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

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

Lianqun Qiu¹, Guilin Tang¹, Shaoying Li¹, Francisco Vega¹, Pei Lin¹, Sa A. Wang¹, Wei Wang¹, Swaminathan P. Iyer², Luis Malpica², Roberto N. Miranda¹, Sergej Konoplev¹, Zhenya Tang¹, Hong Fang¹, L. Jeffrey Medeiros¹, and Jie Xu¹

¹Department of Hematopathology and ²Department of Lymphoma/Myeloma,
The University of Texas MD Anderson Cancer Center, Houston, TX

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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, HF contributed to data collection, data analysis, and reviewed the manuscript; SPI, LM contributed to patient care, reviewed and edited the manuscript; JX designed and supervised the study.

Data Sharing Statement

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

Correspondence:

Jie Xu, MD PhD
Department of Hematopathology
MD Anderson Cancer Center
1515 Holcombe Blvd, Unit 72
Houston, TX 77030
Phone: 713-794-1220
Fax: 713-563-3166
Email: jxu9@mdanderson.org
Abstract

*DUSP22* rearrangement (R) has been associated with a favorable outcome in systemic ALK-negative anaplastic large cell lymphoma (ALCL). However, a recent study reported that patients with *DUSP22*-R ALK-negative ALCL have a poorer prognosis than was reported initially. In the present study, we compare the clinicopathologic 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 the patients with *DUSP22*-NR neoplasms (*p* = 0.049). *DUSP22*-R ALK-negative ALCL cases were more often positive for CD15, CD8, and had less frequent expression of pSTAT3Ty705, PD-L1, granzyme B and EMA (all *p* < 0.05). *TP63* rearrangement (*TP63*-R) was detected in 3 of 66 (5%) ALK-negative ALCL cases tested and none of these cases carried *DUSP22*-R. Overall survival (OS) of patients with *DUSP22*-R ALCL was similar to that of the patients with *DUSP22*-NR neoplasms regardless of International Prognostic Index (IPI) score, stage, age, or stem cell transplantation status (all *p* > 0.05), but was significantly shorter than that of the patients with ALK+ ALCL (median OS 53 months vs undefined, *p* = 0.005). Five-year OS rates were 40% for patients with *DUSP22*-R ALCL versus 82% for patients with ALK+ 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 this time.
**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 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 DUSP22 rearrangement (R) and 2-8% of cases carrying TP63-R [6, 8-11]. TP63 and DUSP22 rearrangements 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 signaling [12, 13]. DUSP22 knockout enhances T-cell activation and T-cell receptor signaling and shows 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 up to 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 ΔNp63. Among the 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 reported initially that patients with DUSP22-R ALK-negative ALCL (n = 22) have a favorable clinical outcome with a 5-year overall survival rate of 90%, similar to that of ALK+ ALCL patients [6]. Later on, two small studies of 5 and 4 patients, respectively, showed a similarly favorable prognosis in patients with DUSP22-R ALCL [9, 10]. Although the case numbers are 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 focus on DUSP22-R ALK-negative ALCL cases to characterize their clinicopathologic and immunophenotypic features and patient 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 World Health Organization classification [1]. The following cases were considered as primary cutaneous ALK-negative ALCL and were excluded from the study: (1) patients with cutaneous disease alone without extracutaneous involvement; (2) 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-STAT3Tyr705, and TIA1. The percentage of lymphoma cells positive for CD15, phospho-STAT3Tyr705 and PD-L1 was read by two hematopathologists (J.X. and S.L.) 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 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, T-cell receptor (TCR) alpha/beta and TCR gamma/delta (Becton-Dickinson Biosciences, San Jose, CA, USA).

**Fluorescence in situ hybridization**

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 cut-off value was 10.7% for **IRF4/DUSP22** rearrangement and 11.1% for **TP63** rearrangement. **IRF4/DUSP22** and **TP63** rearrangements were assessed in a blinded fashion without the knowledge of pathological diagnosis or results of other FISH analysis.

**Statistical analysis**

Statistical analyses were performed using the Graph-Pad Prism 8 and SPSS 26.0 software (IBM Corporation, Armonk, NY). Fisher’s exact test was used to compare the clinicopathologic features between **DUSP22-R** and **DUSP22-NR** groups in patients with ALK-negative ALCL. Overall survival (OS) was calculated from the date of initial diagnosis to the date of death or last follow-up. Progression-free survival (PFS) 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 FISH findings

The results of FISH analysis showed that \textit{DUSP22-R} was positive in 22 (28\%) cases (\textit{DUSP22-R} group), and negative in 59 (72\%) cases (\textit{DUSP22-NR} group). The clinical features of these patients are summarized in Table 1. In the \textit{DUSP22-R} group, there were 15 men and 7 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 9 of 15 (60\%) patients had extra-nodal involvement including skin (6 cases; 40\%), bones (3 cases; 33\%), 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 1 of 8 (13\%) and 1 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) scores of $\geq$ 3. One of 14 (7\%) patients had leukocytosis and 1 of 13 (8\%) patients had absolute lymphocytosis. Anemia was observed in 7 of 14 (50\%) patients and thrombocytopenia was present in 2 of 14 (14\%) patients. An elevated serum lactate dehydrogenase (LDH) level was detected in 7 of 14 (50\%) patients.

In the \textit{DUSP22-NR} group, there were 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 extra-nodal disease. The involved extranodal sites included lung (10 cases; 22\%), skin (9 cases; 20\%), bones (7 cases; 16\%), liver (6 cases; 13\%), soft tissue (5 cases; 11\%), gingiva/oropharynx/nasphoarynx (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 4 of 18 (22%) cases and 8 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 $\geq 3$. Seven of 31 (23%) patients had leukocytosis and no patients (n = 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 LDH level was detected in 16 of 26 (62%) patients.

Compared to patients in $DUSP22$-NR ALK-negative ALCL group, patients with $DUSP22$-R neoplasms were 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 FISH analysis**

FISH analysis was performed to evaluate the status of $TP63$ rearrangement ($TP63$-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 $TP63$-R. There was no significant difference in the frequency of $TP63$-R between the $DUSP22$-R versus $DUSP22$-NR groups ($p = 0.55$).

**Morphologic and immunophenotypic findings**

$DUSP22$-NR ALK-negative ALCL cases in tissue specimens have morphologic features of the so-called “common pattern” as described in ALK+ ALCL. In comparison, the lymphoma cells in $DUSP22$-R neoplasms were relatively more monotonous in appearance, smaller in size, 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 only seen occasionally. A DUPS22-R ALK-negative ALCL case is shown in Fig. 1 (lymph node) and Fig. 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 Fig. 3). Expression of other T-cell-associated antigens was less frequent: TCR αβ (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 7 cases examined were negative for TCR γδ. 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: Golgi-like (perinuclear dot) pattern, membranous/cytoplasmic pattern, and a combination of Golgi and membranous/cytoplasmic pattern (Fig. 4).

By comparison, the immunophenotype of DUSP22-NR ALK-negative ALCL cases was similar except for the following significant differences 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 for nuclear expression of phosphorylated STAT3 (pSTAT3\textsuperscript{Tyr705}). A mean of 2% lymphoma cells in DUSP22-R ALK-negative ALCL cases showed nuclear staining for pSTAT3\textsuperscript{Tyr705}, significantly lower than a mean of 36% observed in DUSP22-NR tumors (\(p = 0.001\); Fig. 5A-C). PD-L1, a downstream molecule regulated by the JAK/STAT3 signaling pathway, was positive in 3% lymphoma cells in DUSP22-R ALCL versus 26% positive cells in DUSP22-NR tumors (\(p = 0.01\)) (Fig. 5D-F).

**Treatment and response**

Sixteen patients with DUSP22-R ALK-negative ALCL and 45 patients with DUSP22-NR neoplasms had treatment information available. 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. Among the 6 patients treated with non-CHOP-based chemotherapy regimens, 4 received brentuximab vedotin-based therapy, 1 received ifosfamide, carboplatin and etoposide (ICE), and 1 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 10 of 37 (27%) patients in the DUSP22-NR group received SCT. There was no significant difference in initial
treatment or SCT rates between patients with *DUSP22*-R versus *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 9 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, 2 of 3 (67%) patients carrying *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 PFS (median 53 vs. 20 vs. 9 months, all $p > 0.05$) between these groups (Fig. 6A-B). Although the patients with *TP63*-R neoplasms tend to have shorter PFS 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 ($\geq 3$ or $< 3$), clinical stage (stage III/IV or I/II), age ($\geq 50$ or $< 50$ years, Fig. 6C; $\geq 40$ or $< 40$ years, data not shown; $\geq 60$ or $< 60$ years, data not shown), and SCT status, there were still no significant differences in OS between the *DUSP22*-R versus *DUSP22*-NR groups (all $p > 0.05$, Fig. 6C-J).

We also compared the OS of patients with *DUSP22*-R ALK-negative ALCL to 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, $p = 0.005$, Fig. 6A). Five-year OS rates were 40% for patients with *DUSP22*-R ALK-negative ALCL as compared to 82% for patients with ALK+ ALCL.
Discussion

*DUSP22* rearrangement 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, NOS, but not in ALK+ ALCL [23, 24]. *DUSP22* rearrangement occurs in 13-30% of ALK-negative ALCL cases [6, 8-11]. Consistent with prior reports, 27% of systemic ALK-negative ALCL cases in the present study harbored *DUSP22* rearrangement. 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 *DUSP22* rearrangement, it seemed reasonable to expect enhanced STAT3 activation in *DUSP22*-R ALCL. Surprisingly, total STAT3 protein level and STAT3 activation (measured by pSTAT3Y705) 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 also has been shown by others [9, 11]. The JAK/STAT3 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 \textit{DUSP22}-R ALCL in this cohort [16]. \textit{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 include 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 \textit{DUSP22} rearrangement [28]. In keeping with prior reports, the expression levels of granzyme B and EMA in our \textit{DUSP22}-R ALK-negative ALCL cases were significantly lower than those in the \textit{DUSP22}-NR cases.

\textit{DUSP22} rearrangement results in inhibition of the JAK/STAT3 pathway, but it may also lead to activation of other pathways, as supported by increased frequency of CD15 observed in the current study. The high CD15 positivity rate in \textit{DUSP22}-R ALK-negative ALCL cases has not been previously reported. Three CD15 staining patterns were observed in \textit{DUSP22}-R ALCL cases: Golgi-like pattern, membranous/cytoplasmic pattern, and combination of Golgi-like and membranous/cytoplasmic pattern. CD15 positivity with a Golgi-like staining pattern is unusual but has been reported in a case of \textit{DUSP22}-R ALK-negative ALCL [29]. Another case report of ALK-negative ALCL showed membranous and Golgi-like staining pattern of CD15, but its \textit{DUSP22} rearrangement status was unknown [30].

Although CD15 has received little attention in \textit{DUSP22}-R ALK-negative ALCL, CD15 expression was observed in ALCL previously. Felgar et al reported CD15 expression in 3 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 2 of 26 (8\%) ALK+ ALCL cases and 7 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. 1 of 3 (33%) case was positive for EMA, 1 of 2 (50%) cases was positive for TIA1 [33]. Retrospectively, we speculate that at least some of 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 further investigated.

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 morphologic and immunophenotypic differences. However, a subset of tumors may occasionally show morphologic 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 rearrangement may be helpful in reaching the correct diagnosis because a positive result for DUSP22 rearrangement points to the diagnosis of ALK-negative ALCL.

The unique morphologic, immunophenotypic, and molecular/genetic features of DUSP22-R ALCL have been consistently reported, suggesting that DUSP22-R cases are a distinct subset of ALK-negative ALCL [9, 11, 15-17]. However, the prognostic significance of DUSP22 rearrangement in ALK-negative ALCL has been controversial. DUSP22 rearrangement 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 the 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 the patients in the *DUSP22*-NR group and substantially poorer than the 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 probes were used in different studies: some were in-house brewed [6, 10, 11] whereas others were commercially purchased [8, 9] as in the current study. It is possible that break-apart FISH probes may not detect all *DUSP22* rearrangements if the breakpoints are located outside of probe coverage or if the rearrangement is though insertion. If true, differences in probes used could alter the results of survival analysis. It is noted 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* and *DUSP22* rearrangements are nearly mutually exclusive [6], although rare cases harboring both rearrangements have been reported [34, 35]. *TP63*-R (n = 6) has been 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 tend to have shorter PFS 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.
In this study, we report a novel finding that CD15 is commonly expressed in \textit{DUSP22}-\textit{ALK}-negative ALCL cases. Our data also suggest that patients with \textit{DUSP22}-\textit{ALK}-negative ALCL do not have an excellent prognosis as earlier studies suggested, but instead have a prognosis similar to patients with \textit{DUSP22}-\textit{NR ALK}-negative ALCL. In addition, we suggest that further investigation is needed before modifications to the treatment of patients with \textit{DUSP22}-\textit{ALK}-negative ALCL are proposed.
References


Table 1. Clinical features of ALK-negative ALCL patients with versus without \textit{DUSP22}-Rearrangement

<table>
<thead>
<tr>
<th>Clinical features at diagnosis</th>
<th>Total (n=81)</th>
<th>\textit{DUSP22}-R (n=22)</th>
<th>\textit{DUSP22}-NR (n=59)</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male: Female</td>
<td>1.9: 1(53/28)</td>
<td>2:1 (15/7)</td>
<td>1.8:1 (38/21)</td>
<td>0.80</td>
</tr>
<tr>
<td>Median age, years (range)</td>
<td>60 (21-95)</td>
<td>52 (33-79)</td>
<td>61 (21-95)</td>
<td>\textbf{0.049}</td>
</tr>
<tr>
<td>B symptoms</td>
<td>57% (33/58)</td>
<td>40% (6/15)</td>
<td>63% (27/43)</td>
<td>0.14</td>
</tr>
<tr>
<td>Lymphadenopathy at diagnosis</td>
<td>84% (57/68)</td>
<td>94% (16/17)</td>
<td>80% (41/51)</td>
<td>0.27</td>
</tr>
<tr>
<td>Extra-nodal involvement</td>
<td>72% (43/60)</td>
<td>60% (9/15)</td>
<td>76% (34/45)</td>
<td>0.32</td>
</tr>
<tr>
<td>Peripheral blood involvement</td>
<td>19% (5/26)</td>
<td>13% (1/8)</td>
<td>22% (4/18)</td>
<td>1.00</td>
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<tr>
<td>Bone marrow involvement</td>
<td>14% (9/64)</td>
<td>6% (1/8)</td>
<td>17% (8/46)</td>
<td>0.43</td>
</tr>
<tr>
<td>Stage III or IV</td>
<td>71% (40/56)</td>
<td>87% (13/15)</td>
<td>66% (27/41)</td>
<td>0.19</td>
</tr>
<tr>
<td>IPI ≥ 3</td>
<td>45% (23/51)</td>
<td>33% (5/15)</td>
<td>50% (18/36)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Laboratory findings at diagnosis</th>
<th>Total (n=81)</th>
<th>\textit{DUSP22}-R (n=22)</th>
<th>\textit{DUSP22}-NR (n=59)</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevated WBC (&gt;11 K/µL)</td>
<td>18% (8/45)</td>
<td>7% (1/14)</td>
<td>23% (7/31)</td>
<td>0.40</td>
</tr>
<tr>
<td>Absolute lymphocytosis (&gt;4.8 K/µL)</td>
<td>2% (1/43)</td>
<td>8% (1/13)</td>
<td>0% (0/30)</td>
<td>0.30</td>
</tr>
<tr>
<td>Anemia*</td>
<td>60% (27/45)</td>
<td>50% (7/14)</td>
<td>65% (20/31)</td>
<td>0.51</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt;140 K/µL)</td>
<td>13% (6/45)</td>
<td>14% (2/14)</td>
<td>13% (4/31)</td>
<td>1.00</td>
</tr>
<tr>
<td>Elevated LDH</td>
<td>58% (23/40)</td>
<td>50% (7/14)</td>
<td>62% (16/26)</td>
<td>0.52</td>
</tr>
<tr>
<td>\textit{TP63} rearrangement by FISH</td>
<td>5% (3/66)</td>
<td>0% (0/19)</td>
<td>6% (3/47)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial treatment</th>
<th>Total (n=81)</th>
<th>\textit{DUSP22}-R (n=22)</th>
<th>\textit{DUSP22}-NR (n=59)</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHOP or modified CHOP</td>
<td>90% (55/61)</td>
<td>81% (13/16)</td>
<td>93% (42/45)</td>
<td>0.18</td>
</tr>
<tr>
<td>Others</td>
<td>10% (6/61)</td>
<td>19% (3/16)</td>
<td>7% (3/45)</td>
<td></td>
</tr>
<tr>
<td>Initial CR</td>
<td>54% (32/59)</td>
<td>75% (12/16)</td>
<td>47% (20/43)</td>
<td>0.08</td>
</tr>
<tr>
<td>Relapse</td>
<td>52% (28/54)</td>
<td>43% (6/14)</td>
<td>55% (22/40)</td>
<td>0.54</td>
</tr>
<tr>
<td>SCT+</td>
<td>29% (15/51)</td>
<td>36% (5/14)</td>
<td>27% (10/37)</td>
<td>0.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total (n=81)</th>
<th>\textit{DUSP22}-R (n=22)</th>
<th>\textit{DUSP22}-NR (n=59)</th>
<th>\textit{P} value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alive</td>
<td>54% (36/67)</td>
<td>50% (9/18)</td>
<td>55% (27/49)</td>
<td>0.79</td>
</tr>
<tr>
<td>Dead</td>
<td>46% (31/67)</td>
<td>50% (9/18)</td>
<td>45% (22/49)</td>
<td></td>
</tr>
<tr>
<td>Median OS, months (range)</td>
<td>36 (1.1-94)</td>
<td>53 (8-85)</td>
<td>36 (1.1-94)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Abbreviations: ALCL, anaplastic large cell lymphoma; \textit{DUSP22}-R, with \textit{DUSP22} rearrangement; \textit{DUSP22}-NR, without \textit{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, \( p < 0.05 \)
Table 2. Immunophenotypic Features of ALK-Negative ALCL with versus without *DUSP22*-Rearrangement

<table>
<thead>
<tr>
<th>Immunophenotype</th>
<th>Total (n=81)</th>
<th>DUSP22-R (n=22)</th>
<th>DUSP22-NR (n=59)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2+</td>
<td>72% (38/53)</td>
<td>77% (13/17)</td>
<td>69% (25/36)</td>
<td>0.75</td>
</tr>
<tr>
<td>CD3+</td>
<td>60% (47/78)</td>
<td>76% (16/21)</td>
<td>55% (31/57)</td>
<td>0.12</td>
</tr>
<tr>
<td>CD4+</td>
<td>82% (56/68)</td>
<td>74% (14/19)</td>
<td>86% (42/49)</td>
<td>0.29</td>
</tr>
<tr>
<td>CD5+</td>
<td>45% (28/62)</td>
<td>35% (6/17)</td>
<td>49% (22/45)</td>
<td>0.40</td>
</tr>
<tr>
<td>CD7+</td>
<td>30% (7/23)</td>
<td>29% (2/7)</td>
<td>31% (5/16)</td>
<td>1.00</td>
</tr>
<tr>
<td>CD8+</td>
<td>13% (8/60)</td>
<td>28% (5/18)</td>
<td>7% (3/42)</td>
<td><strong>0.045</strong></td>
</tr>
<tr>
<td>CD15+</td>
<td>31% (15/48)</td>
<td>80% (12/15)</td>
<td>9% (3/33)</td>
<td><strong>0.0001</strong></td>
</tr>
<tr>
<td>CD25+</td>
<td>60% (9/15)</td>
<td>33% (2/6)</td>
<td>78% (7/9)</td>
<td>0.14</td>
</tr>
<tr>
<td>CD30+</td>
<td>100 (81/81)</td>
<td>100 (22/22)</td>
<td>100 (59/59)</td>
<td>1.00</td>
</tr>
<tr>
<td>CD43+</td>
<td>87% (26/30)</td>
<td>83% (5/6)</td>
<td>88% (21/24)</td>
<td>1.00</td>
</tr>
<tr>
<td>CD45+</td>
<td>81% (39/48)</td>
<td>92% (12/13)</td>
<td>77% (27/35)</td>
<td>0.41</td>
</tr>
<tr>
<td>CD52+</td>
<td>7% (2/29)</td>
<td>40% (2/5)</td>
<td>0% (0/24)</td>
<td>0.44</td>
</tr>
<tr>
<td>CD56+</td>
<td>10% (3/31)</td>
<td>8% (1/12)</td>
<td>11% (2/19)</td>
<td>1.00</td>
</tr>
<tr>
<td>TCR αβ+</td>
<td>55% (18/33)</td>
<td>43% (3/7)</td>
<td>58% (15/26)</td>
<td>1.00</td>
</tr>
<tr>
<td>TCR γδ+</td>
<td>0% (0/17)</td>
<td>0% (0/7)</td>
<td>0% (0/10)</td>
<td>1.00</td>
</tr>
<tr>
<td>BCL2+</td>
<td>75% (15/20)</td>
<td>86% (6/7)</td>
<td>69% (9/13)</td>
<td>0.61</td>
</tr>
<tr>
<td>Granzyne B+</td>
<td>41% (13/32)</td>
<td>0% (0/12)</td>
<td>65% (13/20)</td>
<td>&lt;$0.0001$</td>
</tr>
<tr>
<td>TIA1+</td>
<td>50% (18/36)</td>
<td>30% (3/10)</td>
<td>58% (15/26)</td>
<td>0.26</td>
</tr>
<tr>
<td>Perforin+</td>
<td>50% (5/10)</td>
<td>25% (1/4)</td>
<td>83% (5/6)</td>
<td>0.19</td>
</tr>
<tr>
<td>EMA+</td>
<td>46% (18/39)</td>
<td>18% (2/11)</td>
<td>57% (16/28)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>MUM1+</td>
<td>91% (21/23)</td>
<td>100% (7/7)</td>
<td>88% (14/16)</td>
<td>1.00</td>
</tr>
<tr>
<td>MYC+</td>
<td>43% (22/51)</td>
<td>41% (7/17)</td>
<td>44% (15/34)</td>
<td>0.54</td>
</tr>
<tr>
<td>Mean Ki-67 (n)</td>
<td>88% (35)</td>
<td>91% (11)</td>
<td>85% (24)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

ALCL, anaplastic large cell lymphoma; *DUSP22*-R, with *DUSP22* rearrangement; *DUSP22*-NR, without *DUSP22* rearrangement; The cut-off value for CD15 positivity was ≥ 5%. Bold, p < 0.05
Figure legends

Figure 1. Lymph node biopsy from a *DUSP22*-rearranged (R) ALK-negative ALCL case.
A-B, The nodal architecture is effaced by sheets of monotonous, intermediate size 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, FISH analysis using *IRF4/DUSP22* break-apart probes, ×600. The nuclei showing *DUSP22* rearrangement (with red and green split signals) are indicated by white arrow heads. A-B, hematoxylin-eosin stain, x600 (A) and x600 (B). C-G, immunohistochemistry, x400.

Figure 2. The *DUSP22*-rearranged ALK-negative ALCL 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 as described in Figure 1. B, The lymphoma cells in the bone marrow are positive for CD30 by immunohistochemistry. C, Bone marrow aspirate smear shows 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), CD7 (partial, G), and negative for CD4 and CD26, immunophenotypically similar to the lymphoma cells in the lymph node as described in Fig. 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.

**Figure 3. Flow cytometric immunophenotypic analysis of the lymph node biopsy specimen shown in Figure 1.** 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) alpha/beta (decreased, H), and negative for CD4 (C), CD5 (D), CD26 (F), CD25 (G), and TCR gamma/delta (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 ALK-negative ALCL cases:** Golgi-like pattern (A), membranous/cytoplasmic pattern (B), and combination of Golgi-like and membranous/cytoplasmic pattern (C). D, The percentage of CD15+ lymphoma cells in DUSP22-R ALCL cases is significantly higher than that in the DUSP22-NR cases. A-C, immunohistochemistry, x500. DUSP22-R, with DUSP22 rearrangement; DUSP22-NR, without DUSP22 rearrangement.

**Figure 5. Comparison of STAT3 activation (pSTAT3^{Tyr705}) and PD-L1 expression in ALK-negative ALCL with versus without DUSP22 rearrangement.** A and D, negative pSTAT3^{Tyr705} (A) and PD-L1 (D) expression in a DUSP22-R case. B and E, diffuse positivity of pSTAT3^{Tyr705} (B) and PD-L1 (E) in a DUSP22-NR case. C and F, The percentage of pSTAT3^{Tyr705}+ (C) or PD-L1+ (F) lymphoma cells in DUSP22-R ALCL cases is significantly lower than that in DUSP22-NR cases. DUSP22-R, with DUSP22 rearrangement; DUSP22-NR, without DUSP22 rearrangement.
Figure 6. DUSP22 rearrangement has no prognostic significance in patients with ALK-negative ALCL regardless IPI score, stage, age, or SCT status. A-B, Comparison of OS (A) and PFS (B) among the patients with ALK+ ALCL, DUSP22-R (all were TP63-NR) ALK-negative, DUSP22-NR/TP63-NR (triple-negative) ALK-negative ALCL, and DUSP22-NR/TP63-R ALK-negative ALCL. *, p < 0.05, when comparing ALK+ ALCL vs. DUSP22-R ALK-negative ALCL or comparing ALK+ ALCL vs. DUSP22-NR/TP63-NR ALK-negative ALCL. C and D, in patients with IPI score ≥ 3 (C) and < 3 (D), respectively. E and F, in patients with stage III-IV (E) and stage I-II (F) disease, respectively. G and H, in patients with age ≥ 50 (G) years and < 50 years (H), respectively. I and J, in patients with SCT (I) and without SCT (J), respectively. 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.
(A) Percent Survival vs OS (months)

- ALK+ ALCL
- DUSP22-R, TP63-NR
- DUSP22-NR, TP63-NR
- DUSP22-NR, TP63-R

No. at Risk

- ALK+ 122 70 58 50 34 22 3 2 1
- DUSP22-R, TP63-NR 15 9 5 3 2 1
- DUSP22-NR, TP63-NR 36 16 6 4 3 0
- DUSP22-NR, TP63-R 3 1

(B) Percent Survival vs PFS (months)

- DUSP22-R, TP63-NR
- DUSP22-NR, TP63-NR
- DUSP22-NR, TP63-R

No. at Risk

- DUSP22-R, TP63-NR 15 7 5 3 2
- DUSP22-NR, TP63-NR 32 7 3 2 1
- DUSP22-NR, TP63-R 3 1

(C) IPI ≥ 3
- p = 0.576

(D) IPI < 3
- p = 0.811

(E) Stage III-IV
- p = 0.818

(F) Stage I-II
- p = 0.279

(G) Age ≥ 50
- p = 0.509

(H) Age < 50
- p = 0.655

(I) With SCT
- p = 0.275

(J) Without SCT
- p = 0.882