ALK-positive large B-cell lymphoma showing long-term response to conventional chemoradiotherapy

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We describe a single case of a patient with ALK-positive large B-cell lymphoma (ALK+ LBCL) treated in our hospital. The patient was a 61-year-old man who initially presented with a stage III disease involving systemic lymph nodes (LNs), which was diagnosed with immunoblastic lymphoma. He readily responded to 6 cycles of cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP), achieving a long-term complete response. After 5 and a half years, he relapsed with the involvement of left axillary LNs. A biopsy revealed the proliferation of immunoblasts or plasmablasts, which were positive for ALK immunohistochemistry (IHC) with a granular and restricted cytoplasmic staining pattern. The cells were CD20⁻, cytoplasmic immunoglobulin κ⁺, and CD138⁺. Review of the first biopsy confirmed similar IHC results. Cytogenetic and molecular analyses demonstrated t(2;17)(p23;q23), which generated the CLTC-ALK fusion gene. The relapsed lesions were resolved by regional radiotherapy, and the patient is currently free from lymphoma 13 years after the initial presentation. This case report suggests that the outcome of advanced-stage ALK+ LBCL is not necessarily poor and that the disease can be controlled by conventional chemoradiotherapy.

Key words: ALK-positive large B-cell lymphoma, cytoplasmic granular ALK staining pattern, t(2;17)(p23;q23)/CLTC-ALK, chemoradiotherapy

INTRODUCTION

ALK receptor protein kinase-positive large B-cell lymphoma (ALK+ LBCL) was first described by Delsol et al. in 1997 as a subtype of diffuse large B-cell lymphoma (DLBCL) characterized by the expression of ALK protein but lack of t(2;5)(p23;q35).¹,² Subsequent studies have shown that the lymphoma is very rare, accounting for <1% of all DLBCL cases.³ ALK+ LBCL shows a male predominance, with a male to female ratio of 3.5, and occurs in younger individuals with an average age of 38.4 years.⁴ Patients present with generalized lymphadenopathy, mediastinal mass, or extranodal diseases.³,⁵ Bone marrow involvement is found in 30% of cases.³,⁴ Sixty percent of cases present with stage III/IV disease.²,⁴,⁵

The involved lymph nodes (LNs) are composed of marked infiltrates of lymphoma cells often with
a sinusoidal growth pattern\textsuperscript{1,3-5}. The cells exhibit a monomorphic immunoblast-like cell morphology.\textsuperscript{1,5} Additionally, they are negative for pan–B-cell markers (i.e., CD20 and CD\textsuperscript{79a}) but positive for plasma cell markers, such as CD\textsuperscript{138}, and express monotypic immunoglobulins (Ig) in the cytoplasm, with the heavy chain usually being IgA.\textsuperscript{1,3,4,6} Most importantly, lymphoma cells in the majority of cases express ALK with a restricted cytoplasmic granular staining pattern by immunohistochemistry (IHC),\textsuperscript{1,3-5} which is associated with the generation of ALK-CLTC chimeric protein, resulting from t(2;17)(p23;q23), which fuses CLTC at 17q23 to ALK at 2p23.\textsuperscript{7,8} In the minority of cases, ALK fuses to NPMI, SEC31A, SQSTM1, RANBP2, IGL, or EML4,\textsuperscript{3-5,9,10} leading to variable ALK-staining patterns.\textsuperscript{4,10}

This report describes a single case of a patient with ALK+ LBCL treated in our hospital. The course of the treatment and histopathological and cytogenetic features of the disease are presented in detail.

**CASE REPORT**

A 61-year-old man presented with left neck lymphadenopathy. \textsuperscript{18}F-fluorodeoxyglucose (FDG) positron emission tomography (PET) combined with computed tomography (CT) revealed involvement of the left cervical/supraclavicular, left axillary, paraaortic, and left iliac/inguinal LNs (Figure 1A). His hemoglobin...
level was 16.3 g/dL, white cell count was $7.2 \times 10^3/\mu L$, and platelet count was $245 \times 10^3/\mu L$. The level of lactate dehydrogenase (LD) was 303 U/L (reference range, 100 to 225 U/L), aspartate aminotransferase (AST) was 33 U/L, alanine aminotransferase (ALT) was 30 U/L, total protein was 8.0 g/dL, albumin was 4.1 g/dL, globulin was 3.9 g/dL, creatinine was 0.7 mg/dL, uric acid was 6.4 mg/dL, C-reactive protein (CRP) was 1.5 mg/dL, and soluble interleukin-2 receptor (sIL-2R) was 603 U/mL (reference range, 124 to 496 U/mL). He had no fever, weight loss, or night sweat. His ECOG performance status was 0.

Biopsy of the inguinal LNs revealed diffuse infiltrates of immunoblastic cells that lacked CD20 on their cell surface but expressed Ig κ light chain in their cytoplasm. After the diagnosis of stage III immunoblastic lymphoma, we treated the patient with 6 cycles of cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP), leading to resolution of the lymphoma lesions.

Five and a half years after the completion of CHOP, the patient relapsed with enlargement of the left axillary LNs, in which $^{18}$F-FDG was accumulated with a maximum standardized uptake value (SUVmax) of 6.50; no other LN areas or extranodal sites were affected (Figure 1B). His hemoglobin level was 13.2 g/dL, white cell count was $5.27 \times 10^3/\mu L$, and platelet count was $212 \times 10^3/\mu L$. The level of LD was 238 U/L, AST was 45 U/L, ALT was 54 U/L, total protein was 7.3 g/dL, albumin was 4.4 g/dL, creatinine was 0.8 mg/dL, uric acid was 3.8 mg/dL, CRP was <0.2 mg/dL, and sIL-2R was 282 U/mL. Bone marrow examination revealed normal hematopoietic precursors.

Biopsy showed a similar histopathology to that of the initial biopsy; however, the lymphoma classification remained undetermined at this time. Nevertheless, because the disease was localized within the left axillary region, the patient received radiation therapy consisting of 30 Gy in 15 fractions for the left axillary region followed by booster doses of 16 Gy in 8 fractions for the tumor bed. He is currently free from lymphoma 13 years after the initial presentation (Figure 1C).

HISTOPATHOLOGY OF BIOPSIES
The second LN biopsy from the left axillary legion was subjected to detailed histopathological examination. Under low-power magnification, the specimen was composed of cohesive sheets of tumor cells surrounded by dense collagen bands, showing a nodular appearance (Figure 2A). In areas where the LN architecture was partially preserved, proliferation of the tumor cells within the vessels was found (Figure 2B). Under higher-power magnification, the cells had round nuclei containing single, central, and prominent nucleoli and abundant eosinophilic to amphophilic cytoplasm (Figure 2C). Occasional cells carried eccentric nuclei with perinuclear halo space (Figure 2C, arrows). Mitotic figures were frequent.

IHC using an anti-ALK antibody demonstrated that neoplastic cells had a granular cytoplasmic staining pattern (Figure 2D). The cells were negative for CD20 but slightly positive for CD79a (Figures 2E and F). Restrictive Ig κ light chain positivity in the cytoplasm and CD138 expression in a fraction of the cells confirmed the plasma cell differentiation of lymphoma cells (Figures 2G, H, and I). The Ki-67 proliferation index was 60–80% (Figure 2J). Epithelial membrane antigen (EMA) was weakly positive in a small fraction of the cells. Other negative antigens studied included CD3, CD10, CD21, CD30, CD56, BCL2, AE1/AE3, CAM5.2, CK7, CK20, HMB45, and S-100. Review of the first biopsy confirmed comparable IHC to that of the second biopsy.

Flow cytometry failed to reveal clonal proliferation of the neoplastic B-cells, while polymerase chain reaction (PCR) for rearrangement of the antigen receptor genes generated a single species of PCR products, representing rearrangements of IGK and IGH.
CYTOGENETIC AND MOLECULAR STUDIES

Cytogenetic preparation was done using tumor cells of the second biopsy. G-banding revealed a complex karyotype with multiple structural alterations, including that of the short arm of chromosome 2. We applied the ALK break-apart (BA) probe (Abbott Laboratories, Abbott Park, IL, USA) to the metaphase spreads and found that der(17)t(2;17)(p23;q23) at q23 was labeled by the red-colored telomeric 3’ probe and the green-colored centromeric 5’ probe remained at p23 of der(2)t(2;17)(p23;q23), to which additional chromosomal materials attached at band q37 [der(2)t(2;17)(p23;q23) add(q37)], indicating that the ALK gene was disrupted between the 5’ and 3’ probes (Figure 3A). del(2)(q33) was labeled by an unrearranged ALK signal at p23. Accordingly, interphase nuclei showed a red, green, and yellow (fusion) hybridization signal pattern (Figure 3B).

Total RNA was prepared from cryopreserved biopsy tissue embedded in Tissue-Tek® O. C. T.™ Compound (Sakura Finetek Japan, Tokyo, Japan) using a RNeasy Mini Kit (QIAGEN, Hilden, Germany). Reverse transcription (RT)-PCR using nested primer combinations designed for CLTC and ALK amplified DNA corresponding to the CLTC-ALK fusion mRNA, and RT-PCR amplifying the kinase domain of ALK generated the expected product size of DNA (Figure 2).

Figure 2. Histopathology of the biopsy of the left axillary LNs. A, H&E staining (original magnification, 10× objective lens); B, H&E staining (20×); C, H&E staining (100×); D, anti-ALK immunohistochemistry (40×; inset, 100×); E, anti-CD20 (20×); F, anti-CD79a (20×); G, anti-lg κ light chain (20×); H, anti-lg λ light chain (20×); I, anti-CD138 (20×); and J, anti–Ki-67 (20×). In C, arrows indicate plasmablastic cells carrying eccentric nuclei and perinuclear halo space.
Sanger sequencing of the former RT-PCR products confirmed the in-frame fusion between CLTC exon 31 and ALK exon 20 (Figure 3D).

**DISCUSSION**

Here, we described the case of ALK+ LBCL showing characteristic histopathology except for equivocal EMA positivity. Cytogenetic and molecular studies revealed that t(2;17)(p23;q23) generated the CLTC-ALK fusion gene, accounting for the ALK expression with the granular and restricted cytoplasmic IHC pattern. The patient initially presented with a stage III disease involving systemic LNs, which was classified as high-intermediate risk according to both the International
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Prognostic Index (IPI) and NCCN-IPI scoring scheme, and he readily responded to CHOP chemotherapy. When he relapsed, the disease was confined to the axillary region and was resolved by regional radiotherapy, resulting in long-term survival.

Two-year and 5-year overall survival rates of patients with stage I/II ALK+ LBCL treated with CHOP or CHOP-like regimens were reported to be 76% and 66%, respectively, while those of stage III/IV disease were 27% and 8%, respectively. However, our study suggests that the outcome of advanced-stage ALK+ LBCL is not necessarily poor and that the disease can be controlled by conventional chemoradiotherapy. On the other hand, a review of ALK+ LBCL cases showed that a granular ALK IHC staining pattern, which is the surrogate of the CLTC-ALK fusion gene, was associated with higher response rate to CHOP-like treatment and better survival compared with a non-granular staining pattern. Thus, ALK partners may have to be considered to predict the outcome of each ALK+ LBCL patient.

The diagnosis of ALK+ LBCL is sometimes challenging because lymphoma cells may form cohesive sheets or nests in LN and occasionally express cytokeratin, thus resembling non-hematolymphoid neoplasms. A nodular growth pattern initially misdiagnosed with carcinoma was described previously. In the current case, based on hematoxylin & eosin (H&E) staining observations, we first considered metastatic melanoma, and the initial IHC panel consisted of epithelial cell markers in addition to HMB45 and S-100, resulting in negative results for all antigens tested. Because cytogenetic laboratory results revealed rearrangement of the ALK locus, this diagnostic difficulty was resolved by the demonstration of ALK expression by IHC, finally leading to the correct diagnosis of ALK+ LBCL, in agreement with CD20, cytoplasmic Ig κ, and CD138. We therefore suggest FISH to interphase nuclei to promptly identify the ALK rearrangement along with histopathological examination for the differential diagnosis of ALK+ LBCL.

Because ALK+ LBCL is negative or only weakly positive for CD30 expression, patients theoretically do not benefit from anti-CD30 monoclonal antibody therapy, which is performed for ALK+ anaplastic large cell lymphoma (ALCL). On the other hand, the positive effect of ALK-targeting therapy in ALK-rearranged non-small cell lung cancer (NSCLC) has been extended to non-NSCLC tumors driven by ALK fusion genes, including ALK+ LBCL. The anti-tumor activity of a selective ALK inhibitor, NVP-TAE684, was demonstrated in an ALK+ LBCL cell line (LM1) carrying the CLTC-ALK fusion gene. The inhibitor repressed ALK-activated signaling pathways and induced the apoptosis of LM1 cells, which led to sustained tumor regression in a xenotransplant tumor model. Crizotinib, a first-generation ALK inhibitor, induced a short-term response in a young adult patient with ALK+ LBCL who relapsed after autologous hematopoietic transplantation (HSCT). It is now clear that treatment with crizotinib is limited by the development of drug resistance due to mutations within the ALK fusion gene. A second-generation ALK inhibitor, alectinib, was administered in combination with a cytotoxic drug to a pediatric patient with CLTC-ALK ALK+ LBCL who showed systemic relapse including the bone marrow. The patient achieved a second complete response and underwent allogeneic HSCT (allo-HSCT), suggesting that the alectinib-containing treatment provides a potential bridge to allo-HSCT. Because alectinib was proven to be effective for ALK+ ALCL with favorable toxicity profiles and approved to be used for ALK+ ALCL in the relapsed/refractory setting in Japan and because clinical trials on ALK+ LBCL are impractical due to the paucity of this rare DLBCL subtype, off-label use of alectinib may be considered for relapsed/refractory ALK+ LBCL when the patient is fully informed and consents to the treatment.
In summary, we presented a patient with stage III ALK+ LBCL showing granular cytoplasmic ALK expression and harboring t(2;17)(p23;q23)/CLTC-ALK. The patient readily responded to CHOP and regional radiation therapy, and achieved long-term survival. These results support conventional chemoradiotherapy as the initial treatment for patients with advanced-stage ALK+ LBCL carrying the CLTC-ALK fusion gene.

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従来の化学放射線治療が長期間奏効している ALK 陽性大細胞型 B 細胞リンパ腫の 1 例

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当院で診療した ALK 陽性大細胞型 B 細胞リンパ腫（ALK+ LBCL）の 1 例を報告する。患者は 61 歳の男性で、病期 III 期で発症し、生検の結果、免疫芽球性リンパ腫と診断した。患者は 6 サイクルの CHOP 療法（シクロホスファミド、ドキソルビシン、ビンクリスチン、プレドニゾロン）に速やかに反応し、長期間完全寛解を維持した。5 年半後左腋窩リンパ節に再発した。生検では、免疫芽球または形質芽細胞の形態を示す腫瘍細胞の増殖を認め、ALK 免疫染色で細胞質に限局した顆粒状の染色パターンを示した。腫瘍細胞は CD20 陰性、細胞質κ鎖陽性、CD138 陽性であった。初回生検の免疫染色も同様の結果であった。染色体分析で t(2;17)(p23;q23) 転座を認め、RT-PCR とシークエンシングで CLTC-ALK 融合遺伝子を検出した。再発病変は放射線治療で消失し、初診後 13 年経過した時点でリンパ腫の再燃を認めない。本症例の治療経過は、進行病期 ALK+ LBCL の治療予後は必ずしも不良ではなく、従来の化学放射線治療によってコントロール可能であることを示唆している。

キーワード：ALK 陽性大細胞型 B 細胞リンパ腫、細胞質に限局した顆粒状の ALK 染色パターン、t(2;17)(p23;q23)/CLTC-ALK 転座、化学放射線治療