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Expression of the promyelocytic leukemia zinc-finger in T-lymphoblastic lymphoma and leukemia has strong implications for their cellular origin and greater association with initial bone marrow involvement

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The promyelocytic leukemia zinc-finger (PLZF) is essential for the development of innate T cells (as represented by natural killer T cells) for acquisition of their unique innate immune properties. We evaluated the PLZF protein expression in a variety of immature and mature lymphoid malignancies. PLZF was preferentially expressed in T-lymphoblastic lymphoma/acute lymphoblastic leukemia (T-LBL/ALL) in 50% of the 54 cases. Among 51 cases of peripheral T-cell lymphoma not otherwise specified, only one (2%) expressed PLZF. One mycosis fungoides case expressed PLZF in lymph node involved by tumor. Otherwise, PLZF was not detected in any other type of lymphoma. In T-LBL/ALL, PLZF expression was more common in CD4/CD8 double-negative (67%) or CD8 single-positive subtypes (73%) than in CD4/CD8 double-positive (13%) and CD4 single-positive subtypes (0%) (*P* = 0.001). Importantly, PLZF and CD1a expression were mutually exclusive in T-LBL/ALL (*P* = 0.001). This was also the case for T-cell receptor *β*F1 expression (*P* = 0.000). Most (96%) of the PLZF-positive T-LBL/ALL cases showed initial bone marrow involvement compared with 39% of PLZF-negative cases (*P* = 0.000). Based on these findings, we suggest that T-LBL/ALLs that express PLZF arise from early immature double-negative thymocytes when the T-cell receptor *β* chain has not yet expressed or innate T-cell precursors, and strongly imply bone marrow involvement.

The promyelocytic leukemia zinc-finger (PLZF), a transcription repressor of the BTB/POZ-ZF (broad

complex, Tramtrack, Bric à brac (BTB) or poxvirus and zinc-finger (POZ)-zinc-finger (ZF)) family, was first identified as a fusion partner of retinoic acid receptor-α (RAR-α) in a subset of acute promyelocytic leukemia having t(11;17)(q23;q21).[1–3] PLZF–RAR-α or RAR-α–PLZF fusion proteins function as dominant-negative molecules, contribute to leukemogenesis, and are associated with resistance to retinoid acid in acute promyelocytic leukemia.[4–6]

Under physiologic condition, PLZF is expressed in early hematopoietic stem cells and CD34 (+) human bone marrow progenitor cells and its expression decreases during myeloid differentiation, and is thought to involve in the myeloid development and maintenance of hematopoietic stem cell population.[7–11] PLZF also has a role in skeletal development and spermatogenesis, and it has a tumor-suppressive role in a few solid tumors.[1,12–15]

It was recently found that PLZF is essential for the development of natural killer T (NKT) cells and acquisition of the unique innate-like characteristics of innate T cells.[16–19] Innate immunity is mediated by natural killer (NK) cells, monocytes/macrophages, and granulocytes. In general, this pathway enables the recognition of pathogens via germline-encoded pattern-recognition receptors (eg, the Toll-like receptor) and activation of the immediate immune response, and it does not involve immunological memory. Conversely, B and T cells are responsible for adaptive immunity. These cells have highly variable clonal immune receptors, namely immunoglobulin and T-cell receptors.[20]

The term 'innate T cells' refers to several different T-cell subsets that possess the properties of both innate and adaptive immune cells. They have unique features compared with conventional naive T cells, and share some characteristics with memory/effector T cells. After development and maturation in the thymus, innate T cells already have an activated or memory T-cell phenotype as an inherent property (eg, CD44^{hi}) and the ability to produce both Th1 (eg, IFNγ) and Th2 (eg, IL-4) cytokines immediately on stimulation, similar to memory T cells.[21,22] PLZF is expressed in a variety of innate T cells and is implicated in their development, and it is now considered an innate T-cell determinant.[19]

This lineage-specific expression of PLZF in the immune system and lack of comprehensive information about PLZF expression in lymphoid malignancies led us to investigate the expression of PLZF in immature and mature lymphoid malignancies. Here, we demonstrated that PLZF is uniquely expressed in a substantial percentage of T-lymphoblastic lymphoma/acute lymphoblastic leukemia (T-LBL/ALL). PLZF is thought to be a novel, relatively specific marker for T-LBL/ALL with respect to its cellular origin and early bone marrow involvement.

Materials and methods

Patients and Tissue Samples

Records of patients diagnosed with lymphoid malignancies at Seoul National University Hospital and Asan Medical Center were retrieved. All available materials were reviewed by two hematopathologists (YKJ and JRH) according to current World Health Organization criteria. The population of patients included in our study were summarized in Table 1. Ten samples of non-neoplastic tonsil or

Table 1 PLZF protein expression in lymphoid neoplasms

Lymphoma type	n	PLZF-positive (%)
T-lymphoblastic lymphoma/leukemia	54	27 (50%)
B-lymphoblastic lymphoma/leukemia	29	0
Peripheral T-cell lymphoma not otherwise specified	51	1 (2%)
Angioimmunoblastic T-cell lymphoma	46	0
Anaplastic large cell lymphoma, ALK+	15	0
Anaplastic large cell lymphoma, ALK-	19	0
Extranodal NK/T-cell lymphoma, nasal type	80	0
Enteropathy-associated T-cell lymphoma	6^a	0
Primary cutaneous mature T-cell lymphoma	13^b	1 (8%)^c
Chronic lymphocytic leukemia/small lymphocytic lymphoma	19	0
Mantle cell lymphoma	23	0
Follicular lymphoma	22	0
Diffuse large B-cell lymphoma	58	0
Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue	30	0
Nodal marginal zone B-cell lymphoma	10	0
Burkitt lymphoma	19	0
Hodgkin lymphoma	66	0
Plasmacytoma	28	0

^{a}T-cell receptor βF1 immunohistochemistry staining was negative in three cases.
^{b}T-cell receptor βF1 staining was negative in four cases.
^{c}PLZF staining was positive in lymph nodes involved in mycosis fungoides.

lymph nodes, and fetal and neonatal thymus were also examined as control. Clinical data including age, gender, white blood cell (WBC) count, mediastinal involvement, and bone marrow involvement at the time of presentation were recovered from medical records. The Institutional Review Board in Seoul National University Hospital approved this study.

Immunohistochemistry

For each patient, representative formalin-fixed paraffin-embedded tissue blocks were subjected to immunohistochemistry. Tissue microarrays were manufactured for diffuse large T-cell lymphoma, Hodgkin lymphoma, and a subset of T-LBL/ALL. Core tissues (2 mm in diameter) were taken from individual paraffin blocks (donor blocks) and arranged into a new recipient paraffin block using a trephine apparatus. Immunohistochemical staining was performed using a *Leica* BOND-MAX automated immunostainer (Leica Microsystems, Wetzlar, Germany) and the following antibodies: PLZF (clone D-9, Santa Cruz Biotechnology, Santa Cruz, CA, USA), CD3 (clone F7.2.38, DakoCytomation, Copenhagen, Denmark), CD20 (clone L26, DakoCytomation), CD4 (clone 4B12, Thermo Fisher Scientific, Waltham, MA, USA), CD8 (clone SP16, Thermo Fisher Scientific), T-cell receptor

βF1 (clone 8A3, Thermo Fisher Scientific), TdT (polyclonal rabbit, DakoCytomation), and CD1a (clone MTB1, Novocastra Reagents, Leica Microsystems).

PLZF-specific staining intensity was graded as weak, moderate, or strong in the predominant pattern. The proportion of immunostained cells were classified as 0, 1–25%, 26–50%, 51–75%, or >75%.

Statistical Analysis

Either Pearson's χ^2 test or Fisher's exact test was used to analyze differences between variables. P-values <0.05 were considered to be statistically significant, and all P-values were two sided. All statistical analyses were performed using SPSS version 19.0 for Windows (SPSS, Chicago, IL, USA). Overall survival was measured from the date of diagnosis to the date of death or to the date of the last follow-up visit. Event-free survival was defined as the date of diagnosis to the date of disease progression/relapse, death, or the date of the last follow-up. Survival analysis was performed using the Kaplan and Meier method using the log-rank test.

Results

PLZF Expression in Lymphoid Malignancies

PLZF was immunostained in the nucleus of lymphoid cells. In fetal and neonatal thymi, PLZF-positive cells were occasionally observed in the medulla and cortex (Figure 1a). With age, PLZF-positive cells decreased gradually in number to fewer than five per high-power field in the thymi and lymph nodes (Figure 1b).

The PLZF expression in lymphoid malignancies is summarized in Table 1. PLZF was preferentially expressed in T-LBL/ALL (50%, 27/54 cases). Among 51 cases of peripheral T-cell lymphoma not otherwise specified, only 1 (2%) expressed PLZF. One mycosis fungoides patient showed focal PLZF expression in a lymph node involved by tumor cells. Otherwise, PLZF expression was not detected in any cases of B-lymphoblastic lymphoma/leukemia, other mature T- or NK-cell lymphomas, mature T-cell lymphomas, Hodgkin lymphomas, or plasma cell neoplasm.

Although there were no PLZF-immunostained tumor cells in PLZF-negative lymphoid neoplasm, T-LBL/ALLs showed heterogeneous pattern of PLZF expression in terms of immunostaining intensity and the proportion of tumor cells (Figures 1c–f). As summarized in Table 2, 27 (50%) of 54 T-LBL/ALL cases were completely negative for PLZF (Figure 1f). Eight (15%) patients showed PLZF expression in <25% of tumor cells (Figure 1e), and 17 (32%) exhibited diffuse PLZF expression in more than half

the tumor cells (Figure 1c). Among the 27 PLZF-positive T-LBL/ALLs, 25 cases showed moderate-to-strong PLZF staining intensity (Table 2).

Immunophenotypes of T-LBL/ALL According to PLZF Expression

T-LBL/ALL can be classified into diverse subtypes based on their developmental stages according to the expression of a panel of antigens. In brief, the pro-T and pre-T subtypes are negative for both CD4 and CD8 ('double-negative') and CD1a (−). Cortical T is positive for both CD4 and CD8 ('double-positive') and CD1a (+). Medullary T expresses either CD4 or CD8 ('single-positive') and CD1a (−).[23,24] Additionally, T-cell receptor $\alpha\beta$ expression is observed in late cortical and medullary T-cell lymphoblastic lymphoma/leukemia.[25]

We examined the association between T-LBL/ALL subtype and PLZF expression. All T-LBL/ALL cases included in this study expressed cytoplasmic CD3 and at least one of the makers (eg, TdT, CD1a, CD99, or JL-1), indicating the precursor lymphoblastic nature.[23,26] As shown in Table 3, overall the double-negative subtype was most common (up to 45%); followed by double-positive (28%) and CD8 single-positive (21%), and the CD4 single-positive subtype was least (6%). CD1a was expressed in 9 (17%) of 53 cases with different frequencies according to the T-LBL/ALL subtype ($P=0.002$). None of the T-LBL/ALLs with double-negative subtype expressed CD1a, but 7 (47%) of the 15 double-positive cases and each of the CD4 single-positive (33%, 1/3) and CD8 single-positive (9%, 1/11) cases were CD1a (+) (data not shown). T-cell receptor βF1 was expressed in 14 (28%) of 50 T-LBL/ALLs, and much more frequent in double-positive (69%, 9/13) and CD4 single-positive (67%, 2/3) subtypes compared with double-negative (4%, 1/ 23) and CD8 single-positive (18%, 2/11) cases ($P=0.000$) (data not shown). These expression patterns of CD1a and T-cell receptor βF1 were compatible with those expected.

PLZF expression differed significantly among the different T-LBL/ALL subtypes ($P=0.001$). As summarized in Table 3, 16 (67%) of the 24 double-negative and 8 (73%) of the 11 CD8 single-positive cases were PLZF-positive, whereas 2 (13%) of the 15 double-positive and none of the 3 CD4 single-positive cases expressed PLZF. Moreover, CD1a and PLZF expression was mutually exclusive; namely, PLZF-positive T-LBL/ALLs were all negative for CD1a ($P=0.001$). T-cell receptor βF1 and PLZF expression was also mutually exclusive ($P=0.000$). Overall these data suggest that PLZF is frequently expressed in T-LBL/ALLs of early pro- or pre-T-cell features having double-negative, CD1a (−) and T-cell receptor βF1 (−) immunophenotypes.

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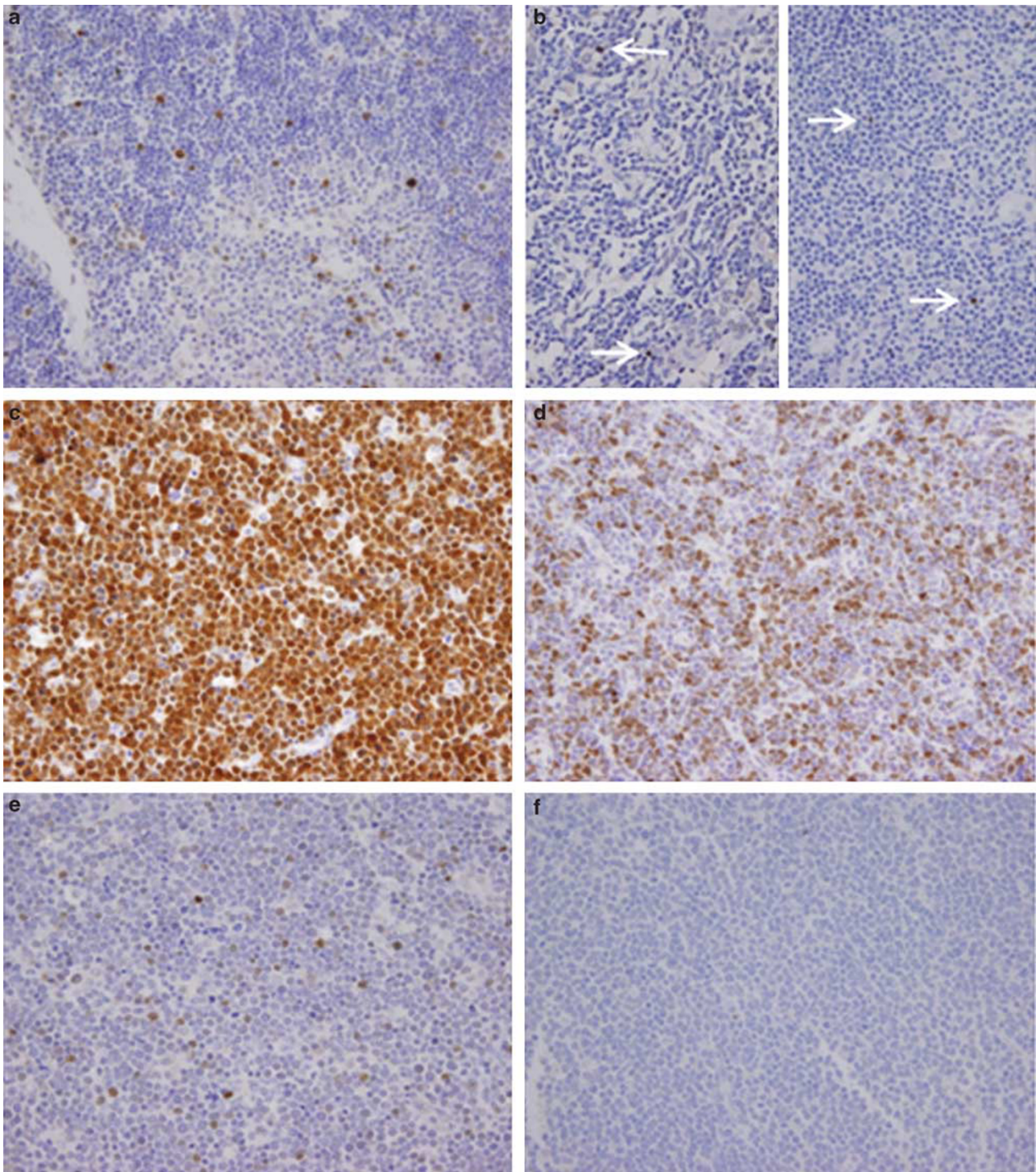
Figure 1 Immunohistochemical expression of PLZF in lymphoid tissue and neoplasms. PLZF was immunostained in the nucleus of lymphoid cells. (**a**) In a fetal thymus at gestational age 22 weeks, some scattered PLZF (+) small to medium-sized cells were observed. In adult thymus (**b**, left) and lymph node (**b**, right), only a few small lymphoid cells were PLZF (+). T-LBL/ALLs showed variable expression of PLZF (**c–f**). Some reveal diffuse strong positivity in the tumor cells (**c**); others have weak-to-moderate intensity in some of the tumor cells (**d**); still others are completely negative for PLZF (**f**). (**e**) Occasionally, PLZF immunostained only in a small portion of tumor cells in T-LBL/ALL. (Original magnification, × 400 in all).

Clinical Features of PLZF-Positive T-LBL/ALL

Clinical characteristics of T-LBL/ALLs according to PLZF expression are presented in Table 4. There were no statistical differences in age, gender, WBC count, or mediastinal involvement between PLZF-positive and -negative T-LBL/ALLs.

Notably, PLZF expression in T-LBL/ALL was strongly associated with bone marrow involvement at presentation with 96% in PLZF-positive *versus*

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Table 2 PLZF expression patterns in T-lymphoblastic lymphoma/leukemia

	Proportion of positive tumor cells					Total
	0	1–25%	26–50%	51–75%	>75%	
Predominant staining intensity						
None	27	0	0	0	0	27 (50%)
Weak	0	0	0	0	2	2 (4%)
Moderate	0	7	0	2	4	13 (24%)
Strong	0	1	2	1	8	12 (22%)
Total						
54	27 (50%)	8 (15%)	2 (4%)	3 (6%)	14 (26%)	54

39% of PLZF-negative cases ($P = 0.000$). This correlation was also significant when analyzed separately in children and adults. All of the four PLZF-positive versus one (14%) of the seven PLZF-negative T-LBL/ALLs had bone marrow involvement in childhood cases ($P = 0.015$). Among adult T-LBL/ALLs, 19 (95%) of the 20 PLZF-positive cases had bone marrow involvement, whereas 8 (50%) of the 16 PLZF-negative cases did ($P = 0.003$). About one-fourth of the patients initially showed a leukemic presentation as defined by >25% of blasts in bone marrow, and they had more frequent PLZF-positivity than the others. However the statistical

Table 3 Immunophenotypes of T-lymphoblastic lymphoma/leukemia according to PLZF expression

	All cases n (%)	PLZF-positive n (%)	PLZF-negative n (%)	P-value
CD4 and CD8				
Double-negative	24/53 (45%)	16/26 (62%)	8/27 (30%)	0.001
Double-positive	15/53 (28%)	2/26 (8%)	13/27 (48%)	
CD4 single-positive	3/53 (6%)	0/26	3/27 (11%)	
CD8 single-positive	11/53 (21%)	8/26 (31%)	3/27 (11%)	
TdT				
Positive	46/53 (87%)	23/26 (89%)	23/27 (85%)	NS
Negative	7/53 (13%)	3/26 (12%)	4/27 (15%)	
CD1a				
Positive	9/53 (17%)	0/26	9/27 (33%)	0.001
Negative	44/53 (83%)	26/26 (100%)	18/27 (67%)	
T-cell receptor β				
Positive	14/50 (28%)	0/25	14 /25 (56%)	0.000
Negative	36/50 (72%)	25/25 (100%)	11/25 (44%)	

Abbreviation: NS, not significant.

Table 4 Clinical features of T-lymphoblastic lymphoma/leukemia according to PLZF expression

	All cases n (%)	PLZF-positive n (%)	PLZF-negative n (%)	P-value
Age (yr), median (range)	25.7 (2–59)	30.7 (8–57)	20 (2–59)	
Age (yr), mean ± s.d.	27.11 ± 15.05	30.67 ± 13.21	24.37 ± 16.49	NS
Age group				NS
Child (⩽15 yr)	14 /54 (26%)	5/27 (19%)	9/27 (33%)	
Adult (>15 yr)	40/54 (74%)	22/27 (82%)	18/27 (67%)	
Age >35 yr	16/54 (30%)	11/27 (41%)	5/27 (19%)	0.074
Gender				NS
M	38/54 (70%)	21/27 (78%)	17/27 (63%)	
F	16/54 (30%)	6/27 (22%)	10/27 (37%)	
WBC (/μl), median (range, × 1000)	6200 (1–134)	5800 (1–134)	6400 (1.6–105)	
WBC (/μl), mean ± s.d.	17 585 ± 29 886	20 812 ± 36 391	14 478 ± 22 191	NS
WBC group				
⩾10⁴/μl	14/53 (26%)	6/26 (23%)	8/27 (30%)	NS
Mediastinal involvement				
Yes	27/51 (50%)	11/23 (48%)	16/31 (52%)	NS
Bone marrow involvement				
Yes	32/47 (68%)	23/24 (96%)	9/23 (39%)	0.000
Relapse				
Yes	24/54 (44%)	13/27 (48%)	11/27 (41%)	NS

Abbreviations: F, female; M, male; NS, not significant; s.d., standard deviation; yr, years.

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difference was insignificant (data not shown). Based on these data, it is highly probable that T-LBL/ALL expressing PLZF might have early bone marrow involvement by tumor cells of any quantity.

When the clinical variables were analyzed according to T-LBL/ALL phenotypes, mediastinal involvement was common among double-positive (80%, 12/15), CD4 single-positive (67%, 2/3), or CD8 single-positive (64%, 7/11) subtypes rather than double-negative (21%, 5/24) ($P = 0.002$). Bone marrow involvement was observed more frequently in cases of double-negative subtype (83%, 20/24) compared with double-positive (42%, 5/12) or single-positive (60%, 6/10) ($P = 0.013$). In addition, 26 (81%) of 32 T-LBL/ALLs lacking T-cell receptor βF1 expression showed bone marrow involvement, whereas only 3 (27%) of 11 T-cell receptor βF1-expressing T-LBL/ALLs did ($P = 0.002$). Similarly, the absence of CD1a expression was significantly related with bone marrow involvement (74% (29/39) in CD1a (−) versus 29% (2/7) in CD1a (+) T-LBL/ALLs, $P = 0.029$).

The relapse rate of T-LBL/ALL cases in this study was 44%; this was similar between the PLZF-positive and -negative groups. The 3-year event-free survival rate was 53% with a median of 21 months. Clinicopathologic factor that adversely affected patient event-free survival in the univariate analysis was age, and initial bone marrow involvement did with marginal significance (Figures 2a–c). However, PLZF expression showed no influence on the event-free survival or overall survival of T-LBL/ALL patients (Figure 2d).

PLZF-Expressing Lymphoid Malignancies Other than T-LBL/ALL

The clinicopathological features of the one PLZF (+) peripheral T-cell lymphoma not otherwise specified case, which showed diffuse strong nuclear PLZF staining in the neoplastic cells (Figure 3c), are as follows. The patient was 73-year-old man presented with multiple systemic lymphadenopathy. Histologically, the lymph node architecture was diffusely effaced. Medium-to-large atypical lymphoid cells proliferated, infiltrated, and formed vague sheet-like patterns. The tumor cells had
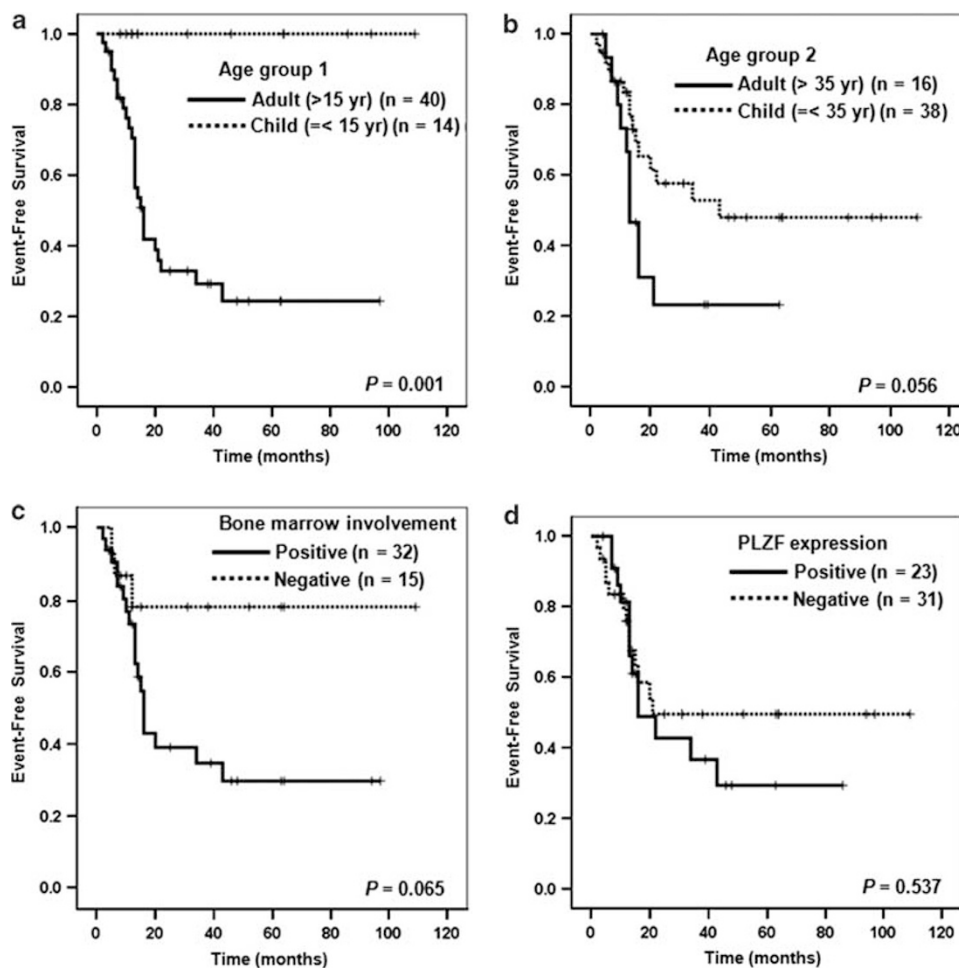
Figure 2 Plots for event-free survival of T-LBL/ALL patients according to the age (**a**, **b**), bone marrow involvement (**c**), or PLZF expression (**d**). Event-free survival time was calculated from the date of diagnosis to the date of disease progression/relapse, death, or the date of the last follow-up. Survival analysis was performed using the Kaplan and Meier method with the log-rank test.
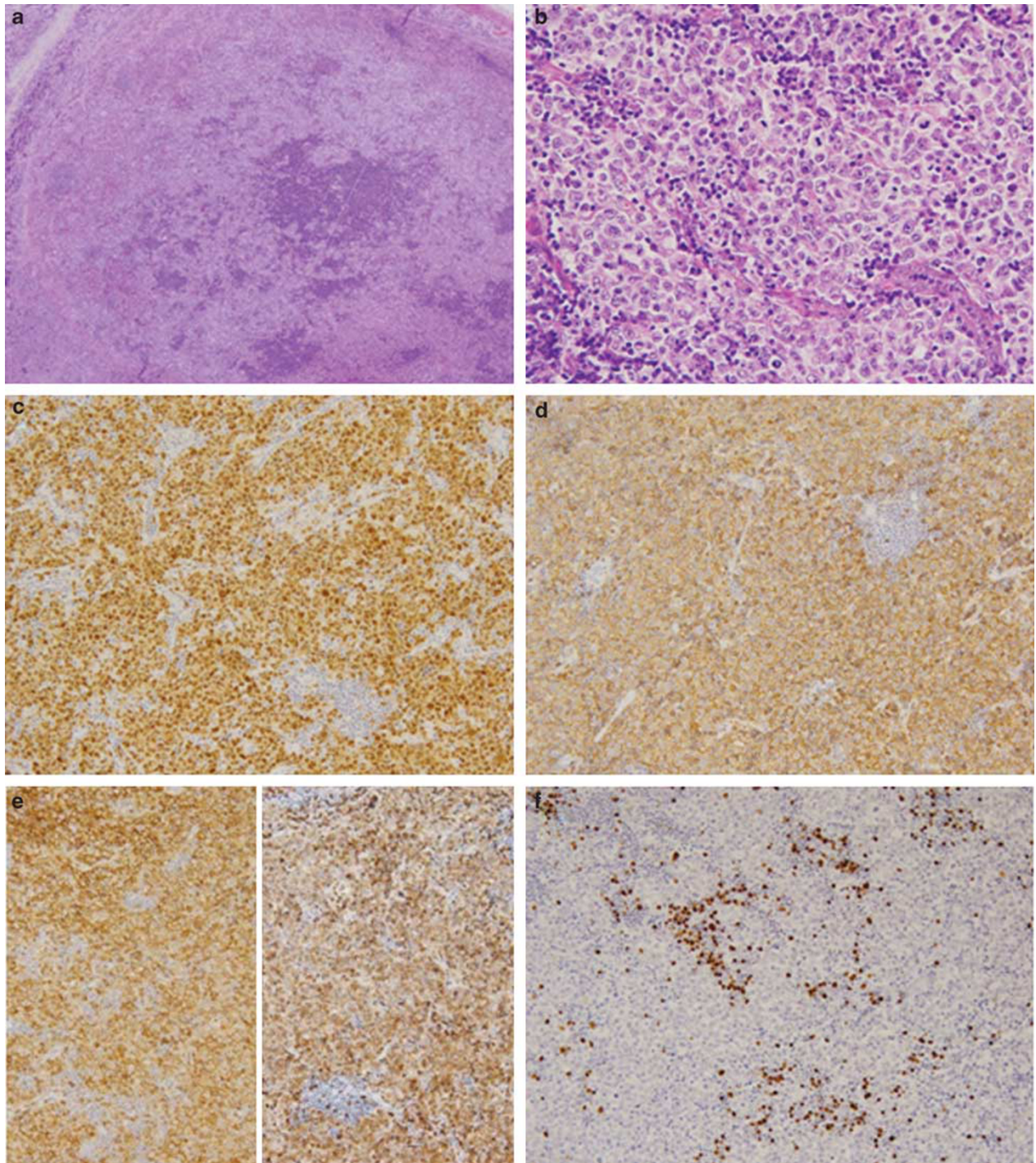
Figure 3 Pathologic features of the PLZF-positive peripheral T-cell lymphoma not otherwise specified case. (**a**) Medium-to-large atypical lymphoid cells diffusely effaced lymph node with a vague sheet-like pattern (H&E, ×40). (**b**) Tumor cells have vesicular nuclei, grooved nuclear membrane, a few prominent nucleoli, and clear to eosinophilic cytoplasm (H&E, ×400). In immunohistochemistry, tumor cells were positive for PLZF (**c**), CD3 (**d**), CD4 (**e**, left), T-cell receptor βF1 (**e**, right). (**f**) EBV was infected in reactive B cells in *in situ* hybridization for EBV-encoded RNA.

vesicular nuclei with grooved nuclear membranes, small but distinct nucleoli, and clear to eosinophilic cytoplasm (Figures 3a and b). The tumor cells were positive for CD3, CD4, BCL2, and T-cell receptor βF1 (Figures 3c–e), and negative for CD8, CD56, CD30, CD10, granzyme B, and BCL6. T-cell monoclonality

was observed by PCR analysis for T-cell receptor γ gene rearrangement, and T-cell receptor Vα24 transcripts (as a determinant for invariant NKT cells) were not detected in reverse transcription (RT)-PCR analysis (data not shown). EBV *in situ* hybridization was positive in some reactive cells corresponding to

B immunoblasts (Figure 3f). He died after 9 months due to disease progression despite of systemic chemotherapy.

In one patient with mycosis fungoides (a 54-year-old man), focal moderate PLZF expression was detected in tumor cells infiltrating a lymph node (data not shown). The tumor cells were CD3 (+), CD4 (+), T-cell receptor βF1 (+), and CD8 (−). He received acitretin and interferon-α combination treatment, and was followed with variable symptoms for a year.

Discussion

In this study, we found that PLZF was preferentially and nearly exclusively expressed in T-LBL/ALL (50%) among lymphoid neoplasm using immunohistochemistry on formalin-fixed paraffin-embedded tissues. We also encountered with a peculiar peripheral T-cell lymphoma not otherwise specified case (2%) and mycosis fungoides case with lymph node involvement that expressed PLZF. Otherwise, PLZF expression was not detected in any cases of the B-lymphoblastic lymphoma/leukemia; other mature T, NK, B-cell, and plasma cell neoplasm; or Hodgkin lymphoma. The absence of PLZF expression in B-lymphoblastic lymphoma/leukemia, the higher frequency of PLZF (+) cases among T-LBL/ALL rather than CD1a, and its specific expression in lymphoma/leukemia of T-cell lineage compared with CD99 suggested that analysis of PLZF expression by immunohistochemistry could have some diagnostic value for determining the precursor T lymphoblastic nature of lymphoid malignancies, when used in combination with other markers.

PLZF is physiologically expressed in early hematopoietic progenitor cells and decreases in its level during differentiation into mature myeloid lineage.[2,3,7,9,11] Recently, an intriguing role of PLZF in immune system has been described.[16,17] Innate T cells bridges the innate and adaptive immune systems, including γδT cells and several subsets of αβT cells. In general, innate T cells (of an αβ T-cell lineage) develop in the thymus from double-positive progenitors. These cells are selected by interactions with hematopoietic cells rather than thymic epithelial cells, and they express T-cell receptors restricted to non-classical MHC class Ib molecules rather than classical MHC class I or II molecules. They develop an activated and/or memory T-cell phenotype, and exhibit immediate effector functions along with cytokine secretion.[21] Representative innate αβT-cell subsets in humans are CD1-restricted NKT and MR1 (MHC class I related)-specific mucosal-invariant T (MAIT) cells. NKT cells include type I NKT cells having invariant T-cell receptor (Vα24-Jα18 and Vβ11 in humans), and type II (non-invariant) NKT cells that have more diverse T-cell receptor chains.[27] PLZF is essential for the development of

invariant NKT cells with acquisition of their unique innate-like properties in a mouse system.[16,17] Recently, our group and others have reported a new distinct subset of innate αβT cells (T-T CD4 T cells), which are selected by MHC class II-expressing thymocytes and also depend on PLZF for their development.[28–30] Moreover, we reported that the generation of T-T CD4 T cells is a physiological process in humans.[18] Up to now, PLZF has been exclusively expressed in a variety innate T cells including innate γδT cells, type I and type II NKT cells, MAIT cells, and T-T CD4 T cells in lymphoid system. Therefore, PLZF expression is regarded as an innate T-cell determinant.[19]

The PLZF expression patterns in lymphoid malignancies observed in this study, in general, are rather reminiscent of the PLZF expression observed in the hematolymphoid system, and cast some important clues to understand the pathogenesis of T cell, particularly immature T-cell neoplasm. We in brief speculate that PLZF (+) T-LBL/ALLs suggest two possible cellular origins, that is, one from innate T cells and the other from early immature T-lymphoid precursors.

In terms of T-LBL/ALL subtypes based on T-cell development, PLZF expression was more common in the double-negative and CD8 single-positive subtypes rather than double-positive or CD4 single-positive. PLZF expressions in a portion of double-negative and CD8 single-positive T-LBL/ALLs were consistent with results from our previous study on PLZF expression in fetal human thymocytes.[18] Although the question about what drives PLZF expression in immature cells committed to innate T cells remains to be answered, PLZF expression has been detected during double-negative stages of thymocytes.[19] This is also consistent with our observation of PLZF positivity in double-negative T-LBL/ALLs. However, it was somewhat puzzling that there were no PLZF (+) CD4 single-positive cases considering that up to 8% of fetal human CD4 single-positive thymocytes were positive for PLZF.[18] A couple of points should be taken into consideration when interpreting this data. First, only a few (just three) cases of CD4 single-positive T-LBL/ALLs were included in this study. Second, neoplastic cells have the potential to aberrantly express any markers compared with normal counterparts.

Other important features of PLZF (+) T-LBL/ALLs are mutually exclusive expressions between CD1a or T-cell receptor βF1 and PLZF, and strong association with initial bone marrow involvement. CD1a, a marker for double-positive cortical thymocytes, and T-cell receptor βF1, which expresses in late cortical and medullary thymocytes, were not found in any cases of PLZF (+) T-LBL/ALL. It was reported that lack of T-cell receptor β expression in cytoplasmic CD3 (+) T-acute lymphoblastic leukemias (ALLs) might represent immature T-ALLs of non-T-restricted precursor stage expressing immature markers such as CD34, CD13, and CD33, and

predominantly with double-negative and CD1a (−) phenotype.[31] PLZF expression was high in multipotent hematopoietic stem cells of bone marrow.[11] Based on these notions, we think that PLZF (+) T-LBL/ALLs might originate from thymocytes of early pro- or pre-T cell stage or more immature T-cell progenitors with double-negative phenotype and lacking CD1a and T-cell receptor βF1 expression, or immature $\gamma\delta$T cells. Therefore, purely on a theoretical basis, we suggest that a large number of T-LBL/ALL might in fact arise from double-negative stages of thymocytes and some of them aberrantly express either both CD4 and CD8 or single expression of CD4 and CD8 along their developmental process toward final maturation.

Of note, PLZF expression in NKT cells is elevated during the early stage of development and decreases as the cells mature.[16,17] In humans, the number of PLZF (+) thymocytes is high during the fetal stage and decreases in the postnatal period.[18] In a mouse system where all CD4 T cells were designed to express PLZF molecule, PLZF was detected in a portion of T-T CD4 T cells.[30] These observations suggest that although PLZF could be a specific marker for innate T cells, it is possible that many innate T cells gradually lose their PLZF expression when mature. Based on this, it is highly conceivable that PLZF expression in mature T-cell lymphomas is very rare and that the PLZF expression is frequent in T-LBL/ALLs in the double-negative phenotype, as in this study.

In this present study, only one peripheral T-cell lymphoma not otherwise specified and one mycosis fungoides patient expressed PLZF, but were negative for Vα24 according to RT-PCR (data not shown). Therefore, it is possible that these cases might represent a peripheral T-cell lymphoma of innate T-cell origin other than invariant NKT cells.

NK-cells, a representative type of innate lymphoid cells, are not known to express PLZF.[19] Consistently, none of the extranodal NK/T-cell lymphomas that predominantly originated from NK cells showed PLZF expression.

In several solid tumors and myeloid cell lines, PLZF inhibited cell proliferation and induced apoptosis.[8,12,13,32] In this study, T-LBL/ALL patients with any bone marrow involvement showed shorter event-free survival, which was consistent with previous report.[33] Although PLZF was a strong indicator for initial bone marrow involvement of any quantity in this study, PLZF expression itself had no influence on the survival of T-LBL/ALL patients. However, the heterogeneity of therapeutic modality restricted the accurate evaluation for prognostic implications of PLZF expression in T-LBL/ALLs.

In summary, we performed the first comprehensive investigation of the expression of PLZF, an innate T-cell determinant, in lymphoid malignancies. We showed that this protein is uniquely expressed in a considerable portion of T-LBL/ALL, but rarely in peripheral T-cell lymphomas. PLZF expression in T-lymphoma/leukemia suggests a possible origin in immature lymphocytes of innate T-cell lineage or early T-lymphoid precursors, and has important clinical implication regarding a greater probability of early bone marrow involvement.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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