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SHORT REPORT

Small cell pattern of ALK-negative anaplastic large cell lymphoma with double-hit rearrangements of *DUSP22* and *TP63*

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Abstract

In ALK-negative anaplastic large cell lymphoma (ALCL), gene rearrangements of *DUSP22* and *TP63* are considered mutually exclusive. The former predicts a favorable prognosis, while the latter is generally unfavorable. We report the first case of ALK-negative ALCL in a leukemic phase with small cell pattern transformation, harboring double-hit rearrangements of the *DUSP22* gene by inv(6)(p25q21) and *TP63* gene by TBL1XR1-TP63 inversion. Despite the resistance to chemotherapies, the patient remained in remission with allogeneic stem cell transplantation over 20 months. Recognizing this pathologically and genetically rare condition is needed for prompt diagnosis and therapeutic decision-making in ALK-negative ALCL.

KEYWORDS
ALK-negative anaplastic large cell lymphoma, DUSP22 gene rearrangement, small cell pattern, TBL1XR1-TP63 inversion, TP63 gene rearrangement

1 | INTRODUCTION

Anaplastic large cell lymphoma (ALCL) includes a prognostically favorable ALK-positive subtype with t(2;5)(p23;p53) and a prognostically poor ALK-negative subtype. Recent studies demonstrated the association of several molecular abnormalities, such as *TP63* rearrangement (*TP63*-R), loss of *TP53*, and IL-2Rα overexpression, with poor outcomes [1, 2]. In contrast, the prognostic impact of *DUSP22* rearrangement (*DUSP22*-R) may be favorable in ALK-negative ALCL [3–5]. Importantly, *DUSP22*-R and *TP63*-R have been primarily considered mutually exclusive [5]. In addition to the typical histologic patterns of CD30-positive large tumor cells and their adhesive nature, accumulating pieces of evidence have disclosed several histologically distinctive variants, including the small cell pattern with frequent leukemic manifestation and poor prognosis, have been reported in ALK-positive ALCL [6–8]. In contrast, ALK-negative ALCL cells generally exhibit

Shinsuke Mizutani and Junya Kuroda contributed equally to this study.

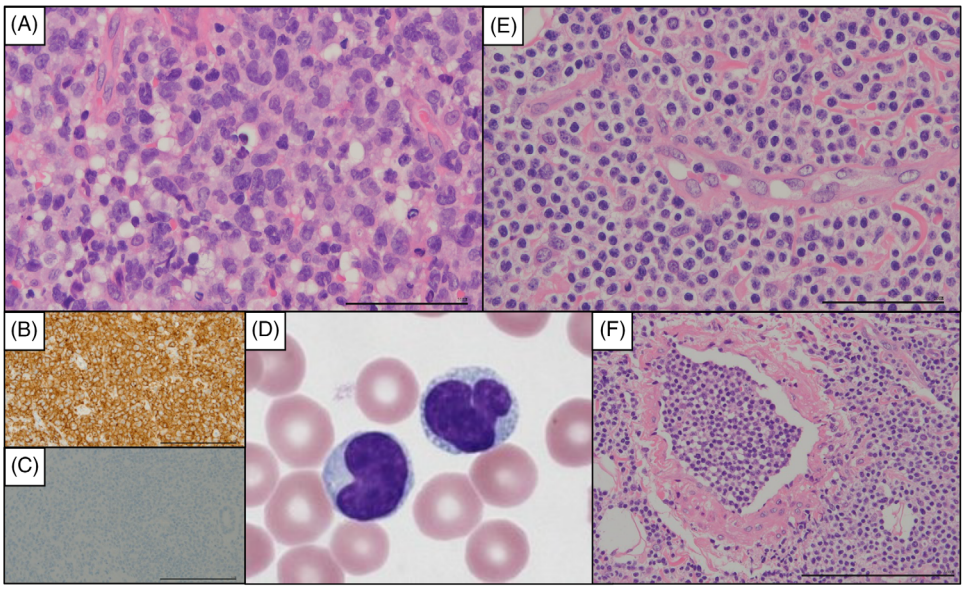

FIGURE 1 Histopathological and cytological findings. (A) Histopathological view of a biopsied specimen from a thoracic subcutaneous nodule at the initial diagnosis revealed the diffuse infiltration of large abnormal lymphoid cells (hematoxylin–eosin [HE] staining, original magnification ×200, scale bar = 200 μm). (B and C) Immunohistochemical analyses of a biopsied specimen from a thoracic subcutaneous nodule at the initial diagnosis showed positive staining for CD30 (×100, scale bar = 500 μm) (B), but negative staining for ALK (×100, scale bar = 500 μm) (C). (D) Small-sized abnormal lymphoid cells with nuclear clefts appeared in peripheral blood at relapse stained by Wright Giemsa staining (×1000). (E) Histopathological view of a biopsied specimen from inguinal lymph node involvement at relapse revealed the diffuse infiltration of small-sized abnormal lymphoid cells (HE staining, ×200, scale bar = 200 μm). (F) A lymphoma cell cluster was observed in the vessel of the inguinal lymph node involvement (HE staining, ×100, scale bar = 500 μm).

the typical large and pleomorphic cell pattern [9]. However, rare cases with small cell pattern ALK-negative ALCL have been recently recognized [10, 11]. Here, we report a first-reported case with a small cell pattern ALK-negative ALCL with concomitant *DUSP22*-R and *TP63*-R.

2 | CASE REPORT

A 57-year-old female patient was referred to our hospital, complaining of a red nodule on the right anterior chest. The biopsied specimen of the nodule disclosed the infiltration of large abnormal CD3/CD7(partially)/TP53/CD30-positive and ALK- and TIA-1-negative lymphoid cells (Figure 1A–C and Figure S1A,B, and data not shown). Giemsa (G-) banding of the tumor cells showed the complex karyotype: 77<4n>,XXX,−3,−6,?inv(6)(p25q21)x2,−7,−7,−9,−10,−11,−11,−12, −13,−13,−14,−15,−15,−16,−16,add(17)(p11.2)x2,+mar1 [6]. Double color fluorescence in situ hybridization (DC-FISH) analysis of formalin-fixed paraffin-embedded tissue revealed the presence of *DUSP22*-R (Figure 2A). Flow cytometry analysis disclosed that abnormal lymphoid cells were positive for CD2, CD4, CD5, and CD30, but were negative for CD3, CD7, or CD8. A systemic workup revealed the additional involvement at the C7 vertebral body and right axillary lymph nodes, while bone marrow (BM) and peripheral blood (PB) were intact. Accordingly, the patient was diagnosed as having ALK-negative ALCL with disease stage IV. Despite the transient remission for 1 month by six cycles of BV-CHP [12], the disease relapsed with the marked leukocytosis of 42.4 × 10^9/L with 39% of CD3- and CD30-positive small-sized abnormal lymphocytes with a nuclear cleft in PB (Figure 1D), splenomegaly, and generalized lymphadenopathy. The biopsied specimen of lymph node involvement showed the diffuse infiltration of CD3/CD30-positive and *DUSP22*-R-positive small abnormal lymphoid cells with blood vessels filled with lymphoma cells (Figure 1E,F). Flow cytometry analysis revealed that abnormal lymphoid cells were positive for CD2, CD3, CD4, CD5, CD7, and CD30, but were negative for CD8. In addition, tumor cells were positive for TP53 and CD7 by immunohistochemical analyses (Figure S1C–F). BM was also infiltrated by small abnormal lymphoid cells. The cytogenetic study by G-banding of tumor cells showed the complex abnormality of 46,XX,?inv(6)(p25q21)[8]/idem,−13,−17,+mar1,+mar2[3] in the lymph node, and 46,XX,?inv(6)(p25q21) in PB. In addition, targeted-capture sequencing of 434 genes using the next-generation sequencing implicated in lymphoid malignancies identified the presence of the inversion of *TBLXR1* at chromosome 3q26.32 and *TP63* at chromosome 3q28 in tumor cells from both lymph node involvement and PB, which was confirmed by visualizing soft clipping reads with the integrated genome viewer (IGV) (Figure 2B), whereas *TP53* mutation was not identified. Collectively, the small-sized abnormal cells in PB were convinced to be the transformed subclone of the initial diagnosis of ALK-negative ALCL. Although the relapsed disease was refractory to a series of salvage therapies, including romidepsin, the combination therapy consisting of gemcitabine, dexamethasone, and cisplatin successfully induced complete response (CR). Then, the patient was

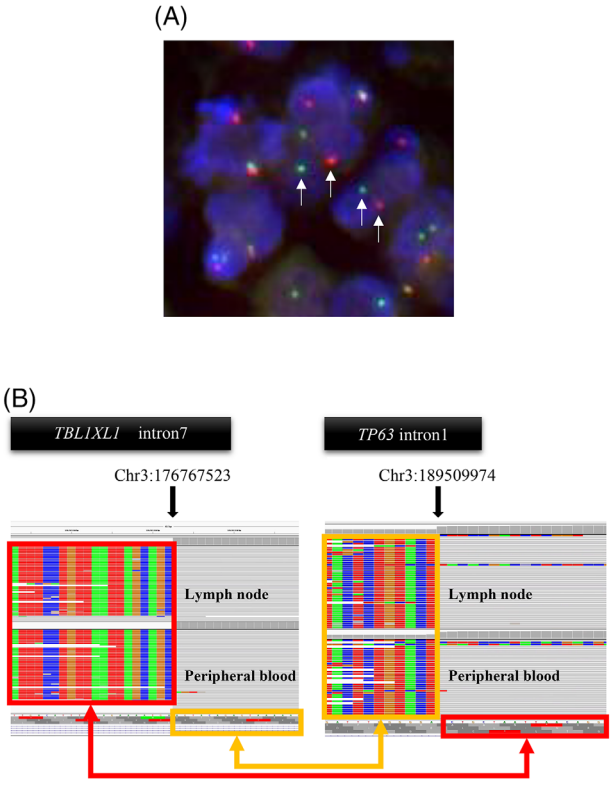

FIGURE 2 Cytogenetic and genetic analyses. (A) Double-color fluorescence in situ hybridization using IRF4 (6p25) break-apart probe (Guangzhou LBP Medicine Science & Technology) showing split signals of *DUSP22* (arrows). (B) Visualizing soft clipping reads with integrated genome viewer on targeted-capture sequence information identified *TBL1XR1-TP63* inversion in tumor cells from both inguinal lymph node and peripheral blood.

subjected to allogeneic peripheral blood stem cell transplantation from the HLA 6/8-matched sibling donor, with a nonmyeloablative regimen consisting of fludarabine (30 mg/m^2) for 6 days plus melphalan (40 mg/m^2) for 2 days. At the time of writing this report, the patient remained in CR for 22 months.

3 | DISCUSSION

In the present case, there are three issues to discuss. The first is the need for acknowledgment of small cell pattern as a leukemic phase in ALK-negative ALCL; the second is the simultaneous presence of *DUSP22*-R and *TP63*-R in ALCL; these issues also need to be combined and discussed. The third is clinical management for this rare pathologic situation. The small cell pattern of ALCL has primarily been restricted to ALK-positive settings [6–8]. Contrastingly, a classical large and pleomorphic morphology is more common with *DUSP22*-R-negative/*TP63*-R-negative subtypes of ALK-negative ALCL. Still, we now recognize a small cell pattern of ALK-negative ALCL in three reported cases, including ours [10, 11]. Regarding cytogenetics, approximately half of the translocation partners of *TP63*-R were *TBL1XR1* in ALK-negative ALCL [13], and *TP63*-R was initially considered mutually exclusive with *DUSP22*-R in ALK-negative ALCL. At the same time, cytologic features of *TP63*-R ALCLs were more variable, and *DUSP22*-rearranged ALK-negative ALCL cells occasionally appeared slightly smaller than those seen in other genetic subtypes [5]. However, we now recognize ALK-negative ALCL with simultaneous *DUSP22*-R and *TP63*-R in four reported cases, including ours (Table S1) [3, 14, 15].

The present case is the first reported case with the mixture of two rare manifestations, that is, a small-sized variant in a leukemic phase in double-hit rearrangements of *DUSP22* and *TP63* in ALK-negative ALCL. In three cases with a small cell pattern with ALK-negative ALCL [10, 11], the present case was the only one with double rearrangements of *DUSP22* and *TP63*. Among these patients, one common cellular characteristic of ALCL cells was the lack of cytotoxic marker typically expressed in ALK-negative ALCL with *DUSP22*-R. In addition, our case was the only one presented with a small cell variant in four cases of ALK-negative ALCL with double-hit rearrangements of DUSP22 and TP63 [3, 14, 15]. It is also intriguing that lymphoma cells of none of the nine patients with ALK-negative ALCL in the leukemic phase exhibit a small cell pattern in ALK-negative ALCL without *DUSP22*-R [1]. More cases are expected to be accumulated to further understand the pathophysiology of ALK-negative ALCL with simultaneous *DUSP22*-R and *TP63*-R ALCL. The therapeutic outcomes of systemic chemotherapy alone seemed unfavorable in small cell patterns, and with the double hit of *DUSP22*-R and *TP63*-R in ALCL [3, 10, 11, 14, 15], thus the poor prognostic impact of *TP63*-R may exceed the favorable prognostic impact of *DUSP22*-R under conventional chemotherapy when co-existed. Our case was the first reported case of a small cell pattern of ALK-negative ALCL with the *DUSP22* and *TP63* gene rearrangements successfully treated by allogeneic stem cell transplantation.

4 | CONCLUSION

In conclusion, recognizing the clinical manifestation and pathophysiologic status of a small cell pattern ALK-negative ALCL with a double hit of *DUSP22*-R and *TP63*-R in a leukemic phase is needed for prompt diagnosis and therapeutic decision-making in ALCL.

AUTHOR CONTRIBUTIONS

Yui Niiyama-Uchibori, Shinsuke Mizutani and Junya Kuroda: Provided the concept and design. **Taku Tsukamoto, Aya Miyagawa-Hayashino, Eiichi Konishi, Kennosuke Karube and Yasuhito Nannya**: Acquired the data. **Yui Niiyama-Uchibori, Shinsuke Mizutani**: Wrote. **Haruya Okamoto, Kennosuke Karube, Yasuhito Nannya and Junya Kuroda**: Reviewed and revised the manuscript. **Daisuke Ide, Akio Onishi, Daishi Kato, Takahiro Fujino and Yuji Shimira**: Provided technical and material support. **Junya Kuroda**: Provided administrative support and supervised the whole study.

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CONFLICT OF INTEREST STATEMENT

Shinsuke Mizutani has received honoraria from Amgen, Astellas, Otsuka Pharmaceutical, Nippon Shinyaku, Chugai Pharmaceutical, Ono Pharmaceutical, Sanofi, and BMS. Takahiro Fujino has received honoraria from Janssen Pharmaceutical, Kyowa Kirin, Chugai Pharmaceutical, and Astellas Pharma. Kennosuke Karube has received research grants from Eisai and Takeda Pharmaceutical, honoraria from Chugai Pharmaceutical, Eisai, Takeda Pharmaceutical, Meiji-Seika Pharma, and Symbio Pharmaceutical. Yasuhito Nannya is a consultant for Otsuka Pharmaceuticals and Bristol Myers Squibb, and has received lecture fees from Otsuka Pharmaceutical, Novartis, Bristol Myers Squibb, Janssen, Astra Zeneca, Astellas, Nippon Shinyaku, Abbvie, and Amgen. Junya Kuroda is a consultant for Janssen Pharmaceutical, Bristol-Myers Squibb (BMS), Pfizer, BeiGene, and Abbvie, and has received research funding from Kyowa Kirin, Chugai Pharmaceutical, Asahikasei, Taiho Pharmaceutical, Otsuka Pharmaceutical, Sumitomo Pharma, Eisai, Japan Blood Product, Regeneron Pharmaceutical, Takeda Pharmaceutical, and Mochida Pharmaceutical, and has received honoraria from Janssen Pharmaceutical, Kyowa Kirin, Chugai Pharmaceutical, Ono Pharmaceutical, Sanofi, BMS, Abbvie, and Novartis. The remaining authors declare they have no conflicts of interest with this article.

FUNDING INFORMATION

No funding was received for this work.

DATA AVAILABILITY STATEMENT

Data supporting this study's findings are available from the corresponding author upon reasonable request. However, due to privacy or ethical restrictions, the data are not publicly available.

ETHICS STATEMENT

The study was conducted in compliance with the Declaration of Helsinki, and the study protocol was approved by the institutional review board (IRB) of Kyoto Prefectural University of Medicine (protocol code ERB-G-113-2) and IRBs of participating institutes.

PATIENT CONSENT STATEMENT

Informed consent was obtained from the patient for the study's procedures and the publication of the case report.

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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