

DUSP22 rearrangement is associated with a distinctive immunophenotype but not outcome in patients with systemic ALK-negative anaplastic large cell lymphoma

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Received: April 11, 2022.

Accepted: September 7, 2022.

Prepublished: December 1, 2022.

<https://doi.org/10.3324/haematol.2022.281222>

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Abstract

DUSP22 rearrangement (R) has been associated with a favorable outcome in systemic ALK-negative anaplastic large cell lymphoma (ALCL). However, a recent study found that patients with *DUSP22*-R ALK-negative ALCL have a poorer prognosis than was reported initially. In this study, we compared the clinicopathological features and outcomes of patients with ALK-negative ALCL with *DUSP22*-R (n=22) versus those without *DUSP22*-R (*DUSP22*-NR; n=59). Patients with *DUSP22*-R ALCL were younger than those with *DUSP22*-NR neoplasms ($P=0.049$). *DUSP22*-R ALK-negative ALCL cases were more often positive for CD15, CD8, and less frequently expressed pSTAT3^{Tyr705}, PD-L1, granzyme B and EMA (all $P<0.05$). *TP63* rearrangement (*TP63*-R) was detected in three of the 66 (5%) ALK-negative ALCL cases tested and none of these cases carried the *DUSP22*-R. Overall survival of patients with *DUSP22*-R ALCL was similar to that of the patients with *DUSP22*-NR neoplasms regardless of International Prognostic Index score, stage, age, or stem cell transplantation status (all $P>0.05$), but was significantly shorter than that of the patients with ALK-positive ALCL (median overall survival 53 months vs. undefined, $P=0.005$). Five-year overall survival rates were 40% for patients with *DUSP22*-R ALCL versus 82% for patients with ALK-positive ALCL. We conclude that *DUSP22*-R neoplasms represent a distinctive subset of ALK-negative ALCL. However, in this cohort *DUSP22*-R was not associated with a better clinical outcome. Therefore, we suggest that current treatment guidelines for this subset of ALK-negative ALCL patients should not be modified at present.

Introduction

Anaplastic large cell lymphoma (ALCL) is a mature T-cell neoplasm characterized by large pleomorphic neoplastic cells with kidney-shaped nuclei (so-called “hallmark” cells) and uniform, strong CD30 expression. Based on the presence or absence of an anaplastic lymphoma kinase gene (*ALK*) rearrangement and resultant ALK expression, ALCL is further classified into ALK-positive (+) and ALK-negative types.¹ Although ALK-negative ALCL is morphologically indistinguishable from ALK+ ALCL, patients with systemic ALK-negative ALCL are usually older and have a more aggressive clinical course and poorer outcome with 5-year overall survival (OS) rates of <50% compared with 80-90% for patients with ALK+ ALCL.²⁻⁷

ALK-negative ALCL is a genetically heterogeneous entity, with 13-30% of cases harboring a *DUSP22* rearrangement (R) and 2-8% of cases carrying a *TP63*-R.^{6,8-11} *TP63*-R and

DUSP22-R are nearly mutually exclusive. *DUSP22*, also known as c-Jun N-terminal kinase (JNK) pathway-associated phosphatase (JKAP), is a tumor suppressor gene located on chromosome 6p25.3. *DUSP22* encodes the dual-specificity phosphatase-22 which plays a role in inhibiting T-cell receptor (TCR) signaling.^{12,13} *DUSP22* knock-out enhances T-cell activation and TCR signaling and produces enhanced T-cell-mediated immune responses in a mouse model.¹² Restoring expression of *DUSP22* in *DUSP22*-deficient malignant T cells inhibits cellular expansion by stimulating apoptosis and impairs clonogenicity and tumorigenicity.¹³ *DUSP22*-R often results from t(6;7)(p25.3;q32.3) and is associated with as much as a 50-fold reduction of *DUSP22* expression.¹⁴ *DUSP22*-R ALK-negative ALCL cases appear to represent a distinctive subset of ALK-negative ALCL cases with unique morphology, immunophenotype, and molecular signature.^{9,11,15-17} *TP63* is located at chromosome 3q28. *TP63*-R results in a

p63 fusion protein with structural homology to oncogenic Δ Np63. Among ALK-negative ALCL patients, the subgroup with *TP63*-R neoplasms has a poor prognosis.⁶

The prognostic significance of *DUSP22*-R in ALK-negative ALCL is currently controversial. A Mayo Clinic group initially reported that patients with *DUSP22*-R ALK-negative ALCL (n=22) have a favorable clinical outcome with a 5-year OS rate of 90%, similar to that of ALK+ ALCL patients.⁶ Later on, two small studies of five and four patients, respectively, showed a similarly favorable prognosis in patients with *DUSP22*-R ALCL.^{9,10} Although the case numbers were limited in these studies, others have wondered if the treatment guidelines for patients with *DUSP22*-R ALK-negative ALCL should be modified. However, a recent study by Hapgood and colleagues reported a 5-year OS of 40% for their cohort of 12 patients with *DUSP22*-R ALK-negative ALCL.¹¹

In this study, we used fluorescence *in situ* hybridization (FISH) to determine the status of *DUSP22* and *TP63* in cases of ALK-negative ALCL. We further focused on *DUSP22*-R ALK-negative ALCL cases to characterize their clinicopathological and immunophenotypic features and the patients' outcomes.

Methods

Case selection

We searched the database of the Department of Hematopathology at the MD Anderson Cancer Center from January 1, 2007 through December 31, 2021 for cases of systemic ALK-negative ALCL. The diagnosis of ALK-negative ALCL was based on criteria specified in the 5th edition of the World Health Organization classification.¹ The following cases were considered as primary cutaneous ALK-negative ALCL and were excluded from the study: (i) patients with cutaneous disease alone without extracutaneous involvement; and (ii) patients with concurrent cutaneous disease and involvement of regional lymph nodes but no other extracutaneous involvement.^{1,18} For comparison of survival, we compared this cohort with a group of patients with ALK+ ALCL seen at our institution during the same time interval. Some data for the group of ALK+ ALCL patients have been published previously.¹⁹ Clinical information was obtained by review of medical records. This study was approved by the Institutional Review Board.

Immunophenotypic analysis

Immunohistochemical studies were performed as described previously.²⁰ The antibodies used were specific for: ALK1, BCL2, CD2, CD3, CD4, CD5, CD7, CD8, CD15, CD20, CD30, CD43, CD45, CD56, EMA, granzyme B, Ki-67, MUM1, MYC, PAX5, PD-L1, perforin, phospho-STAT3^{Tyr705}, and TIA1.

The percentages of lymphoma cells positive for CD15, phospho-STAT3^{Tyr705} and PD-L1 were read by two hematopathologists (JX and SL) with estimation to the closest 5%, and then the average of the two reads was used for the final reading of each case.

Flow cytometry immunophenotypic analysis was performed using either a FACSCanto II or a FACSCalibur cytometer (Becton-Dickinson Biosciences, San Jose, CA, USA) as described previously.²¹ The panel of antibodies employed included CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD25, CD30, CD45, CD52, CD56, TCR α/β and TCR γ/δ (Becton-Dickinson Biosciences, San Jose, CA, USA).

Fluorescence *in situ* hybridization

FISH analysis was performed on formalin-fixed, paraffin-embedded tissue sections using *IRF4/DUSP22* dual-color break-apart probes (3'*IRF4/DUSP22* – centromeric, labeled with red; 5'*IRF4/DUSP22* – telomeric, labeled with green; CytoTest, Rockville, MD, USA) and *TP63* dual-color break-apart probes (Cytocell, Cambridge, UK) according to the manufacturers' instructions. Two hundred interphase nuclei were analyzed.²² The cutoff value was 10.7% for the *IRF4/DUSP22*-R and 11.1% for the *TP63*-R. *IRF4/DUSP22*-R and *TP63*-R were assessed in a blinded fashion without knowledge of the pathological diagnosis or results of other FISH analyses.

Statistical analysis

Statistical analyses were performed using Graph-Pad Prism 8 and SPSS 26.0 software (IBM Corporation, Armonk, NY, USA). The Fisher exact test was used to compare clinicopathological features between the *DUSP22*-R and *DUSP22*-NR groups in patients with ALK-negative ALCL. OS was calculated from the date of initial diagnosis to the date of death or last follow-up. Progression-free survival was calculated from the date of diagnosis to the date of progression/relapse or, if no progression/relapse, the date of death or last follow-up. Survival was analyzed using the Kaplan-Meier method and was compared using the log rank test. A *P* value of less than 0.05 was considered statistically significant.

Results

Clinical and fluorescence *in situ* hybridization findings

The results of FISH analysis showed that a *DUSP22*-R was present in 22 (28%) cases (*DUSP22*-R group), and absent in 59 (72%) cases (*DUSP22*-NR group). The clinical features of these patients are summarized in Table 1. The *DUSP22*-R group comprised 15 men and seven women with a median age of 52 years (range, 33–79 years) at the time of diagnosis. Six (40%) patients with available data had B symptoms. Lymphadenopathy was identified in 16 of 17

(94%) patients, and nine of 15 (60%) patients had extranodal involvement including skin (6 cases; 40%), bone (3 cases; 20%), soft tissue (2 cases; 13%), liver (1 case; 7%), lung (1 case; 7%), and muscle (1 case; 7%). Peripheral blood and bone marrow involvement were seen in one of eight (13%) and one of 18 (6%) patients, respectively. Fifteen patients were fully staged, and 13 (87%) had stage III or IV disease. Five of 15 (33%) patients had an International Prognostic Index (IPI) score of ≥ 3 . One of 14 (7%) patients had leukocytosis and one of 13 (8%) patients had absolute lymphocytosis. Anemia was observed in seven of 14 (50%) patients and thrombocytopenia was present in two of 14 (14%) patients. An elevated serum lactate dehydrogenase level was detected in seven of 14 (50%) patients.

The *DUSP22*-NR group consisted of 38 men and 21 women with a median age of 61 years (range, 21-95 years) at the time of diagnosis. Twenty-seven (63%) patients had B symptoms. Forty-one of 51 (80%) patients had lymphadenopathy and 34 of 45 (76%) patients had extranodal disease. The involved extranodal sites included lung (10 cases; 22%), skin (9 cases; 20%), bone (7 cases; 16%), liver (6 cases; 13%), soft tissue (5 cases; 11%), gingiva/oropharynx/nasopharynx (3 cases; 7%), spleen (2 cases; 4%), muscle (2 cases; 4%), kidney (1 case; 2%), and stomach (1 case; 2%). Peripheral blood and bone marrow involvement were seen in four of 18 (22%) cases and eight of 46 (17%) patients, respectively. Forty-one patients were fully staged, and 27 (66%) had stage III or IV disease. Eighteen of 36 (50%) patients had an IPI scores of ≥ 3 . Seven of 31 (23%) patients had leukocytosis and no patients (of 30) had absolute lymphocytosis. Anemia was observed in 20 of 31 (65%) patients and thrombocytopenia was present in 4 of 31 (13%) patients. An elevated serum lactate dehydrogenase level was detected in 16 of 26 (62%) patients. Compared to patients in the *DUSP22*-NR ALK-negative ALCL group, patients with *DUSP22*-R neoplasms were

fully staged, and 27 (66%) had stage III or IV disease. Eighteen of 36 (50%) patients had an IPI scores of ≥ 3 . Seven of 31 (23%) patients had leukocytosis and no patients (of 30) had absolute lymphocytosis. Anemia was observed in 20 of 31 (65%) patients and thrombocytopenia was present in 4 of 31 (13%) patients. An elevated serum lactate dehydrogenase level was detected in 16 of 26 (62%) patients. Compared to patients in the *DUSP22*-NR ALK-negative ALCL group, patients with *DUSP22*-R neoplasms were

Table 1. Clinical features of patients with ALK-negative anaplastic large cell lymphoma with or without a *DUSP22*-rearrangement.

	Total (N=81)	<i>DUSP22</i> -R (N=22)	<i>DUSP22</i> -NR (N=59)	P value
Clinical features at diagnosis				
Male:female (N/N)	1.9:1 (53/28)	2:1 (15/7)	1.8:1 (38/21)	0.80
Age in years, median (range)	60 (21-95)	52 (33-79)	61 (21-95)	0.049
B symptoms, % (P/T tested)	57 (33/58)	40 (6/15)	63 (27/43)	0.14
Lymphadenopathy at diagnosis, % (P/T tested)	84 (57/68)	94 (16/17)	80 (41/51)	0.27
Extra-nodal involvement, % (P/T tested)	72 (43/60)	60 (9/15)	76 (34/45)	0.32
Peripheral blood involvement, % (P/T tested)	19 (5/26)	13 (1/8)	22 (4/18)	1.00
Bone marrow involvement, % (P/T tested)	14 (9/64)	6 (1/18)	17 (8/46)	0.43
Stage III or IV, % (P/T tested)	71 (40/56)	87 (13/15)	66 (27/41)	0.19
IPI ≥ 3 , % (P/T tested)	45 (23/51)	33 (5/15)	50 (18/36)	0.36
Laboratory findings at diagnosis, % (P/T tested)				
Elevated WBC, $>11 \times 10^9/L$	18 (8/45)	7 (1/14)	23 (7/31)	0.40
Absolute lymphocytosis, $>4.8 \times 10^9/L$	2 (1/43)	8 (1/13)	0 (0/30)	0.30
Anemia*	60 (27/45)	50 (7/14)	65 (20/31)	0.51
Thrombocytopenia, $<140 \times 10^9/L$	13 (6/45)	14 (2/14)	13 (4/31)	1.00
Elevated LDH	58 (23/40)	50 (7/14)	62 (16/26)	0.52
<i>TP63</i> rearrangement by FISH, % (P/T tested)	5 (3/66)	0 (0/19)	6 (3/47)	0.55
Initial treatment, % (P/T tested)				
CHOP or modified CHOP	90 (55/61)	81 (13/16)	93 (42/45)	0.18
Others	10 (6/61)	19 (3/16)	7 (3/45)	
Initial CR, % (P/T tested)	54 (32/59)	75 (12/16)	47 (20/43)	0.08
Relapse, % (P/T tested)	52 (28/54)	43 (6/14)	55 (22/40)	0.54
SCT+, % (P/T tested)	29 (15/51)	36 (5/14)	27 (10/37)	0.73
Outcome				
Alive, % (P/T tested)	54 (36/67)	50 (9/18)	55 (27/49)	0.79
Dead, % (P/T tested)	46 (31/67)	50 (9/18)	45 (22/49)	
OS, months, median (range)	36 (1.1-94)	53 (8-85)	36 (1.1-94)	0.64

DUSP22-R: with *DUSP22* rearrangement; *DUSP22*-NR: without *DUSP22* rearrangement; IPI: International Prognostic Index; WBC: white blood cell; *female <12.0 g/dL, male <14.0 g/dL; LDH: lactate dehydrogenase; FISH: fluorescence *in situ* hybridization; CHOP: cyclophosphamide, doxorubicin, vincristine, and prednisone; CR: complete response; SCT: stem cell transplant; OS: overall survival. Bold: statistically significant, i.e., $P < 0.05$. P/T tested: positive/total cases tested.

younger (median age 52 vs. 61 years, $P=0.049$). There were no other significant differences in clinical features between patients in these two groups (all $P>0.05$) (Table 1).

***TP63* rearrangement by fluorescence *in situ* hybridization analysis**

FISH analysis was performed to evaluate the status of *TP63*-R in 66 cases (19 *DUSP22*-R and 47 *DUSP22*-NR) with material available. Three (~5%) cases were positive for *TP63*-R and all were in the *DUSP22*-NR group (Table 1). None ($n=19$) of the *DUSP22*-R ALK-negative ALCL cases tested carried a *TP63*-R. The difference in the frequency of *TP63*-R between the *DUSP22*-R and *DUSP22*-NR groups was not statistically significant ($P=0.55$).

Morphological and immunophenotypic findings

DUSP22-NR ALK-negative ALCL cases in tissue specimens have morphological features of the so-called “common pattern” described in ALK+ ALCL. In comparison, the lymphoma cells in *DUSP22*-R neoplasms were relatively more monotonous in appearance, smaller, and more often had central nuclear pseudoinclusions (“doughnut” cells). Hallmark cells were present in all cases of *DUSP22*-R ALCL, but large pleomorphic cells were seen only occasionally. A *DUSP22*-R ALK-negative ALCL case is shown in Figure 1

(lymph node) and Figure 2 (bone marrow and peripheral blood).

The lymphoma cells of *DUSP22*-R ALK-negative ALCL cases were positive for CD45 (12/13; 92%), BCL-2 (6/7; 86%), CD43 (5/6; 83%), CD2 (13/17; 77%), CD3 (16/21; 76%) and CD4 (14/19; 74%) (Table 2 and Figure 3). Expression of other T-cell-associated antigens was less frequent: TCR α/β (3/7; 43%), CD52 (2/5; 40%), CD5 (6/17; 35%), CD25 (2/6; 33%), CD7 (2/7; 29%) and CD8 (5/18; 28%). Cytotoxic markers were positive in only a few cases of *DUSP22*-R ALK-negative ALCL including TIA1 (3/10; 30%) and perforin (1/4; 25%) and granzyme B was consistently negative ($n=12$). Small subsets of cases were positive for EMA (2/11; 18%) and CD56 (1/12; 8%). All seven cases examined for TCR γ/δ were negative. The proliferation index as assessed by Ki67 was high (~90%).

CD15 was positive in 12 of 15 (80%) *DUSP22*-R cases examined. A mean of 26% lymphoma cells in *DUSP22*-R cases were positive for CD15. Three types of CD15 staining pattern were observed: a Golgi-like (perinuclear dot) pattern, a membranous/cytoplasmic pattern, and a combination of the Golgi and membranous/cytoplasmic patterns (Figure 4).

The immunophenotype of *DUSP22*-NR ALK-negative ALCL cases was similar except for the following significant dif-

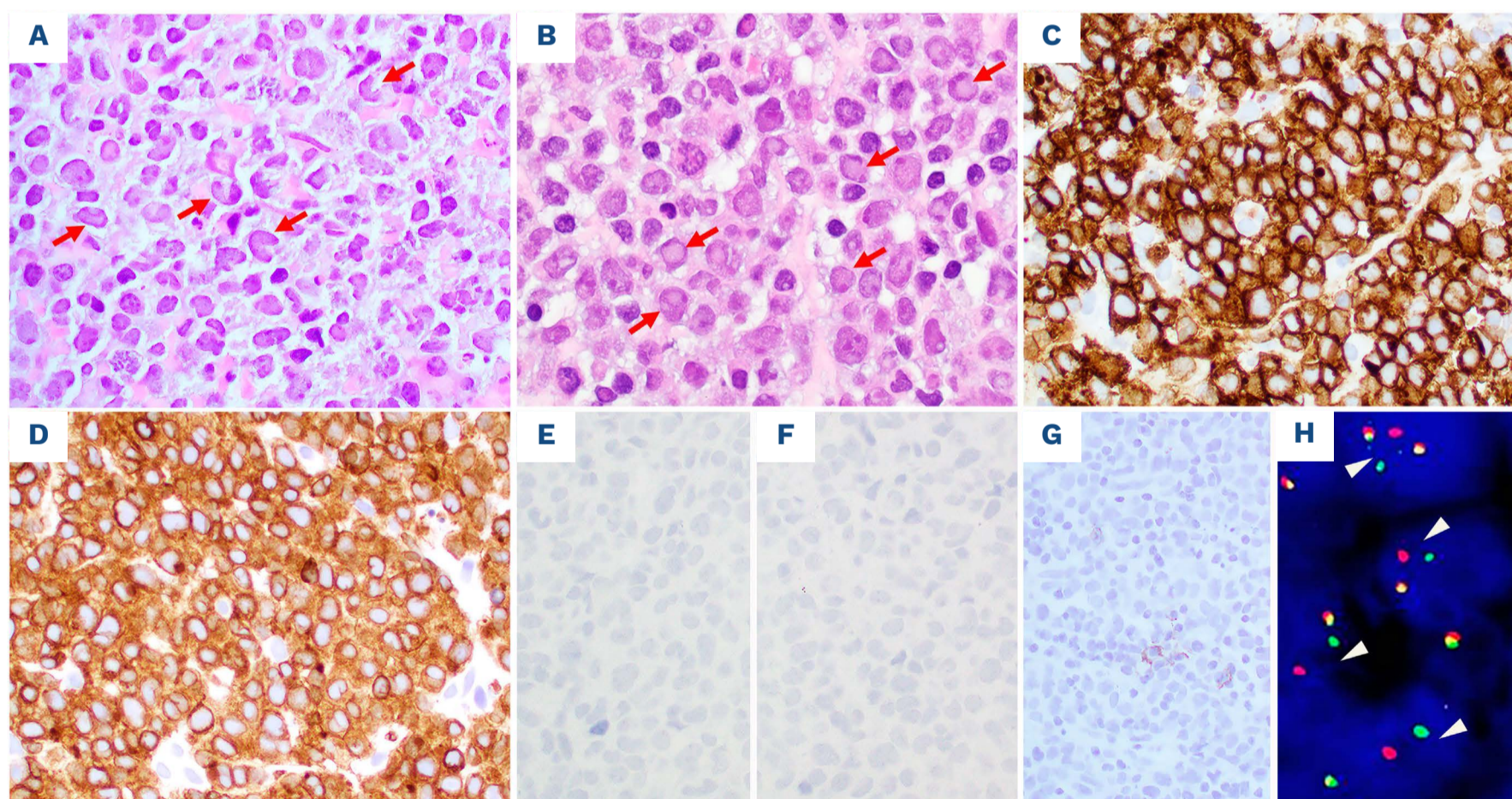


Figure 1. Lymph node biopsy from a case with *DUSP22*-rearranged ALK-negative anaplastic large cell lymphoma. (A, B) The nodal architecture is effaced by sheets of monotonous, intermediate sized lymphoma cells. Some lymphoma cells show kidney-shaped nuclei (hallmark cells) (arrows, A) or central nuclear pseudoinclusions (“doughnut” cells) (arrows, B). (C-G) The lymphoma cells are strongly and diffusely positive for CD30 (C) and CD3 (D), and are negative for ALK1 (E), granzyme B (F) and EMA (G). (H) Fluorescence *in situ* hybridization analysis using *IRF4/DUSP22* break-apart probes, $\times 600$. The nuclei showing a *DUSP22* rearrangement (with red and green split signals) are indicated by white arrow heads. (A, B) Hematoxylin-eosin stain, $\times 600$ (A) and $\times 600$ (B). (C-G) Immunohistochemistry, $\times 400$.

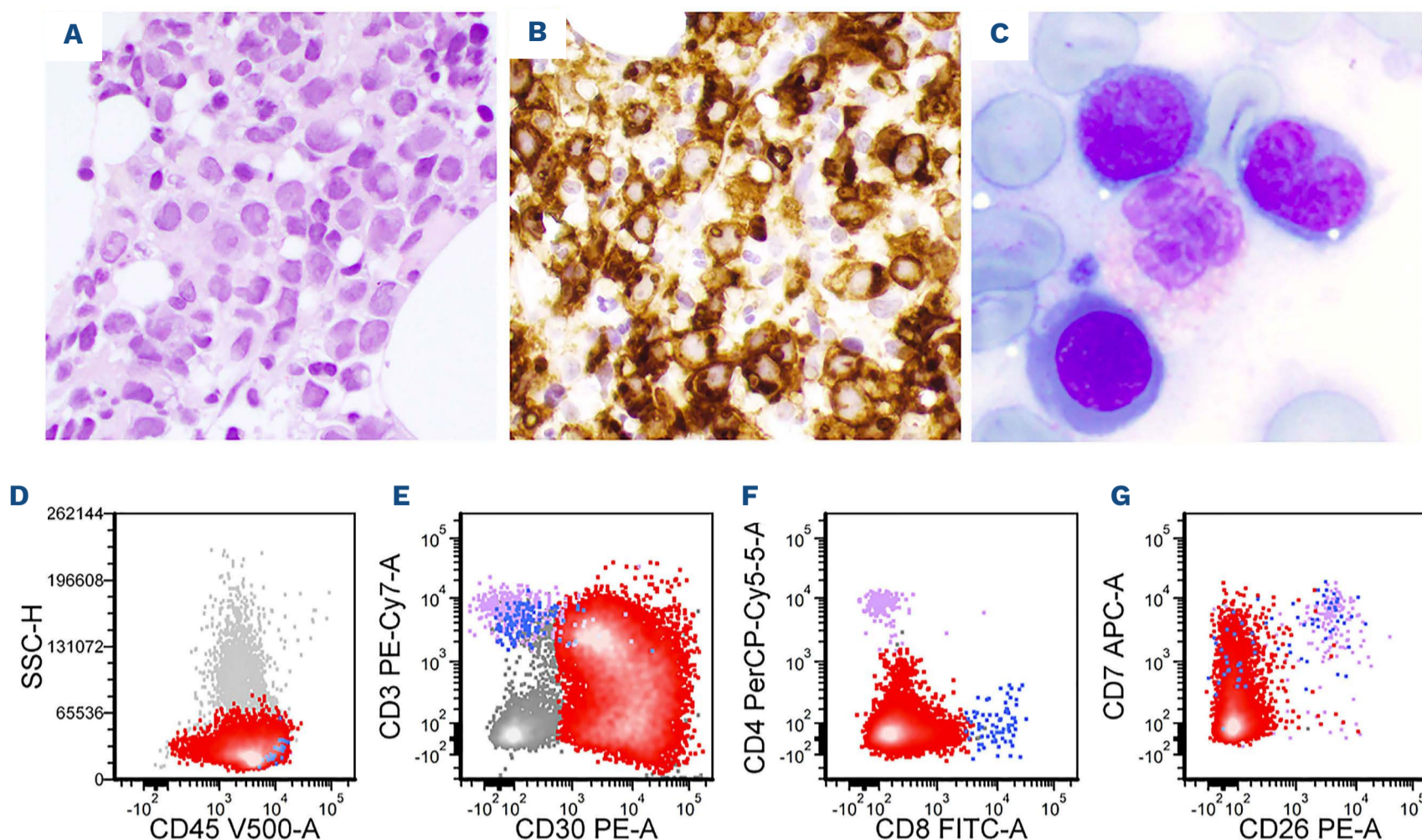


Figure 2. The *DUSP22*-rearranged ALK-negative anaplastic large cell lymphoma shown in Figure 1 also involves bone marrow and peripheral blood. (A) This bone marrow core biopsy specimen shows a hypercellular bone marrow infiltrated by lymphoma cells in an interstitial pattern. The lymphoma cells are morphologically similar to those in the lymph node described in Figure 1. (B) The lymphoma cells in the bone marrow are positive for CD30 by immunohistochemistry. (C) Bone marrow aspirate smear showing lymphoma cells, mostly small to intermediate size, with irregular nuclear contours and basophilic cytoplasm. A lymphoma cell shows kidney-shaped nuclei. (D-G) Flow cytometric immunophenotypic analysis of the peripheral blood shows a large population (75%) of lymphoma cells (red dots) which are positive for CD45 (D), CD30 (E), CD3 (partial/decreased, E), CD8 (dim, F), and CD7 (partial, G), and negative for CD4 and CD26, immunophenotypically similar to the lymphoma cells in the lymph node described in Figure 2. The purple and blue dots represent background benign CD4⁺ and CD8⁺ T cells, respectively. (A) Hematoxylin-eosin stain, x500. (B) Immunohistochemistry, x500. (C) Wright-Giemsa stain, x1000.

ferences that included CD15 being usually negative (9% vs. 80%, $P=0.0001$), lower frequency of CD8 (7% vs. 28%, $P=0.045$), and more common expression of granzyme B (65% vs. 0%, $P<0.0001$) and EMA (57% vs. 18%, $P=0.04$).

STAT3 activation and PD-L1 expression

Activation of STAT3 was examined by assessing nuclear expression of phosphorylated STAT3 (pSTAT3^{Tyr705}). A mean of 2% lymphoma cells in *DUSP22*-R ALK-negative ALCL cases showed nuclear staining for pSTAT3^{Tyr705}, a value significantly lower than the mean of 36% observed in *DUSP22*-NR tumors ($P=0.001$) (Figure 5A-C). PD-L1, a downstream molecule regulated by the JAK/STAT3 signaling pathway, was positive in 3% lymphoma cells in the *DUSP22*-R ALCL group compared to 26% positive cells in *DUSP22*-NR tumors ($P=0.01$) (Figure 5D-F).

Treatment and response

Treatment information was available for 16 patients with *DUSP22*-R ALK-negative ALCL and 45 patients with *DUSP22*-NR neoplasms. All patients were treated with chemotherapy regimens over the time interval of this

study, with or without consolidation with stem cell transplant (SCT). Fifty-five of 61 (90%) patients were treated with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or modified CHOP: 13 of 16 (81%) patients in the *DUSP22*-R group and 42 of 45 cases (93%) in the *DUSP22*-NR group. Of the six patients treated with non-CHOP-based chemotherapy regimens, four received brentuximab vedotin-based therapy, one received ifosfamide, carboplatin and etoposide (ICE), and one received etoposide, methylprednisolone, high-dose cytarabine and cisplatin (ESHAP) in combination with gemcitabine and vinorelbine. After initial induction chemotherapy, 12 of 16 (75%) patients in the *DUSP22*-R group and 20 of 43 patients (47%) in the *DUSP22*-NR group achieved complete remission. Patients with *DUSP22*-R ALCL tended to have a higher initial complete response rate than the patients with *DUSP22*-NR neoplasms, but this difference did not reach statistical significance ($P=0.08$). Five of 14 (36%) patients in the *DUSP22*-R group and ten of 37 (27%) patients in the *DUSP22*-NR group underwent SCT. There was no significant difference in initial treatment or SCT rates between

Table 2. Immunophenotypic features of ALK-negative anaplastic large cell lymphoma with or without a *DUSP22*-rearrangement

Immunophenotype	Total (N=81)	<i>DUSP22</i> -R (N=22)	<i>DUSP22</i> -NR (N=59)	P value
	% (Positive/Total tested)			
CD2+	72 (38/53)	77 (13/17)	69 (25/36)	0.75
CD3+	60 (47/78)	76 (16/21)	55 (31/57)	0.12
CD4+	82 (56/68)	74 (14/19)	86 (42/49)	0.29
CD5+	45 (28/62)	35 (6/17)	49 (22/45)	0.40
CD7+	30 (7/23)	29 (2/7)	31 (5/16)	1.00
CD8+	13 (8/60)	28 (5/18)	7 (3/42)	0.045
CD15+	31 (15/48)	80 (12/15)	9 (3/33)	0.0001
CD25+	60 (9/15)	33 (2/6)	78 (7/9)	0.14
CD30+	100 (81/81)	100 (22/22)	100 (59/59)	1.00
CD43+	87 (26/30)	83 (5/6)	88 (21/24)	1.00
CD45+	81 (39/48)	92 (12/13)	77 (27/35)	0.41
CD52+	7 (2/29)	40 (2/5)	0 (0/24)	0.44
CD56+	10 (3/31)	8 (1/12)	11 (2/19)	1.00
TCR αβ+	55 (18/33)	43 (3/7)	58 (15/26)	1.00
TCR γδ+	0 (0/17)	0 (0/7)	0 (0/10)	1.00
BCL-2+	75 (15/20)	86 (6/7)	69 (9/13)	0.61
Granzyme B+	41 (13/32)	0 (0/12)	65 (13/20)	<0.0001
TIA1+	50 (18/36)	30 (3/10)	58 (15/26)	0.26
Perforin+	50 (5/10)	25 (1/4)	83 (5/6)	0.19
EMA+	46 (18/39)	18 (2/11)	57 (16/28)	0.04
MUM1+	91 (21/23)	100 (7/7)	88 (14/16)	1.00
MYC+	43 (22/51)	41 (7/17)	44 (15/34)	0.54
Mean Ki-67, % (N)	88 (35)	91 (11)	85 (24)	0.16

DUSP22-R: with *DUSP22* rearrangement; *DUSP22*-NR: without *DUSP22* rearrangement. The cutoff value for CD15 positivity was ≥5%. Bold: statistically significant, i.e., $P < 0.05$

patients with *DUSP22*-R or *DUSP22*-NR ALK-negative ALCL (all $P > 0.05$) (Table 1).

Outcome

After a median follow-up of 19.2 months (range, 1.1–94 months), 31 of 67 (46%) patients with clinical follow-up data died, including nine of 18 (50%) patients in the *DUSP22*-R group and 22 of 49 (45%) patients in the *DUSP22*-NR group. In the *DUSP22*-NR group, two of three (67%) patients carrying a *TP63*-R died. Comparing the patients with *DUSP22*-R, *DUSP22*-NR/*TP63*-NR (so called “triple-negative”), and *DUSP22*-NR/*TP63*-R ALK-negative ALCL, no significant differences were observed in OS (median 53 vs. 36 vs. 21 months, all $P > 0.05$) or in progression-free survival (median 53 vs. 20 vs. 9 months, all $P > 0.05$) between these groups (Figure 6A, B). Although the patients with *TP63*-R neoplasms tended to have shorter progression-free survival than those without *TP63*-R, this difference did not reach statistical significance. After excluding the *TP63*-R cases, the OS of the *DUSP22*-R and *DUSP22*-NR groups was further compared after stratifying patients by IPI score (≥3 or <3), clinical stage (stage III/IV or I/II), age (≥50 or <50 years [Figure 6C]; ≥40 or <40 years,

data not shown; ≥60 or <60 years, *data not shown*), and SCT status, there were still no significant differences in OS between the *DUSP22*-R and *DUSP22*-NR groups (all $P > 0.05$) (Figure 6C–J).

We also compared the OS of patients with *DUSP22*-R ALK-negative ALCL to that of a group of patients with ALK+ ALCL. The OS of patients with *DUSP22*-R ALK-negative ALCL was significantly poorer than that of patients with ALK+ ALCL (median OS 53 months vs. undefined, $P = 0.005$) (Figure 6A). The 5-year OS rates were 40% for patients with *DUSP22*-R ALK-negative ALCL as compared to 82% for patients with ALK+ ALCL.

Discussion

The *DUSP22*-R has been reported most often in cases of systemic ALK-negative ALCL and primary cutaneous ALCL, occasionally in lymphomatoid papulosis and rarely in peripheral T-cell lymphoma, not otherwise specified, but not in ALK+ ALCL.^{23,24} A *DUSP22*-R occurs in 13–30% cases of ALK-negative ALCL.^{6,8–11} Consistent with prior reports, 27% of systemic ALK-negative ALCL cases in the present study

harbored a *DUSP22*-R. We also showed that patients with *DUSP22*-R ALCL were ~10 years younger than patients with *DUSP22*-NR neoplasms. Except for age, the present study showed no significant differences in other clinical features between patients with *DUSP22*-R versus *DUSP22*-NR ALK-negative ALCL.

Constitutive activation of the JAK/STAT3 signaling pathway is a central pathogenic feature of ALK+ as well as ALK-negative ALCL. *DUSP22* has been shown to inhibit STAT3

signaling.²⁵ Since *DUSP22* expression was significantly decreased in the presence of a *DUSP22*-R, it seemed reasonable to expect enhanced STAT3 activation in *DUSP22*-R ALCL. Surprisingly, total STAT3 protein level and STAT3 activation (measured by pSTAT3^{Y705}) in *DUSP22*-R ALCL was significantly decreased, at least partially due to significantly reduced expression of *STAT3* and other genes in the JAK/STAT3 pathway.¹⁶ Lack of STAT3 activation in *DUSP22*-R ALCL has also been shown by others.^{9,11} The JAK/STAT3

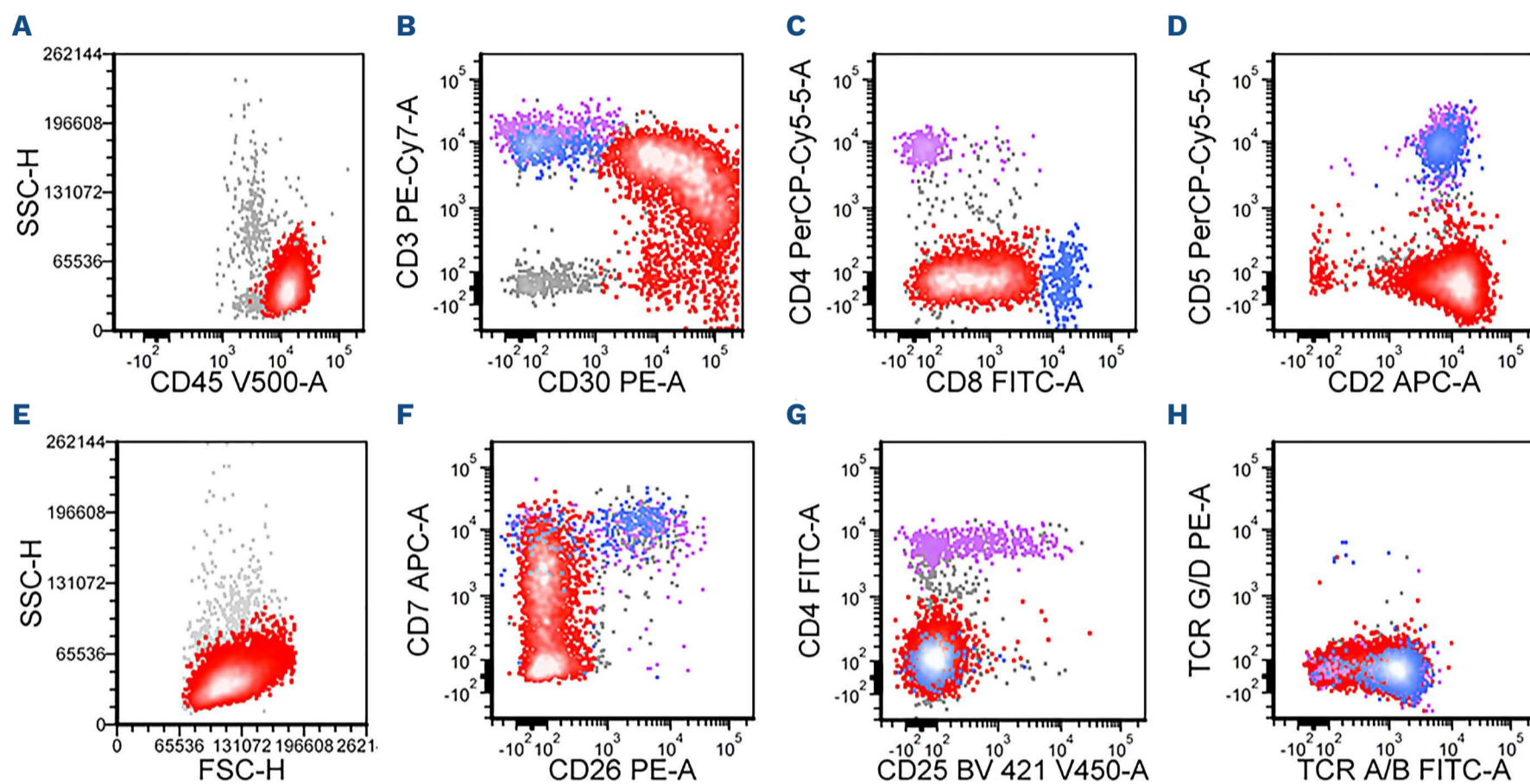


Figure 3. Flow cytometric immunophenotypic analysis of the lymph node biopsy specimen shown in Figure 1. (A-H) The lymphoma cells (red dots) are positive for CD45 (A), CD30 (B), CD3 (decreased, B), CD8 (partial, C), CD2 (D), CD7 (partial/decreased, F), and T-cell receptor (TCR) α/β (decreased, H), and negative for CD4 (C), CD5 (D), CD26 (F), CD25 (G), and TCR γ/δ (H). The lymphoma cells are medium-sized by forward scatter (E). The purple and blue dots represent background benign CD4⁺ and CD8⁺ T cells, respectively.

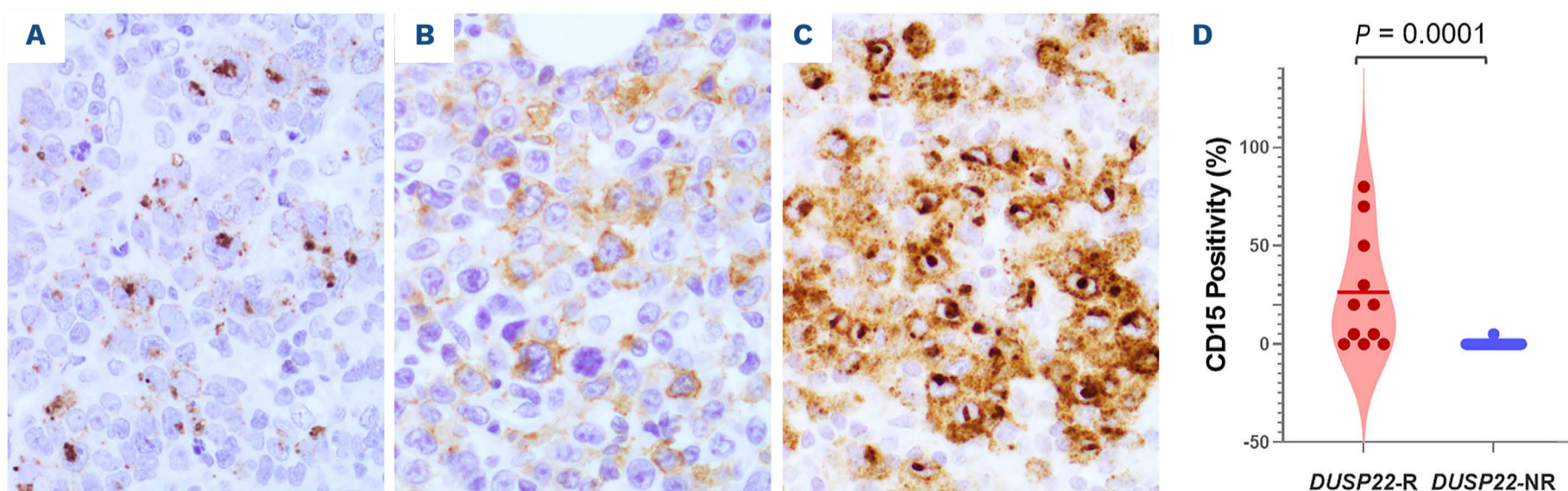


Figure 4. CD15 expression with three staining patterns in *DUSP22*-rearranged cases of ALK-negative anaplastic large cell lymphoma. (A) Golgi-like pattern. (B) Membranous/cytoplasmic pattern. (C) Combination of the Golgi-like and membranous/cytoplasmic patterns. (D) The percentage of CD15⁺ lymphoma cells in *DUSP22*-rearranged cases of anaplastic large cell lymphoma cases is significantly higher than that in the cases without a *DUSP22* rearrangement. (A-C) Immunohistochemistry, x500. *DUSP22*-R: with *DUSP22* rearrangement; *DUSP22*-NR: without *DUSP22* rearrangement.

pathway regulates expression of many downstream molecules including PD-L1.^{20,26,27} Therefore, one would predict that lack of pSTAT3 in *DUSP22*-R ALCL would result in absent PD-L1 expression, as shown here and elsewhere.^{11,16} Expression of other known STAT3 target genes, such as granzyme B and CD25, was also low in *DUSP22*-R ALCL in this cohort.¹⁶ *DUSP22*-R ALCL cases were nearly always negative or infrequently expressed cytotoxic markers and EMA.^{6,9,11,15} Based on gene expression profiling, ALK-negative ALCL cases included two distinct subgroups: cytotoxic and non-cytotoxic. The non-cytotoxic subgroup showed high expression of CD30 but not perforin or granzyme B, with half of cases harboring a *DUSP22*-R.²⁸ In keeping with prior reports, the levels of expression of granzyme B and EMA in our *DUSP22*-R ALK-negative ALCL cases were significantly lower than those in the *DUSP22*-NR cases. *DUSP22*-R results in inhibition of the JAK/STAT3 pathway, but it may also lead to activation of other pathways, as supported by the increased frequency of CD15 observed in the current study. A high CD15 positivity rate in *DUSP22*-R ALK-negative ALCL cases has not been previously reported. Three CD15 staining patterns were observed in

DUSP22-R ALCL cases: a Golgi-like pattern, a membranous/cytoplasmic pattern, and a combination of the Golgi-like and membranous/cytoplasmic patterns. CD15 positivity with a Golgi-like staining pattern is unusual but has been reported in a case of *DUSP22*-R ALK-negative ALCL.²⁹ Another case report of ALK-negative ALCL showed membranous and Golgi-like staining pattern of CD15, but *DUSP22*-R status was unknown.³⁰ Although CD15 has received little attention in *DUSP22*-R ALK-negative ALCL, CD15 expression was observed in ALCL previously. Felgar *et al.* reported CD15 expression in three of 17 (18%) T- or null cell ALCL with unknown ALK status; these cases were all negative for TIA1, suggesting to the authors that the CD15⁺ ALCL cases may be different from other ALCL cases.³¹ Gorczyca *et al.* reported CD15 positivity in two of 26 (8%) ALK⁺ ALCL cases and seven of 30 (23%) ALK-negative CD30⁺ T-cell lymphomas.³² Four CD15⁺ ALK-negative ALCL cases were submitted to the 2005 Society of Hematopathology/European Association for Hematopathology Workshop: one of three (33%) cases was positive for EMA, and one of two (50%) cases was positive for TIA1.³³ Retrospectively, we speculate that at least some of

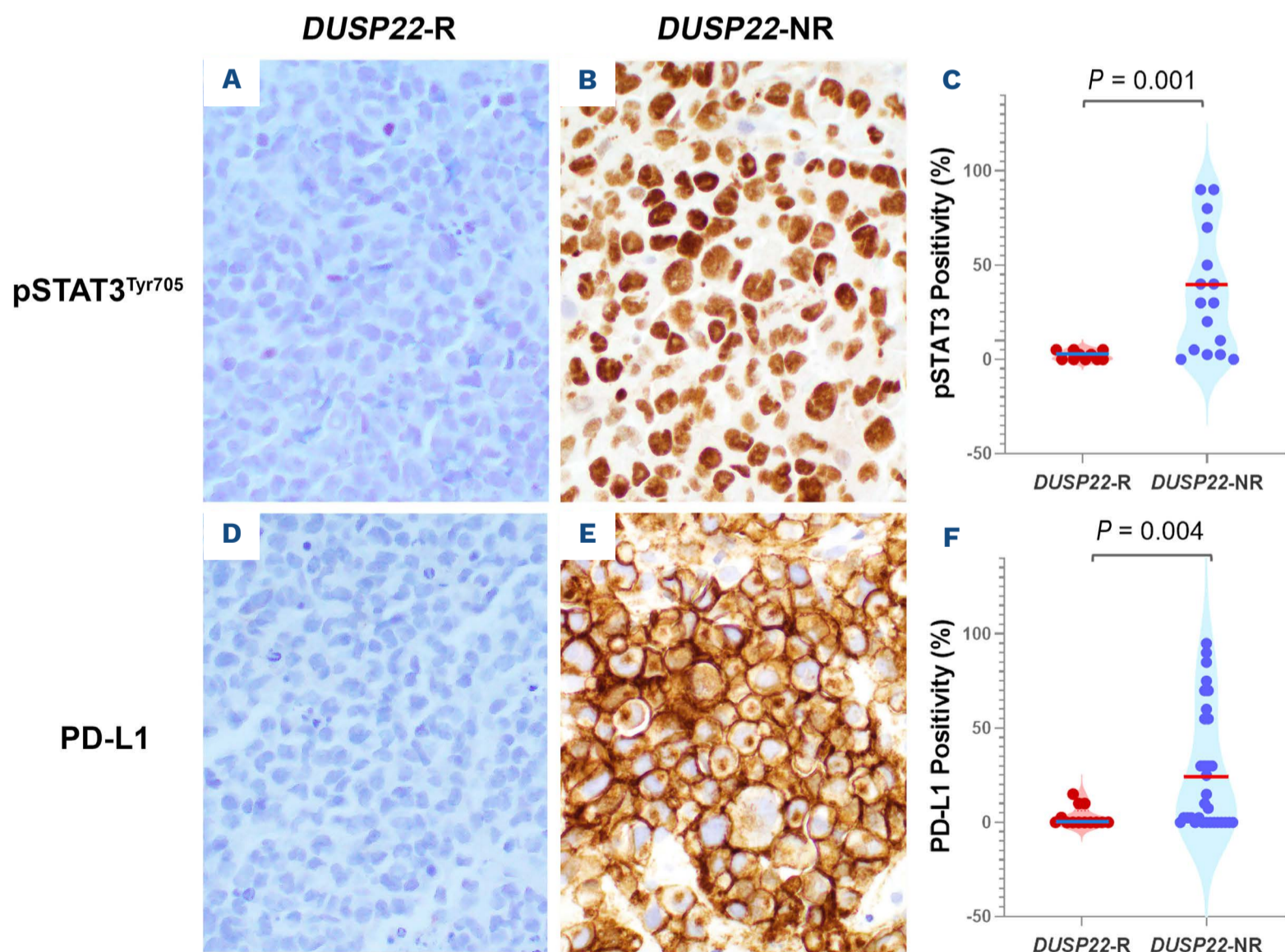


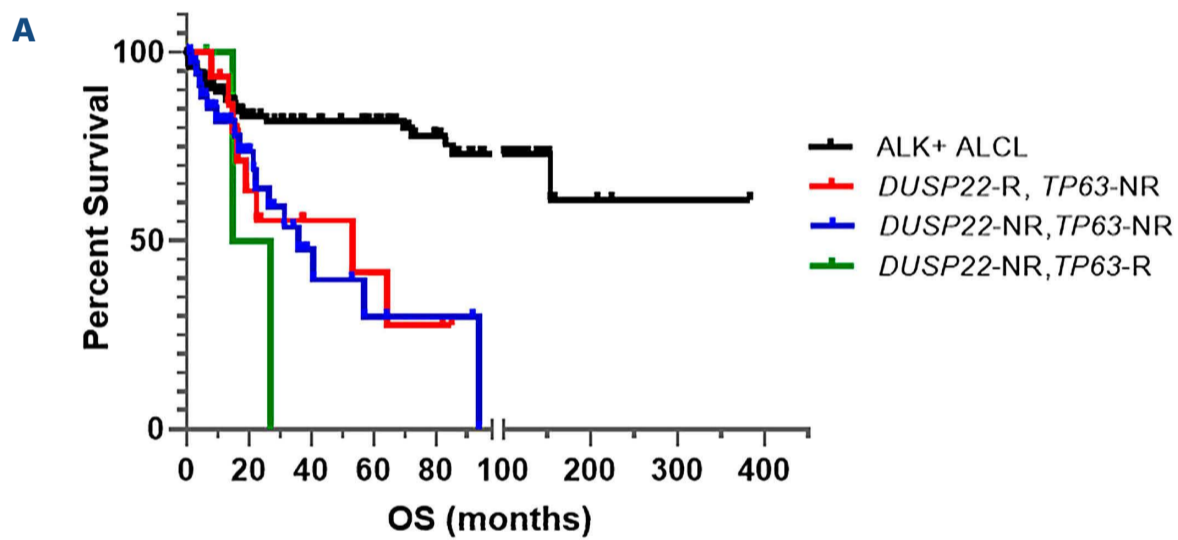
Figure 5. Comparison of STAT3 activation (pSTAT3^{Tyr705}) and PD-L1 expression in ALK-negative anaplastic large cell lymphoma with or without a *DUSP22* rearrangement. (A, D) Negative pSTAT3^{Tyr705} (A) and PD-L1 (D) expression in a *DUSP22*-rearranged case. (B, E) Diffuse positivity of pSTAT3^{Tyr705} (B) and PD-L1 (E) in a case without a *DUSP22*-rearrangement. (C, F) The percentage of pSTAT3^{Tyr705}-positive (C) or PD-L1-positive (F) lymphoma cells in *DUSP22*-rearranged cases of anaplastic large cell lymphoma is significantly lower than that in cases without a *DUSP22* rearrangement. *DUSP22*-R: with *DUSP22* rearrangement; *DUSP22*-NR: without *DUSP22* rearrangement.

previously reported CD15⁺ ALK-negative ALCL cases were likely neoplasms with *DUSP22*-R. The potential explanation for CD15 expression in *DUSP22*-R ALCL is unknown and needs to be investigated further.

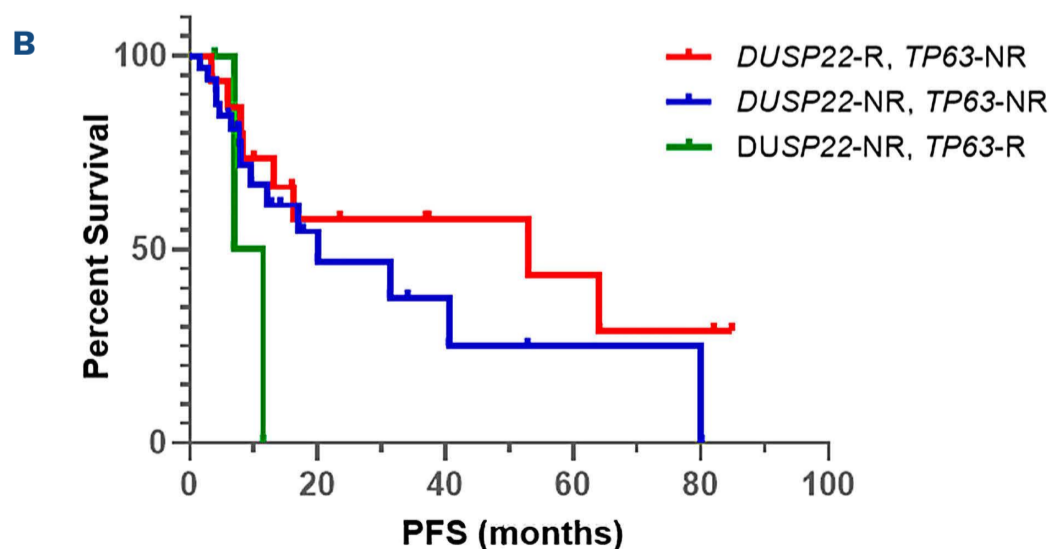
CD15 has been a valuable marker for distinguishing classic Hodgkin lymphoma from other CD30⁺ lymphomas including ALK-negative ALCL. The differential diagnosis between ALK-negative ALCL and classic Hodgkin lymphoma is usually not difficult based on their morphological and immunophenotypic differences. However, a subset of tumors may occasionally show morphological and/or immunophenotypic overlap, posing a diagnostic challenge. For the CD30⁺ CD15⁺ cases with overlapping features of ALCL and classic Hodgkin lymphoma, FISH for *DUSP22*-R may be helpful in reaching the correct diagnosis because a positive result for *DUSP22*-R points to the diagnosis of ALK-negative ALCL.

The unique morphological, immunophenotypic, and mol-

ecular/genetic features of *DUSP22*-R ALCL have been reported consistently, suggesting that *DUSP22*-R cases are a distinct subset of ALK-negative ALCL.^{9,11,15-17} However, the prognostic significance of *DUSP22*-R in ALK-negative ALCL has been controversial. *DUSP22*-R was initially reported to be associated with a favorable clinical outcome in systemic ALK-negative ALCL patients with a 5-year OS rate of 90%, similar to that of patients with ALK+ ALCL.⁶ However, in a recent study from Vancouver, the 5-year OS of *DUSP22*-R ALCL patients was only 40%.¹¹ Unlike earlier studies with small cohorts, the present study of 81 ALK-negative ALCL patients shows that patients with *DUSP22*-R neoplasms have a relatively poorer outcome, similar to that of patients in the *DUSP22*-NR group and substantially poorer than that of ALK+ ALCL patients. The discrepancies observed between studies on the outcome of *DUSP22*-R cases could potentially be attributable to some missed breakpoints. Different FISH



No. at Risk	0	20	40	60	80	100	200	300	400	
ALK+	122	70	58	50	34	22	3	2	1	P = 0.005* p = 0.002* p = 0.818 p = 0.279 p = 0.256
<i>DUSP22</i> -R, <i>TP63</i> -NR	15	9	5	3	2	1	-	-	-	
<i>DUSP22</i> -NR, <i>TP63</i> -NR	36	16	6	4	3	0	-	-	-	
<i>DUSP22</i> -NR, <i>TP63</i> -R	3	1	-	-	-	-	-	-	-	



No. at Risk	0	20	40	60	80	100	
<i>DUSP22</i> -R, <i>TP63</i> -NR	15	7	5	3	2	-	P = 0.275 p = 0.150 p = 0.080
<i>DUSP22</i> -NR, <i>TP63</i> -NR	32	7	3	2	1	-	
<i>DUSP22</i> -NR, <i>TP63</i> -R	3	1	-	-	-	-	

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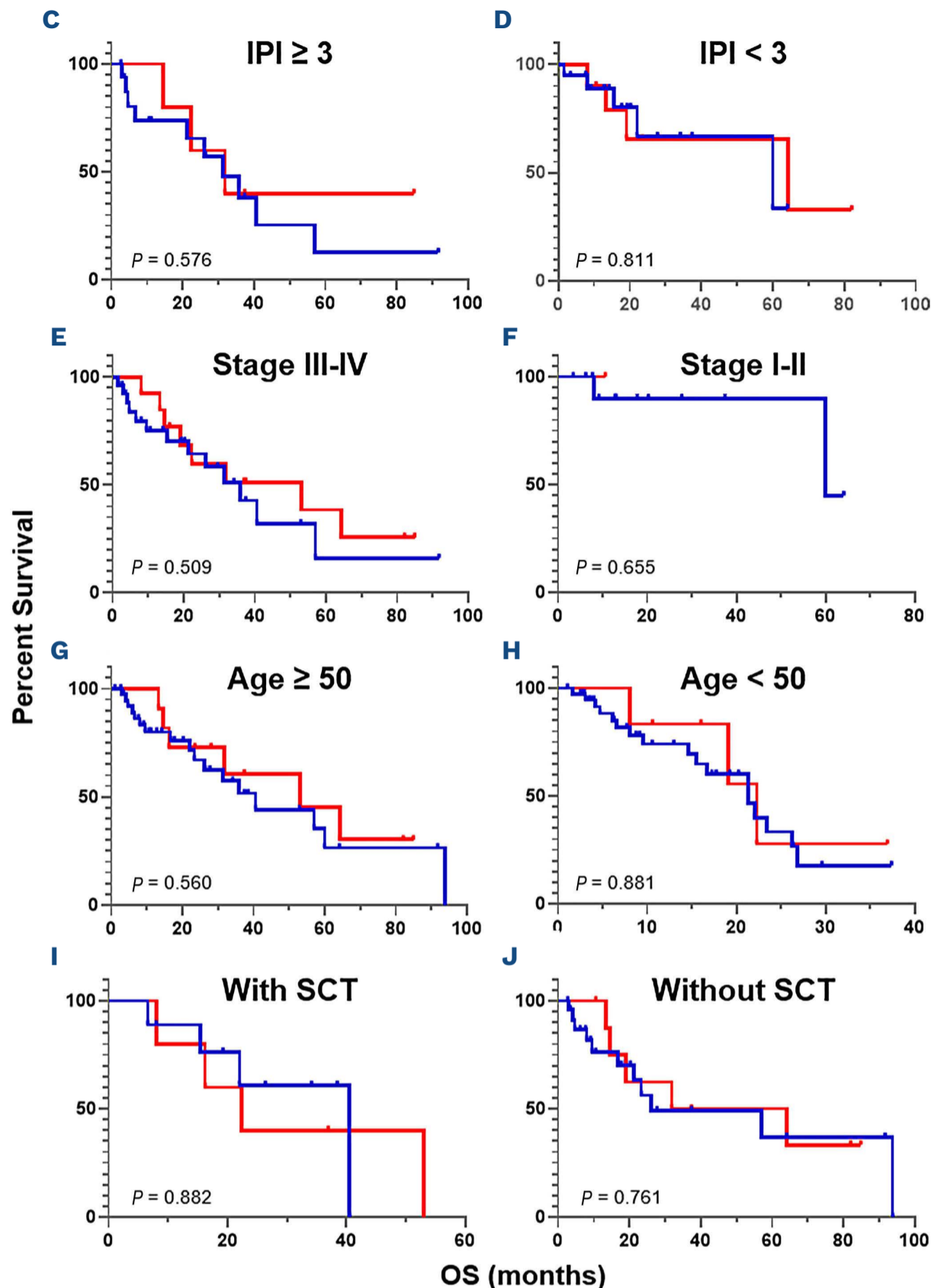


Figure 6. *DUSP22* rearrangement has no prognostic significance in patients with ALK-negative anaplastic large cell lymphoma regardless of International Prognostic Index score, stage, age, or transplantation status. (A, B) Comparison of overall survival (A) and progression-free survival (B) among the patients with ALK+ anaplastic large cell lymphoma (ALCL), *DUSP22*-R (all were *TP63*-NR) ALK-negative ALCL, *DUSP22*-NR/*TP63*-NR (triple-negative) ALK-negative ALCL, and *DUSP22*-NR/*TP63*-R ALK-negative ALCL. * $P < 0.05$, when comparing ALK+ ALCL versus *DUSP22*-R ALK-negative ALCL or comparing ALK+ ALCL versus *DUSP22*-NR/*TP63*-NR ALK-negative ALCL. (C, D) Comparison of overall survival in patients with International Prognostic Index score ≥ 3 (C) and < 3 (D). (E, F) Comparison of overall survival in patients with stage III-IV (E) and stage I-II (F) disease. (G, H) Comparison of overall survival in patients aged ≥ 50 years (G) and < 50 years (H). (I, J) Comparison of overall survival in patients with stem cell transplantation (I) and without (J). *DUSP22*-R: with *DUSP22* rearrangement; *DUSP22*-NR: without *DUSP22* rearrangement; *TP63*-R: with *TP63* rearrangement; *TP63*-NR: without *TP63* rearrangement; IPI: International Prognostic Index; OS: overall survival; PFS: progression-free survival; SCT: stem cell transplant. (C-J) *TP63*-R cases were excluded.

probes were used in different studies: some were prepared in-house^{6,10,11} whereas others were purchased,^{8,9} as in the current study. It is possible that break-apart FISH probes may not detect all *DUSP22*-R if the breakpoints are located outside of the probe coverage or if the rearrangement is through insertion. If true, differences in probes used could alter the results of survival analyses. We note that patients with *DUSP22*-R ALK-negative ALCL in this cohort tended to have a higher initial complete response rate than the patients with *DUSP22*-NR neoplasms, although this difference did not reach statistical significance. This result might be due to the relatively small size of this cohort and larger-sized studies are needed for further investigation.

In this cohort, ~5% of patients with ALK-negative ALCL had *TP63*-R, consistent with the reported frequency (2-8%) of *TP63*-R cases. All *TP63*-R cases in this study were negative for *DUSP22*-R. *TP63*-R and *DUSP22*-R are nearly mutually exclusive,⁶ although rare cases harboring both rearrangements have been reported.^{34,35} In a past study *TP63*-R (n=6) was reported to be associated with poorer prognosis in patients with ALK-negative ALCL.⁶ The present study shows no association between *TP63*-R and OS, but the patients with *TP63*-R neoplasms tended to have shorter progression-free survival than those without *TP63*-R. Given the limited number of *TP63*-R cases in our cohort, the prognostic significance of *TP63*-R cannot be determined.

In summary, our data support the idea that *DUSP22*-R ALK-negative ALCL is a distinctive subset of ALCL. As suggested by others, our data showed that these neoplasms have minimal STAT3 activation and PD-L1 expression, and no/low expression of cytotoxic markers. We report a novel finding in this study that CD15 is commonly expressed in *DUSP22*-R ALK-negative ALCL cases. Our data also suggest that patients with *DUSP22*-R ALK-negative ALCL do not have an excellent prognosis as earlier studies suggested, but instead have a prognosis similar to that of patients with *DUSP22*-NR ALK-negative ALCL. We therefore suggest that further investigation is needed before modifications to the treatment of patients with *DUSP22*-R ALK-negative ALCL are proposed.

Disclosures

No conflicts of interest to disclose

Contributions

LQ, LJM and JX collected and analyzed data and wrote the manuscript; GT and ZT analyzed FISH studies; SL, FV, PL, SW, WW, RNM, SK, and HF contributed to data collection, data analysis, and reviewed the manuscript; SPI, LM contributed to the patients' care, and reviewed and edited the manuscript; JX designed and supervised the study.

Data-sharing statement

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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