Clinicopathological analysis of splenic red pulp low grade B-cell lymphoma

Short running title: BRAF mutation in SPLL-U in Japan

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List of abbreviations:

HCL, hairy cell leukemia

SPLL-U, splenic B-cell lymphoma/leukemia, unclassifiable

HCL-v, hairy cell leukemia-variant

SDRPL, splenic diffuse red pulp small B-cell lymphoma

SPLL-U-NS, narrow-sense SPLL-U

WHO, World Health Organization

SMZL, splenic marginal zone lymphoma

PCR, polymerase chain reaction

LCH, Langerhans cell histiocytosis

Abstract

Primary splenic low-grade B-cell lymphoma of the red pulp comprises hairy cell leukemia (HCL) and splenic B-cell lymphoma/leukemia, unclassifiable (SPLL-U). SPLL-U is a rare disease that includes subtypes of a hairy cell leukemia-variant (HCL-v), splenic diffuse red pulp small B-cell lymphoma (SDRPL), and other types that are known as narrow-sense SPLL-U (SPLL-U-NS). Notably, limited information is available regarding the *BRAF* mutation (V600E) and cyclin D3 expression in subtypes of SPLL-U. Therefore, we performed a pathological analysis of the *BRAF* mutation (V600E) and characterized pathological features of SPLL-U. We reviewed the pathological findings of 12 SPLL-U cases. The 12 cases considered included 2 cases of HCL-v, 6 cases of SPLL-U-NS, and 4 undetermined cases. The *BRAF* mutation (V600E) was detected in 3 cases, which were all SPLL-U-NS. Cases with the *BRAF* mutation (V600E) have increased levels of CD103 expression and decreased cyclin D3 and cyclin D1 expression compared with cases that lacked the *BRAF* mutation. These findings suggest that the *BRAF* mutation might play a significant role in SPLL-U. Therefore, the significance of the *BRAF* mutation should be evaluated via genomic or transcriptional analyses of a large cohort of SPLL-U patients.

Keywords: Splenic B-cell lymphoma/leukemia, unclassifiable; Hairy cell leukemia-

variant; *BRAF* mutation (V600E); cyclin D3; cyclin D1

1. Introduction

According to the revised 2017 World Health Organization (WHO) classification (4th edition), the main types of primary splenic B-cell lymphoma include splenic marginal zone lymphoma (SMZL), hairy cell leukemia (HCL), and splenic B-cell lymphoma/leukemia, unclassifiable (SPLL-U),[1] although rare subtypes have also been described.[2,3] Subtypes of SPLL-U include splenic diffuse red pulp small B-cell lymphoma (SDRPL) and hairy cell leukemia-variant (HCL-v).[1] Subtypes of SPLL-U that are not classified as SDRPL or HCL-v are known as narrow-sense SPLL-U (SPLL-U-NS).[1]

The parenchyma of the spleen is comprised of white and red pulp. In the white pulp, lymphocytes around the central artery form a cylindrical shape. The red pulp consists of the splenic cord and splenic sinusoid. In the splenic cord, there are lymphocytes, erythrocytes, macrophages, and dendritic cells. In SMZL, tumor cells infiltrate, and surround the germinal center of the white pulp of the spleen.[1] In contrast, HCL and SPLL-U are B-cell lymphomas, which infiltrate the red splenic marrow of the spleen.[1]

Whole-exome sequencing has revealed that the *BRAF* mutation (V600E) was present in all patients with HCL examined.[4] HCL cells were also positive for CD103 and CD11c when immunostained.[1] These are useful and highly specific diagnostic markers for HCL. In contrast, among SPLL-U types, more than half of the cases of SDRPL evaluated expressed cyclin D3, which was identified via immunohistochemical analysis.[5] However, there are few reports regarding the *BRAF* mutation (V600E) and cyclin D3 expression in patients with other subtypes of SPLL-U. Therefore, we performed a pathological analysis of the *BRAF* mutation (V600E) and characterized the pathological features of SPLL-U.

2. Methods

2.1 Patients and samples

We analyzed 12 patients who were diagnosed with SPLL-U using specimens obtained via splenectomy at Kurume University between April 2005 and February 2018. The diagnosis of each specimen was reviewed by two hematopathological experts (H.M. and K.O.) based on the revised 2017 WHO classification (4^{th} edition).[1] Cases of mantle cell lymphoma, follicular lymphoma, chronic lymphocytic leukemia/small cell lymphoma, marginal zone B-cell lymphoma, and HCL were excluded. Clinicians at each hospital determined whether hairy cell leukemia-like cells were present. Pathological findings, including the presence of hairy cells, immunohistochemical analyses, and *BRAF* mutational status were collected from the results of routine diagnostic procedures, which were recorded in medical charts. This study was carried out in accordance with the recommendations of the Declaration of Helsinki and approved by the ethics review committee of Kurume University.

2.2 Immunochemical staining

Tissue samples were processed as formalin-fixed, paraffin-embedded tissues, in accordance with standard institutional procedures. Antibodies (clones) used for immunohistochemistry included anti-CD20 (L-26; DakoCytomation, Glostrup, Denmark), anti-BCL2 (clone 124; DakoCytomation), anti-BCL6 (P1F6; Leica Microsystems, Wetzlar, Germany), anti-CD103 (EPR4166(2); Abcam, Cambridge, UK), anti-cyclin D3 (DCS22'; Abcam), anti-CD19 (LE-CD19; DakoCytomation), anti-CD11c (EP1347Y; Abcam), and anti-cyclin D1 (EP12; DakoCytomation). Tissue samples were considered positive if > 30% of the lymphoma cells were positive for these antibodies.

2.3 DNA isolation and analysis of the *BRAF* mutation

The mutational status of *BRAF* was determined using the Sanger technique.[6] DNA was prepared from whole sections of several paraffin-embedded tissues. DNA samples were extracted using a commercial kit (KAPA Express Extract kit, KAPA BIOSYSTEMS, Wilmington, MA, USA). The *BRAF* exon 15 region of the gene was amplified via semi-nested polymerase chain reaction (PCR) using KAPA2G Robust HotStart ReadyMix (for the first PCR; KAPA BIOSYSTEMS) and AmpliTaq Gold DNA Polymerase Master Mix (for the second PCR; Applied Biosystems, Foster City, CA, USA). The primer BRAF-15-RV was used as the anti-sense primer along with sense primers BRAF-15-FW (for the first PCR) and BRAF-15-FW2 (for the second PCR). The conditions used for the first PCR were as follows: initial denaturation occurred at 95°C for 3 min; followed by 40 cycles at 95°C for 15 s, 60°C for 15 s, and 72°C for 30 s; a final extension step occurred at 72°C for 10 min. The second PCR consisted of 40 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The primer sequences were as follows: sense primers, BRAF-15-FW (outer primer), 5'-taaactcttcataatgcttgctctgat-3'; and BRAF-15-FW2 (inner primer), 5'-cataatgcttgctctgataggaaaatgag-3'; and anti-sense primer BRAF-15-RV, 5'-aactcagcagcatctcagggccaa-3'. The amplified PCR products were evaluated on a 3% agarose gel, stained with ethidium bromide and visualized under ultraviolet light. The PCR product produced was approximately 241 bp.

Sanger sequencing was performed to confirm the presence of the *BRAF* V600E mutation. The sequencing reaction was carried out using the ABI BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems) and cycling parameters were as follows: 96°C for 1 min, followed by 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The

BRAF-15-FW2 primer was used for the sequencing reaction. The resulting products were run on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems).

3. Results

Fig. 1 includes a flowchart used to differentially diagnose primary splenic low-grade B-cell lymphoma, which was based on the 2017 WHO classification scheme. First, we evaluated whether the main lesion was located in the white pulp or red pulp. Cases in which primary lesions were located in the white pulp were classified as SMZL.[1] In cases where the primary locus of the lesion was in the red splenic cord, it was necessary to evaluate the presence or absence of neoplastic hairy cells, the *BRAF* mutation (V600E), and the presence of bone marrow infiltration.[1] If there were neoplastic hairy cells, cases with *BRAF* mutation (V600E) were classified as HCL; those without the *BRAF* mutation (V600E) were classified as HCL-v of SPLL-U. In cases without hairy cells, the diagnosis was made on the basis of the status of bone marrow infiltration. Cases in which tumor cells invaded the sinusoids in the bone marrow were classified as SDRPL of SPLL-U. Cases in which tumor cells did not show invasion of the sinusoidal pattern within bone marrow, and cases without bone marrow infiltration, were classified as SPLL-U-NS or SPLL-U. Based on these criteria, two cases (Cases 1 and 2) were diagnosed as HCL-v, 0 cases as SDRPL, and 6 cases (Cases 3–8) as SPLL-U-NS. The remaining four cases (Cases 9–12) could not be classified into subtypes because details necessary for the classification of the cases were unavailable.

Of the 12 cases described above, eight reported the prognoses of patients considered. Of the eight cases, only 1 died within the forceps period (Table 1, Fig. S1). The patient who died had been treated with Rituximab monotherapy after splenectomy;

however, a sufficient therapeutic effect could not be obtained. Later, salvage therapy was performed, but the disease could not be controlled, and the patient died 15.1 months after onset. There was no significant difference in OS between tissues with or without the BRAF mutation (V600E). Table 1 includes pathological findings and clinical data for the 12 cases considered. HCL-v (Cases 1 and 2) was negative for CD103 and cyclin D1, but it was positive for cyclin D3 and CD11c. Among the six cases of SPLL-U-NS identified, three had the *BRAF* mutation (V600E). Further, cyclin D3 expression was detected in two of the six cases, CD11c expression was detected in four, and cyclin D1 expression was identified in one. Moreover, in five of six SPLL-U-NS cases, cyclin D3 and/or cyclin D1 expression was detected, which seemed to be mutually exclusive. Fig. 2 shows histopathological images from Case 2 (HCL-v), Case 3 (SPLL-U-NS, BRAF mutation (V600E) (+)), and Case 6 (SPLL-U-NS, BRAF mutation (V600E) (-)).

Table 2 shows associations between the *BRAF* mutation and pathological features. Cases with the *BRAF* mutation (V600E) had higher levels of CD103 expression ~~(p = 0.018)~~, lower levels of cyclin D3 expression, and higher levels of cyclin D1 expression than cases that lacked the *BRAF* mutation.

Table 3 describes the treatment details. Clinical information of 8 cases was obtained. One patient only underwent splenectomy, two patients underwent splenectomy and rituximab monotherapy, two patients underwent splenectomy and R-CHOP therapy, and two patients underwent splenectomy and cladribine therapy. For the one patient who died of primary disease, splenectomy and rituximab monotherapy were performed, followed by bendamustine and EPOCH therapy. (Table3)

4. Discussion

This study examined the pathological characteristics, including the *BRAF* mutational status, of SPLL-U in Japan and its statistical association with CD103, cyclin D3, and CD11c expression. According to the 2017 WHO classification, the *BRAF* mutation (V600E) is a genetic abnormality defining HCL.[1] BRAF is a serine/threonine-phosphorylating enzyme that regulates MAPK signaling.[7] It has been reported that the *BRAF* mutation (V600E) may be found in melanoma and colon cancer, which comprise solid cancers rather than hematologic malignancies.[1,8] The *BRAF* mutation (V600E) has been reported in Langerhans cell histiocytosis (LCH) and Langerhans cell sarcoma in hematopoietic tumors other than HCL[9-11]; notably, the mutation has been reported in approximately 50% of LCH cases.[9-10] Further, there is no significant difference in the long-term prognoses of patients that either have or lack the *BRAF* mutation (V600E).[-12] In the present study examining the eight cases that included patient prognosis, the presence of the *BRAF* mutation (V600E) also did not significantly affect long-term prognosis. In the present study, three of six SPLL-U-NS cases had the *BRAF* mutation (V600E). However, because of the few cases of SPLL-U included in this study, which was a result of the rare occurrence of the disease, the results regarding the mutational analysis of *BRAF* require confirmation. Hence, a study should be performed using a larger cohort to verify the results of this study.

As per the definition set forth in the revised 2017 WHO classification (4th edition), HCL cells are defined as round and cytoplasm-rich cells with hair-like circumferential projections that invade peripheral blood, bone marrow, and splenic red pulp[1]. It has been reported that the hairy projections have a different pattern depending on the dry state of the smear slide and are observed in naturally dried specimens but

disappear in specimens prepared by forced drying [13]. In this study, the preparation method of specimens was determined by each medical facility. Although not all cases were evaluated using air-dried specimens, cases 3 and 4 did not show circumferential hairy projections typical of peripheral blood, bone marrow, and spleen sample cells. In this study, we diagnosed HCL only after the hairy cells were confirmed according to the revised 2017 WHO classification (4th edition). Therefore, cases 3 and 4 were classified as SPLL-U. In the future, we hope that the evaluation of naturally dried specimens will be unified in Japan as it is in Western countries.

The patient in case 5 had lesions only in the spleen, and smear preparation was difficult. SPLL-U was diagnosed because it was difficult to confirm the presence of hairy cells. In terms of surface markers, HCL is very difficult to distinguish. In the future, if peripheral blood or bone marrow infiltration is observed in case 5, it may be diagnosed as HCL.

Immunohistochemical analysis revealed that cyclin D3 was expressed in 24 of 33 cases of SDRPL. There have been no previous reports that have identified cyclin D3 expression in HCL-v or SPLL-U-NS. In the current study, cyclin D3 expression was observed in both cases of HCL-v and in two of the six cases of SPLL-U-NS considered. Nevertheless, cyclin D3 could not be used to differentially diagnose subtypes of SPLL-U, although further analysis of the expression of cyclin D3 in splenic lymphoma should be performed.

The *BRAF* mutation V600E in SPLL-U cases was associated with higher levels of CD103 and cyclin D1 expression, and with lower levels of cyclin D3 expression. The physiopathological mechanism by which *BRAF* mutations might induce or reduce expression of CD103, cyclin D3, or cyclin D1 is thus far unknown. In general, cases of

splenic low-grade B-cell lymphoma with red pulp lesions, the *BRAF* mutation, CD103 expression, and cyclin D1 expression, and without cyclin D3 expression are primarily considered to be HCL.[1] However, evidence of the presence of hairy cells in the cases was not found, which is essential for the diagnosis of HCL. Notably, these cases might indicate specific subtypes of lymphoma cells that are identical to HCL, regardless of the loss of the hairy cell morphology. Hence, detailed investigation at the genomic and transcriptional level, which considers clinical features of HCL, is needed.

Cases of SPLL-U-NS express either cyclin D1 or cyclin D3 in a mutually exclusive manner, and both proteins are involved in cell proliferation.[14-16] Activation of cyclin D1 has been reported post-activation of MAPK via protein kinase C.[14] The tumorigenesis of Burkitt lymphoma occurs via mutation of ID3-TCF3-Cyclin D3, which mediates MYC translocation.[15,17] Activation of cyclin

D3 activates mTOR signaling.[15] Moreover, a highly cytostatic effect was obtained by knocking down cyclin D3 in a pancreatic duct adenocarcinoma cell line with siRNA.[16] As the involvement of cyclin D1 and cyclin D3 in the tumorigenesis of SPLL-U has not been elucidated, further analysis is needed to determine the precise roles of the proteins.

In conclusion, the *BRAF* mutation may play a significant role in the progression of SPLL-U and HCL. The significance of the *BRAF* mutation in SPLL-U should be confirmed using a large cohort via genomic and transcriptional analyses.

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure Statement

The authors declare that they have no conflict of interest.

Author Contributions

That all authors have contributed significantly, and are in agreement with the content of the manuscript. H.M. and K.O. were responsible for conception and design; J.S. and K.K. provided study materials or patients; F.A. and T.F. performed collection and assembly of data; K.Y., E.Y., M.S., and H.S. and J.T. performed data analysis and interpretation; T.S. and H.M. wrote the manuscript; and all authors approved the final manuscript.

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Figure legends

Fig. 1 Flowchart showing the differential diagnosis for primary splenic low-grade B-cell lymphoma. First, we evaluated whether the main lesion is in the white pulp or red pulp. Cases with the primary locus of lesions in the white pulp were classified as splenic marginal zone lymphoma (SMZL). In cases where the main locus of the lesion was in the red splenic cord, it was necessary to evaluate the presence or absence of neoplastic hairy cells, the *BRAF* mutation (V600E), and the presence of bone marrow infiltration. If there were neoplastic hairy cells, cases with the *BRAF* mutation (V600E) were classified as hairy cell leukemia (HCL), while those without the *BRAF* mutation (V600E) were classified as hairy cell leukemia-variant (HCL-v). In cases without hairy cells, diagnosis was made on basis of the status of bone marrow infiltration. Cases in which tumor cells invaded the sinusoids in the bone marrow were classified as splenic diffuse red pulp small B-cell lymphoma (SDRPL). Cases in which tumor cells did not show invasion of the sinusoidal pattern in the bone marrow and cases without bone marrow infiltration were

classified as narrow sense splenic B-cell lymphoma/leukemia, unclassifiable (SPLL-U-NS).

Fig. 2 Pathological findings of splenic B-cell lymphoma/leukemia, unclassifiable (SPLL-U). Hematoxylin-eosin staining and immunohistochemical staining of CD103, cyclin D3, and cyclin D1. Case 2 (hairy cell leukemia variant) was negative for CD103 and cyclin D1, and was positive for cyclin D3. Case 3 (narrow sense splenic B-cell lymphoma/leukemia, unclassifiable [SPLL-U-NS] with the *BRAF* mutation) was positive for CD103 and cyclin D1, and was negative for cyclin D3. Case 6 (SPLL-U-NS without the *BRAF* mutation) was negative for CD103 and cyclin D1, and was positive for cyclin D3.

Supporting Information list:

Fig. S1 Overall survival (OS) of primary splenic low-grade B-cell lymphoma of the red pulp, with or without the BRAF mutation (V600E).

Fig.1

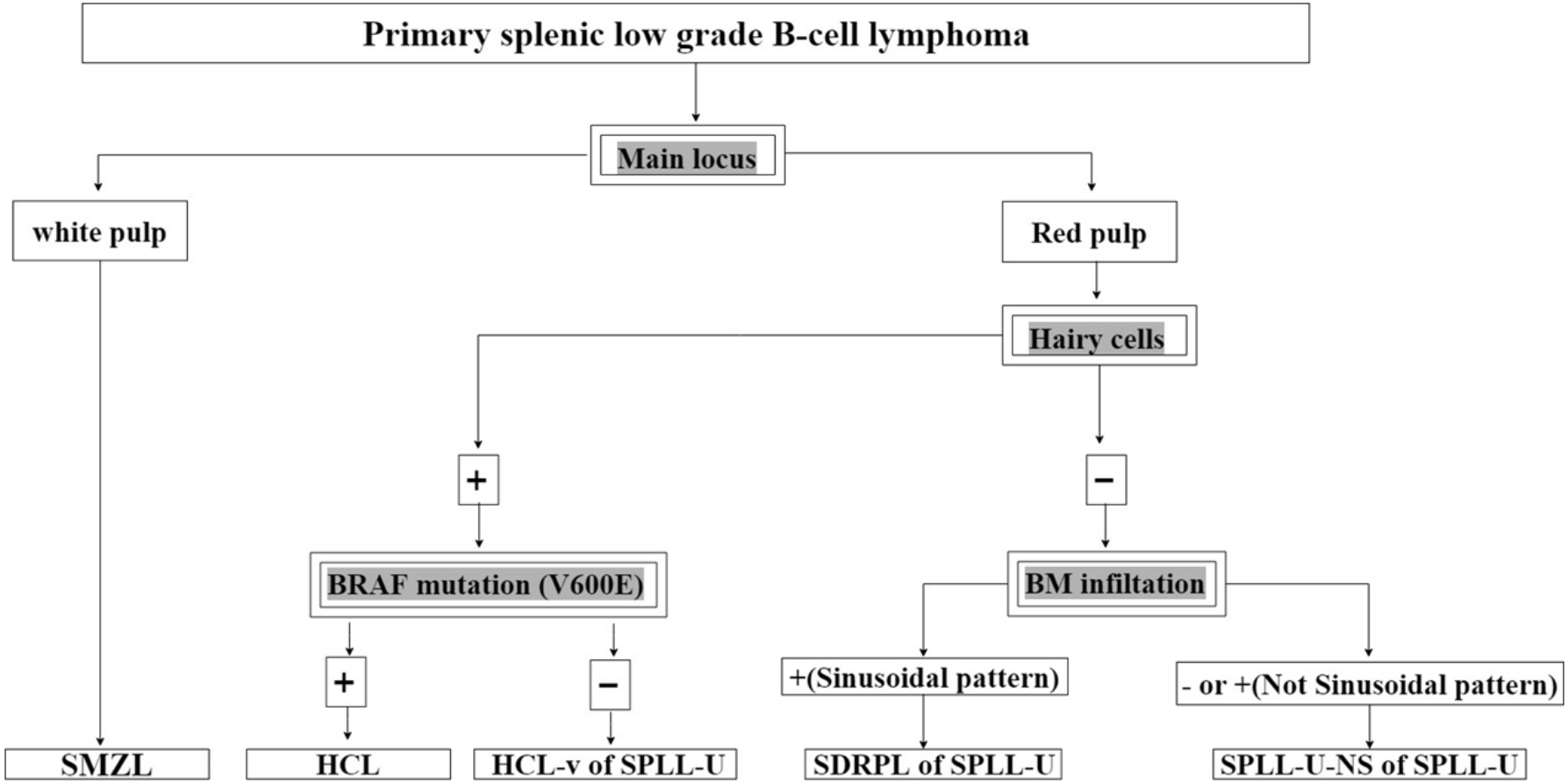
Primary splenic low grade B-cell lymphoma
Main locus
white pulp
Red pulp
Hairy cells
+
−
BRAF mutation (V600E)
BM infiltation
+
−
+(Sinusoidal pattern)
- or +(Not Sinusoidal pattern)
SMZL
HCL
HCL-v of SPLL-U
SDRPL of SPLL-U
SPLL-U-NS of SPLL-U

Fig.2

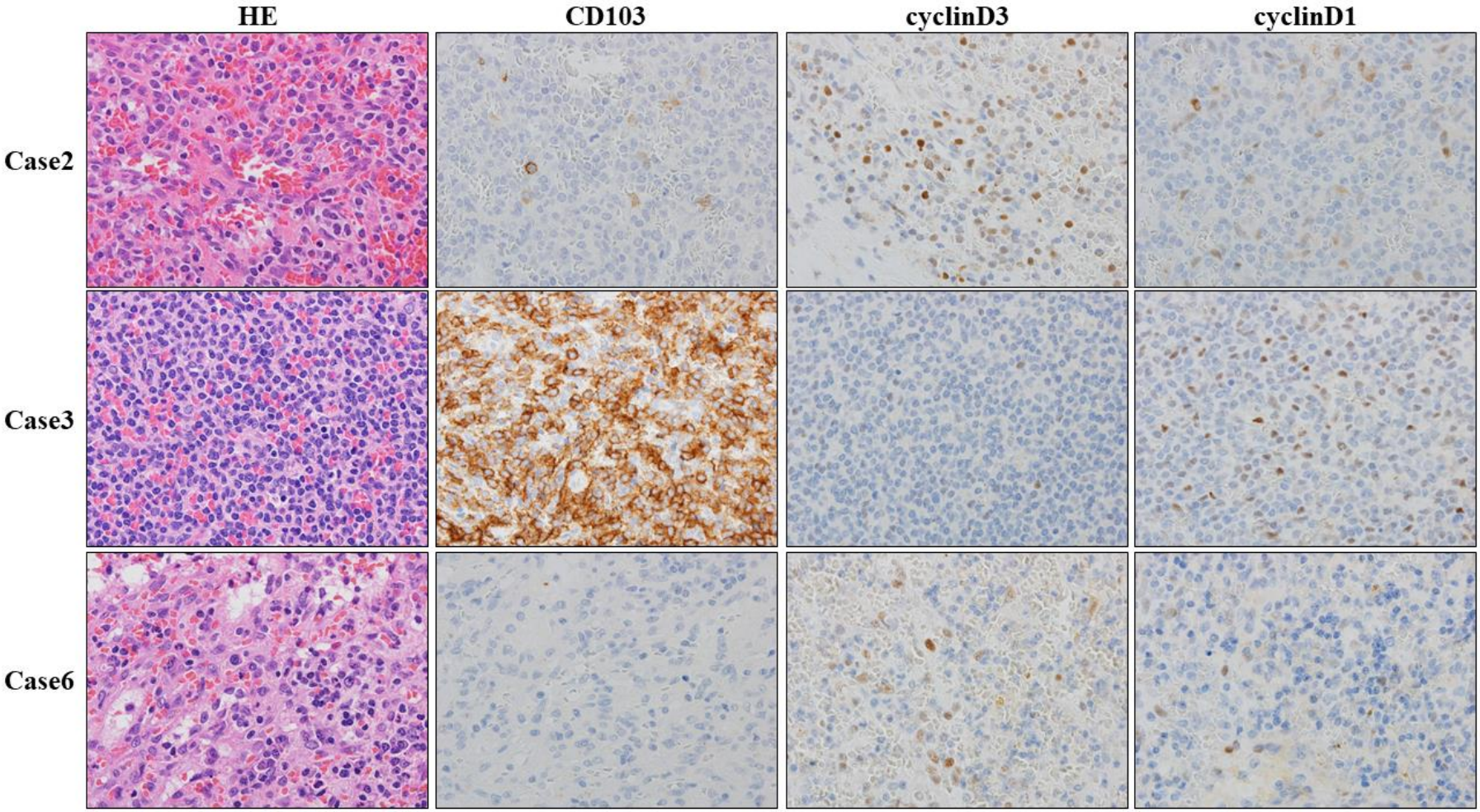

Fig. S1

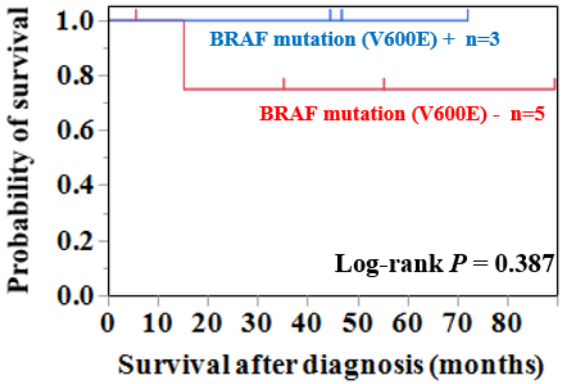
Probability of survival
1.0
0.8
0.6
0.4
0.2
0.0
BRAF mutation (V600E) + n=3
BRAF mutation (V600E) - n=5
Log-rank P = 0.387
0 10 20 30 40 50 60 70 80
Survival after diagnosis (months)

Table 1. Diagnostic findings and immunohistochemical analysis of primary splenic low grade B-cell lymphoma with red pulp lesion

Case No.	Age	Sex	Hairy cells	BM infiltation	BRAF mutation (V600E)	Diagnosis	CD103	CyclinD3	CD20	CD19	CD11c	CD5	CyclinD1	bcl-2	bcl-6	IPI	WBC (/μL)	Lymphocyte (/μL)	Hb (g/dL)	Plate ($\times 10^3$)	LDH higher than limit	sIL-2R(U/mL)	CRP (mg/dL)	Ann Arbor stage	Extranodal sites≧ 2	B-symptom	Hepatomegaly	ECOG Performance status	Alive	OS observation period (months)
1	56	F	+	+ (diffuse)	-	HCL-v	-	+	+	+	+	-	-	+	-	Low-intermediate risk	17610	13207	10.7	203	+	1211	0.66	Ⅳ	-	n.a.	-	n.a.	Alive	32.5
2	66	M	+	+ (diffuse)	-	HCL-v	-	+	+	+	+	-	-	+	-	n.a.	7000	3990	14.3	760	-	322	0.1	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
3	48	F	-	+ (diffuse)	+	SPLL-U-NS	+	-	+	+	+	-	+	+	-	n.a.	2630	1683	11.9	121	-	n.a.	0.02	n.a.	n.a.	n.a.	n.a.	n.a.	Alive	46.7
4	58	M	-	+ (diffuse)	+	SPLL-U-NS	+	-	+	+	+	-	+	-	-	Low-intermediate risk	2160	1121	9	157	+	11737	0.59	Ⅳ	-	-	-	0	Alive	44.4
5	73	F	-	-	+	SPLL-U-NS	+	-	+	+	+	-	-	n.a.	n.a.	Low risk	5200	1898	13.5	74	-	5633	0.08	Ⅰ	-	-	-	0	Alive	72.0
6	59	M	-	-	-	SPLL-U-NS	-	+	+	+	-	-	-	+	+	Low-intermediate risk	4500	1080	15.7	840	+	1500	n.a.	Ⅳ	-	-	-	1	Alive	35.1
7	71	M	-	+ (diffuse)	-	SPLL-U-NS	-	+	+	+	-	-	-	+	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
8	62	F	-	+ (diffuse)	-	SPLL-U-NS	-	-	+	+	+	+	-	+	-	Low-intermediate risk	3700	1813	7.1	75	+	2110	0.99	Ⅳ	-	-	-	1	Dead	15.1
9	72	F	-	+ (n.a.)	-	Undetermined (SDRPL vs SPLL-U-NS)	-	+	+	+	+	-	-	+	-	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
10	57	M	-	+ (n.a.)	-	Undetermined (SDRPL vs SPLL-U-NS)	+	+	+	+	-	-	-	+	-	High risk	2380	1261	7.2	191	-	4720	6.1	Ⅳ	-	+	-	2	Alive	89.4
11	74	F	-	n.a.	-	Undetermined (SDRPL vs SPLL-U-NS)	-	-	+	+	-	+	-	+	-	Low risk	6220	2457	9.4	103	-	2100	3.16	Ⅳ	-	+	-	1	n.a.	n.a.
12	63	F	n.a.	n.a.	-	Undetermined	-	+	+	+	-	-	-	-	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Alive	5.5

HCL-v, hairy cell leukemia variant in splenic B-cell lymphoma / leukemia, unclassifiable;

SDRPL, splenic diffuse red pulp small B-cell lymphoma in splenic B-cell lymphoma / leukemia, unclassifiable;

SPLL-U-NS, splenic B-cell lymphoma / leukemia, unclassifiable- narrow sense; n.a., not analyzed

Table 2. BRAF mutation and its association with pathological findings of SPLL-U

	BRAFV600E^{mut}	BRAF ^{wt}
Age, >60	1/3 (33.3%)	3/6 (66.7%)
Sex [M:F]	1:2	4:5
CD103	3/3 (100%)	1/9 (8.3%)
Cyclin D3	0/3 (0%)	7/9 (77.8%)
CD11c	3/3 (100%)	4/9 (44.4%)
Cyclin D1	2/3 (66.7%)	0/9 (0%)

Table 3. Treatments of primary splenic low grade B-cell lymphoma with red pulp lesion

Case No.	Age	Sex	Hairy cells	BM infiltation	BRAF mutation (V600E)	Diagnosis	Treatments
1	56	F	+	+ (diffuse)	-	HCL-v	n.a.
2	66	M	+	+ (diffuse)	-	HCL-v	Splenectomy only
3	48	F	-	+ (diffuse)	+	SPLL-U-NS	Splenectomy + Rituximab
4	58	M	-	+ (diffuse)	+	SPLL-U-NS	Splenectomy + Cladribine
5	73	F	-	-	+	SPLL-U-NS	Splenectomy + Rituximab
6	59	M	-	-	-	SPLL-U-NS	Splenectomy + R-CHOP
7	71	M	-	+ (diffuse)	-	SPLL-U-NS	n.a.
8	62	F	-	+ (diffuse)	-	SPLL-U-NS	Splenectomy +Rituximab→Benda→EPOCH
9	72	F	-	+ (n.a.)	-	Undetermined (SDRPL vs SPLL-U-NS)	n.a.
10	57	M	-	+ (n.a.)	-	Undetermined (SDRPL vs SPLL-U-NS)	Splenectomy + Cladribine
11	74	F	-	n.a.	-	Undetermined (SDRPL vs SPLL-U-NS)	n.a.
12	63	F	n.a.	n.a.	-	Undetermined	Splenectomy + R-CHOP

HCL-v, hairy cell leukemia variant in splenic B-cell lymphoma / leukemia, unclassifiable;

SDRPL, splenic diffuse red pulp small B-cell lymphoma in splenic B-cell lymphoma / leukemia, unclassifiable;

SPLL-U-NS, splenic B-cell lymphoma / leukemia, unclassifiable- narrow sense; n.a., not analyzed

R-CHOP, Rituximab + Cyclophosphamide + Hydroxydaunorubicin + vincristine + Prednisolone

Benda, Bendamustine; EPOCH, Etoposide + Prednisolone + vincristine + Cyclophosphamide + Hydroxydaunorubicin