolerogenic host immune response to be surrogate markers for aggressiveness of diffuse large B-cell lymphoma (DLBCL).[7, 8] In addition, breakthroughs in cancer immunotherapy have made clinical application of modifying the host

immune response feasible.[9] Recently, either breaking immune tolerance or rescuing cytotoxic T-cell function showed effective anti-tumor response in patients with DLBCL.[10, 11] The fundamentals of anti-tumor cellular immunity are that immature dendritic cells phagocytize tumor cell antigens, and following maturation they present tumor antigens to tumor-specific T cells. $CD8^+$ cytotoxic T cells can directly kill tumor cells and $CD4^+$ T cells may activate the innate immunity to boost this anti-tumor response.[6, 12] Since dendritic cells elicit both tumor-specific $CD4^+$ and $CD8^+$ T cells and both types of T cells are indispensable for lymphoma immunity,[13-15] assessment of $CD45RO^+$ effector or memory T cells may reflect the final common pathway of cell-mediated anti-tumor response.

In DLBCL, tumor-specific antigen is the unique idiotype (Id) antigen expressed by the neoplastic clone.[16] Through the presentation to T cells by self MHC molecules, this Id antigen could be potentially recognized as a target by host immunity. The expression of MHC molecules in DLBCL cells can be a surrogate marker for lymphoma-specific antigens.[15, 17] Dendritic cells are the most potent antigen-presenting cells. By expressing high levels of MHC and co-stimulatory molecules, dendritic cells can present tumor-associated antigens to elicit T-cell-mediated tumor destruction.[18] In humans, dendritic cells are heterogeneous as reflected by different precursor populations, anatomical localization and functions.[12] We have found that increased peritumoral distribution of $S100^+$ cells with dendritic cytoplasmic processes is a favorable prognosticator for patients with DLBCL.[19]

In non-CNS DLBCL the composition of infiltrating immune cells correlates with patient survival,[20] but similar studies performed on cases of PCNSL are sparse.[17, 21, 22] The CNS is an immune-privileged sanctuary where immune recruitment may not be as efficient as in other locations and may result in cancer progression. We conducted this study to analyze the immune composition of PCNSL and compare the findings with non-CNS DLBCL in immunocompetent patients.

Material and methods

Tissue samples

Tissue samples from 32 untreated patients with primary central nervous system diffuse large B-cell lymphoma (PCNSL) accessioned from 1991 to 2003 were recruited from the archives at National Taiwan University Hospital as reported previously.[23] All cases of PCNSL were confined within the CNS (stage IE), HIV-unrelated, and fulfilled the World Health Organization (WHO) criteria for diagnosis.[24] All patients had no evidence of immunodeficiency. The majority of our PCNSL cases had total or sub-total tumor excision. The specimens were fixed in 10% neutral formalin and embedded in paraffin. Clinical and pathologic data including patient gender and age, Ann Arbor stage, ECOG performance status, cerebrospinal fluid (CSF) protein level, serum level of lactate dehydrogenase (LDH), tumor site, overall survival in months, and treatment modalities were obtained by chart review. There were 18 male and 14 female patients (mean age: 58.7 years; range: 16-78 years). Upon diagnosis, 26 patients with PCNSL received high-dose methotrexate–based polychemotherapy (BOMES: BCNU, vicristin, methotrexate, ectoposide and methylprednisolone, or modified regimen) with additional radiotherapy in 16.[23] Radiotherapy alone or CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) regimen with radiotherapy was given in one each. Four patients with advanced age or co-morbidity received supportive care only. All the patients were followed for a mean duration of 25.2 months (range, 0.2-116.3 months).

For comparison, tissue samples from 30 untreated patients with low-stage (I/II) non-CNS DLBCL accessioned from February 1995 to December 1998 were recruited from the archives at National Cheng Kung University Hospital; these patients were also reported previously.[19] Eleven patients had stage I disease and the others had stage II disease. Nine biopsy specimens were from lymph nodes and the remaining cases were extra-nodal: gastrointestinal tract (n = 10), soft tissue (n = 4), nasopharynx (n = 2), nasal cavity (n = 1), tonsils (n = 1), spleen (n = 1), mediastinum (n = 1), and breast (n = 1). There were 11 male and 19 female patients with a mean age of 61.2 years (range, 30-79 years). All patients were treated with a curative CHOP or CHOP-like regimen.[19] For selected patients,

surgical intervention and/or radiotherapy preceded chemotherapy. None of the patients received rituximab. All the patients were followed for mean follow-up length of 28.8 months (range: 0.5-87 months). There were no statistically significant differences in clinical characteristics between PCNSL and non-CNS DLBCL (Table 1). Studies were carried out under a laboratory protocol approved by the institutional review board (NCKUH-ER-100-351) and were performed in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Immunohistochemical methods

Immunohistochemical analysis was performed on deparaffinized formalin-fixed, paraffin-embedded tissue sections after microwave-enhanced epitope retrieval. After endogenous activity had been blocked, the sections were incubated with primary antibodies for 2 h at room temperature and then detected using streptavidin-biotinylated peroxidase-conjugated reagents (LSAB+ kit; Dako, Carpinteria, CA) with 3-amino-9-ethylcarbazole as the chromogen and hematoxylin for the counterstain. The selected primary antibodies included HLA-DR (LN3, Novocastra, 1:20) expression for tumor cells, DC-LAMP (dendritic cell-specific lysosomal-associated membrane protein, 104.G4, Immunotech, 1:10) and S100 (polyclonal, DAKO, 1:400) for dendritic cells, CD45RO (UCHL1, DAKO, 1:100) for effector or memory T cells, and granzyme B (GrB-7, DAKO, 1:50) for cytotoxic T cells, as previously described.[6, 19, 23] Appropriate positive and negative controls were used. One "host-response" subtype of DLBCL contains infiltrating dendritic cells, which express S100 molecule but not CD1a.[25] DC-LAMP is a marker of mature dendritic cells.[19] The germinal center immunophenotype was determined using the Hans algorithm.[26] Images were photographed using an Olympus DP12 Digital Microscope Camera (Olympus Co, Tokyo, Japan) and processed by Adobe Photoshop version 7.0 software (Adobe Systems Incorporated, San Jose, CA, USA).

Measurements

As previously described, quantitative evaluation of $S100^+$ cells with dendritic cytoplasmic processes was accessed by counting at 10 high-power fields (HPF, X400 magnifications) in higher density areas.[19] $CD45RO^+$ and granzyme B^+ T cells were assessed by counting at 10 fields under oil immersion (X1000). For cases showing a difference in cell density between intratumoral and peritumoral areas, both areas were evaluated. Peritumoral infiltration (rimming) pattern was defined as more immune cells infiltrating around the tumor edge than within tumor beds with the difference being statistically significant ($P < 0.05$, paired *t* test), as previously described.[19] For HLA-DR, unequivocal membrane staining in ≥30% of tumor cells was graded as positive. For DC-LAMP, because only a few cases or a few target cells were positive, simply positive or negative expression rather than the numbers per case was used in the statistical calculation. The criteria were defined as unequivocally positive cell(s) in 2 or more separate HPFs or ≧2 positive cells in one HPF (Supplementary Figure S1).[19]

Statistical analysis

The following statistical tests were used to examine the relationships and correlations between variables: a χ^2 test (or two-tailed Fisher's exact test when expected number < 5) for categorical variables, a Mann-Whitney U test (also called Wilcoxon rank-sum test) for continuous variables, and Kendall's tau (*T*) correlation test for two variables. Bonferroni correlation was used to avoid spurious positive results in multiple comparisons. The overall survival was measured from initial diagnosis to death from any cause, with follow-up data of surviving patients assessed at the last contact date. Estimates of overall survival distribution were calculated using the Kaplan-Meier method.[27] Time-to-event distributions were compared using the log-rank test.[28] A Cox regression model was used to test the simultaneous influence on overall survival of covariates with *p*-value < 0.1 in the univariate analysis.[29] All *p*-values are two-sided. SPSS 17.0 (SPSS, Inc., Chicago, IL) was used for all analyses.

Results

Differences between PCNSL and non-CNS DLBCL

In PCNSL, tumor cells less frequently expressed HLA-DR than did those in non-CNS DLBCL (Fig. 1A). One case of PCNSL and 9 cases of non-CNS DLBCL were positive for HLA-DR (P = 0.003, χ^2-test, Table 1). PCNSL had less immune-cell infiltration. One of 32 PCNSL cases and 5 of 26 non-brain DLBCL cases had DC-LAMP$^+$ infiltration (P = 0.080, Table 1). PCNSL had significantly less S100$^+$ cell infiltration in tumor beds (P = 0.002, Mann-Whitney U test, Fig. 1B) and around the tumor edge (P = 0.005). There were fewer cytotoxic T cells in the PCNSL tumor beds (granzyme B$^+$: P = 0.083, Fig. 1C) and fewer effector T cells around the tumor edge (CD45RO$^+$: P = 0.026, Fig. 1D). The rimming patterns of S100$^+$ cells and CD45RO$^+$ T cells also appeared to be less frequent in PCNSL than in non-CNS DLBCL, but the differences were not significant. Patients with PCNSL had poorer overall survival than did patients with low-stage non-CNS DLBCL (P = 0.032, log-rank test) (Fig. 2A).

Prognostic factors in PCNSL

For the PCNSL group, older patient age (≥ 70 years, P = 0.008, Fig. 2B), a high serum LDH level (> normal, P < 0.001, Fig. 2C), MUM1 expression (P = 0.030, Fig. 2D), BCL2 expression (P = 0.010), and less cytotoxic T-cell infiltration in tumors (granzyme B$^+$, P = 0.004, Fig. 2E) were unfavorable prognosticators in univariate analysis (Table 2). Some of the clinical factors have been discussed previously.[23] Patients with less S100$^+$-cell infiltration in tumor beds showed a trend toward poorer survival (P = 0.062, Fig. 2F). In the multivariate model, the older patient age (P = 0.022), high LDH level (P = 0.005), BCL2 expression (P = 0.009), and fewer intratumoral granzyme B$^+$ cytotoxic T cells (P = 0.032) were poor prognostic factors (Table 2). Patient performance on the ECOG scale, cerebrospinal fluid (CSF) protein levels, and deep brain involvement did not significantly affect survival (Table 2), nor did different chemotherapy or radiotherapy regimens (data not shown).

Correlation of PCNSL immune cell infiltration and tumor characteristics

In PCNSL a higher number of infiltrating immune cells correlated with tumor characteristics, including HLA-DR expression and germinal center phenotype. HLA-DR expression of tumor cells correlated positively with $S100^+$-cell infiltration at the tumor edge ($r = 0.370$, $P = 0.050$). BCL6 expression correlated positively with infiltration of $S100^+$ cells within the tumor bed ($r = 0.473$, $P = 0.008$) and around the edge ($r = 0.437$, $P = 0.019$). Interestingly, a germinal center phenotype correlated with more $CD45RO^+$ T cells around the tumor edge ($r = 0.398$, $P = 0.032$) and the $CD45RO^+$ T-cell rimming pattern ($r = 0.515$, $P = 0.006$). In addition, correlations between immune cells were in accordance with immune recruitment, as in non-CNS DLBCL.[19] Intratumoral $S100^+$ cell infiltration correlated with intratumoral granzyme B^+ T-cell infiltration ($r = 0.532$, $P = 0.004$), more $CD45RO^+$ T cells around the tumor edge ($r = 0.676$, $P < 0.001$), and a $CD45RO^+$ T-cell rimming pattern ($r = 0.480$, $P = 0.010$). Peritumoral $S100^+$-cell infiltration showed similar correlations. There was no significant correlation between BCL2 expression and immune response composition including the germinal center phenotype. In comparison with BCL2-negative PCNSL cases, the BCL2-positive group had a lower number of active caspase 3-positive cells (BCL2-negative group, 2.75% ± 0.26%; BCL2-positive group, 1.01% ± 0.82%). There was a marginal significant difference (P = 0.086, Supplementary Figure S2).

Discussion

In DLBCL, clinicopathologic features, tumor locations, immune-cell infiltration, and molecular biology are important prognostic factors.[19, 20, 24, 30, 31] Regarding tumor location, extranodal locations are reported to affect patients' survival.[32, 33] For CNS and testis, immune escape due to lost expression of HLA genes is proposed as a potential explanation for their poor prognoses.[17] In this comparison of PCNSL with non-CNS DLBCL, we found that patients with PCNSL had poorer survival. In addition, PCNSL tumors had poorer HLA-DR expression, less infiltration of $S100^+$ cells, and less peritumoral infiltration of $CD45RO^+$ effector T cells. On average, the infiltration of granzyme B^+ cytotoxic cells in

PCNSL was marginally lower than in non-CNS DLBCL. These data suggest that PCNSL induces a lower immunogenicity and less subsequent infiltration of dendritic cells and effector T cells. Similarly, Rimsza *et al* reported that lost expression of MHC class II genes (eg, HLA-DR) in non-CNS DLBCL correlated with poorer survival and fewer tumor-infiltrating $CD8^+$ T cells.[15] For PCNSL, the results of this study suggest that more intratumoral $S100^+$ cells and granzyme B^+ cytotoxic cells were beneficial for patient survival, consistent with our previous study of non-CNS DLBCL.[19] The granzyme B^+ cytotoxic cell infiltration in PCNSL was also a favorable factor in multivariate analysis.

In contrast, there are studies in the literature that indicate an unfavorable role for cytotoxic T cells. In one report, a higher level of tumor-infiltrating activated cytotoxic T lymphocytes (CTLs) was correlated with the frequent loss of HLA class I and II expression in DLBCL of the brain and testis.[22] It was believed that the immune system in these sites caused a higher selective pressure and a loss of HLA class I expression as a mechanism of immune escape. The infiltration of granzyme B-, TIA-, and perforin-positive T cells correlated with poor outcome in nodal and systemic DLBCL.[34, 35] Muris *et al* suggested that a strong CTL-mediated immune response leads to apoptosis-resistant tumor clones that are also therapy-resistant.[34] The discrepancy between our results and those of other studies may be ascribed to different methods, differences in study populations, or the different functional status of cytotoxic lymphocytes. The interaction between DLBCL and host immune cells is dynamic.[36] These contradictory reports might be a consequence of the dual role of the immunosurveillance and immunoediting of the host immune response.[36] It might also reflect the different host immune status of tumor elimination, equilibrium, and escape. Our study enrolled low-stage cases only and, therefore, may include fewer tumor samples with immune escape. Furthermore, the animal studies with perforin-knockout mice also support a role for CTLs in inhibiting spontaneous lymphoma development.[36, 37] Clinically, a perivascular T-cell infiltrate predicts better survival for patients with PCNSL,[38] although the immunologic role is unclear.

Five clinical parameters associated with poor prognoses in PCNSL have been suggested by International Extranodal Lymphoma Study Group: age >60 years, an ECOG performance status score

>1, elevated serum LDH level, high CSF protein concentration, and involvement of deep regions of the brain.[39] The first three factors are the same as those for the systemic International Non-Hodgkin's Lymphoma Prognostic Factors index.[32] In this study, patient age and elevated LDH level were significant prognostic factors, but ECOG performance status, CSF protein level, and deep brain involvement were not. These differences could be attributable to the relatively small number of patients in this study.

Immunophenotyping DLBCL into germinal-center-B (GCB)-like and non-germinal-center-B (non-GCB)-like tumors has been reported to predict survival,[26, 40, 41] although this classifier may be irrelevant for PCNSL.[42] Here, we found that PCNSL tumor cell expression of MUM1 and BCL2 negatively influenced survival. In addition, the infiltration of effector T-cells ($CD45RO^+$) correlated with GCB-like tumors. Other studies have reported that lymphocyte infiltration and the absolute lymphocyte count in GCB-like and non-GCB-like tumors predicted survival.[43, 44] More immune cell infiltration in GCB-like phenotype may contribute to better survival. Here and in the literature,[1, 20] patients with PCNSL have a worse prognosis than those with non-CNS counterparts. In addition to the more frequent expression of B-cell differentiation markers,[23, 45] the poorer immune response and lost MHC expression may also play roles in the poorer prognosis of patients with PCNSL.

Components of the host immune response are potential therapeutic targets.[46, 47] The basic theory suggests that HLA-DR expressed by lymphoma cells interacts with dendritic cells ($S100^+$) and then recruits effector T cells ($CD45RO^+$) and cytotoxic T cells (granzyme B^+) to attack tumor cells. However, tumor cells might prevent anti-tumor immunization by presenting interfering self-antigen to dendritic cells at the steady state, suppressing effector T-cell responses, and modifying regulatory T-cell (Treg) responses to oppose effector T-cell activity.[48] PCNSL likely bears these features that lead to multiple relapses and hence prevent from long-term remission.

The principal antigen-presenting cells in CNS are composed of dendritic cells and microglia. The former (consisting of conventional and plasmacytoid subsets) are derived from common myeloid

precursor cells of bone marrow, while the latter, resident immune cells of the brain, arise from yolk sac-based precursors, which are distinct from bone marrow-derived monocytic cells.[49] Whether microglia can become dendritic cells is still under investigation. Since microglia express low level of MHC-II, Fc and mannose receptors and co-stimulatory molecules, they are less efficient antigen-presenting cells than monocyte-derived dendritic cells.[49] In addition, microglia have a poor antigen-presenting capacity.[50] Whether activated microglia participate in antigen presentation and the initiation of adaptive immune responses remains unclear. In the steady-state brain, dendritic cells are absent or few in number, while recruitment of dendritic cells is enhanced by a compromised blood-brain barrier such as tumorigenesis.[50] Thus, we focused on the dendritic cells but not microglia. We have found in our previous study that S100 is a surrogate marker for dendritic cells.[19] However, S100 is negative for microglia, which instead are best recognized by lipocortin 1 (LC1).[51-53] On the contrary, there may be potential pitfalls of using S100 to identify dendritic cells in CNS tissue in which both astrocytes and neurons are strongly S100 reactive. To prevent from the pitfall, when we evaluated the $S100^+$ cells in CNS, we were very careful to avoid the hypocellular tumor regions. As shown in Supplementary Figure S3, the $S100^+$ cells showed a stronger signal than brain tissue. We can clearly differentiate $S100^+$ stromal cells from neurons and astrocytes.

Our findings show that there are clear differences in immune cell infiltration between PCNSL and non-CNS DLBCL. Tumor characteristics correlated with immune cell infiltration and may therefore contribute to the prognosis of PCNSL. Although immunosuppression may contribute to an immune-privilege milieu of CNS, we found that PCNSL had less infiltration of plasmacytoid dendritic cells (Supplementary Figure S4), which are immunosuppressive cells. Together with the less infiltration of $S100^+$ cells and effector T cells, it seems that the poorer prognosis of PCNSL may be attributed to decreased immunotoxicity rather than increased immunosuppression in the CNS milieu. These differences in immune response offer a basis for anti-PCNSL therapy and adjuvant dendritic-cell and T-cell immunotherapy may further boost treatment responses in PCNSL patients.

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Supplementary Information

Supplementary Materials and Methods

Supplementary Figures S1-S4

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Table 1. Summary of clinicopathological features between primary central nervous system (CNS) diffuse large B-cell lymphoma (PCNSL) and non-CNS (peripheral) diffuse large B-cell lymphoma (DLBCL)

	PCNSL	**Non-CNS DLBCL**	***p*-value**
	n = 32 (%)	n = 30 (%)	
Parameters	mean ± SD	mean ± SD	
Age (years, median, range)	61.5 (16-78)	65 (30-79)	0.413
Gender (Male)	18 (56.3%)	11 (36.7%)	0.137
Stage I/II	32/0	11/19	<0.001

Follow-up time (month, range)	25.2 (0.2-116.3)	28.8 (0.5-87)	0.213
Death	22 (68.8%)	11 (36.7%)	***0.021***
LDH level (>normal limit)	15 (53.6%)	16 (53.3%)	1.000
Germinal center phenotype	12 (37.5%)	6 (25.0%)	0.394
HLA-DR expression (≥30%)	1 (3.2%)	9 (34.6%)	***0.003***
Presence of DC-LAMP$^+$ dendritic cells	1 (3.1%)	5 (19.2%)	0.080
No. of S100$^+$ cells in tumor beds	9.9 ± 9.0	27.6 ± 23.5	***0.002***
No. of S100$^+$ cells in tumor edge	5.7 ± 14.6	16.4 ± 17.4	***0.005***
S100$^+$ cell rimming pattern	1 (3.1%)	4 (16.0%)	0.157
No. of CD45RO$^+$ T-cells in tumor beds	29.8 ± 24.5	32.8 ± 20.9	0.320
No. of CD45RO$^+$ T-cells in tumor edge	23.0 ± 35.0	32.9 ± 29.2	***0.026***
CD45RO$^+$ T-cell rimming pattern	4 (13.3%)	5 (20.0%)	0.761
No. of granzyme B$^+$ T-cells in tumor beds	8.6 ± 14.5	13.3 ± 17.9	0.083

SD: standard deviation. Peritumoral rimming pattern: more immune cells infiltrating around the tumor edge than in tumor bed, as previously described.[19]

Table 2. Unfavorable clinicopathologic factors of primary central nervous system diffuse large B-cell lymphoma (PCNSL)

	Unfavorable	**Univariate**	**Multivariate**		
Parameters	Factor / n (%)	*p*-value	*p*-value	HR	(95% CI)
Gender	Female	0.073			-
	14 (43.8)				
Age (years)	≥70	***0.008***	***0.022***	0.022	(0.001-0.580)
	7 (21.8)				
ECOG score	High (3-4)	0.122			-
	16 (50)				
Cerebrospinal fluid	High	0.995			-
(CSF) protein	5 (45.5)				
Lactate dehydrogenase	High (>normal)	***<0.001***	***0.005***	0.004	(0.000-0.196)
(LDH) level	15 (53.6)				
Deep brain involvement	Yes	0.353			-
	19 (59.4)				
Germinal center phenotype	No	0.770			-
	20 (62.5)				
CD10 expression	No	0.860			-
	24 (75.0)				
BCL6 expression	No	0.839			-
	14 (43.8)				
MUM1 expression	Yes	***0.030***	0.059	0.071	(0.005-1.109)

	23 (71.9)				
BCL2 expression	Yes	***0.010***	***0.009***	146.4	(3.53-6077.6)
	7 (29.2)				
HLA-DR expression	No	0.793			-
	29 (96.7)				
Presence of DC-LAMP$^+$	No	0.325			-
	31 (96.9)				
No. of S100$^+$ cells	<10/HPF	***0.062***	0.443	2.047	(0.328-12.8)
in tumor beds	19 (59.4)				
No. of S100$^+$ cells	<6/HPF	0.164			-
in tumor edge	24 (80)				
Peritumoral S100$^+$ cell	No	0.325			-
rimming pattern	31 (96.9)				
No. of CD45RO$^+$ T cells	<30/HPF	0.280			-
in tumor beds	19 (63.3)				
No. of CD45RO$^+$ T cells	<23/HPF	0.361			-
in tumor edge	23 (76.7)				
Peritumoral CD45RO$^+$	No	0.834			-
T-cell rimming pattern	26 (86.7)				
No. of granzyme B$^+$ T cells	<9/HPF	***0.004***	***0.032***	10.98	(1.235-97.6)
in tumor beds	21 (67.7)				

HR: hazard ratio; CI: confidence interval; ECOG: Eastern Cooperative Oncology Group performance status scale; DC-LAMP: dendritic cell-lysosomal-associated membrane protein; HPF: high-power field. The edge between tumor cells and brain parenchyma was present in 30 cases. Peritumoral

rimming pattern: more immune cells infiltrating around the tumor edge than in tumor bed, as previously described.[19]

Figure Legends

Figure 1. Comparisons of HLA-DR expression and immune cell infiltration between PCNSL (left column) and peripheral (non-CNS) DLBCL (right column). In comparison with peripheral DLBCL, PCNSL shows fewer cases with HLA-DR expression (A, 400X), fewer $S100^+$ cell infiltration in tumor beds (B, 400X), fewer granzyme B^+ cytotoxic T-cell infiltration in tumor beds (C, 400X), and fewer $CD45RO^+$ T-cell in tumor edge (D, 100X). The cells positive for granzyme B (C, right panel) or CD45RO (D, right panel) are negative for PAX5 by double staining with granzyme B/PAX5 (C, inset, 400X) or CD45RO/PAX5 (D, inset, 400X) (granzyme B, in red-cytoplasmic granular; CD45RO, in red-cytoplasmic; PAX5, in brown-nuclear).

Figure 2. Overall survival curves. (A) PCNSL patients have worse overall survival than non-CNS DLBCL (p=0.032, log-rank test). (B)-(E) For PCNSL, the poor prognostic factors are old age (B), a high serum LDH level (>normal, C), MUM1 expression (D), and less cytotoxic T-cell infiltration in tumors (granzyme B^+, E). (F) $S100^+$ cell infiltration in tumor beds has a trend toward poorer survival (p=0.062).

Fig. 1

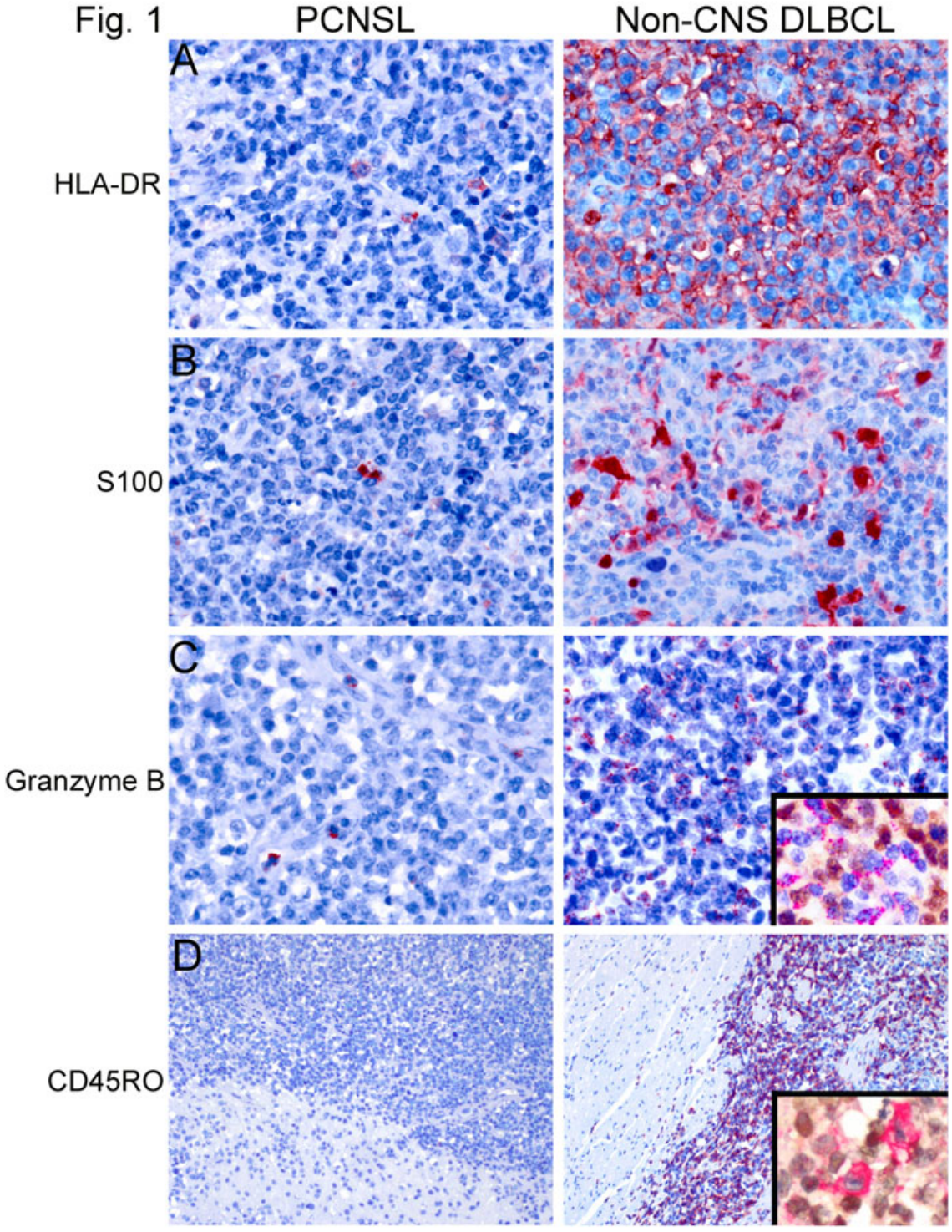

Fig. 2