Brentuximab vedotin for refractory anaplastic lymphoma kinase-negative anaplastic large cell lymphoma in leukemic phase with *RUNX3* overexpression

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Abstract

Anaplastic lymphoma kinase (ALK)-negative anaplastic large cell lymphoma (ALCL) is an aggressive CD30-positive non-Hodgkin lymphoma. ALK-ALCL rarely manifests with extensive bone marrow and peripheral blood involvement (known as "leukemic phase"). A 54-year-old woman was diagnosed with ALK-ALCL in leukemic phase, characterized by an extremely poor prognosis. Lymphoma cells in this case showed chromosomal translocation 1p36.1-encoded RUNX3 and overexpression of its protein. She was refractory to CHOP and salvage chemotherapy. Fortunately, she achieved complete remission with three cycles of Brentuximab vedotin (BV) and underwent umbilical cord blood transplantation. However, she died due to treatment-related mortality on day 129. The autopsy findings showed no lymphoma cells. Treatment strategy for ALK-ALCL is controversial, but the efficacy of BV in CD30-positive peripheral T-cell lymphoma not only as salvage regimens, but also in first line, has been reported in recent years. BV may be an effective option for ALK-ALCL in leukemic phase.

Introduction

Anaplastic lymphoma kinase (ALK)-negative anaplastic large cell lymphoma (ALCL) is a CD30-expressing aggressive T-cell lymphoma that has been entered as a provisional entity in the WHO 2008 classification representing 2 to 3% of non-Hodgkin lymphomas (NHLs) and 12% of T-cell NHLs.[1] The prognosis of ALK-ALCL patients is poor, with 5-year overall survival (OS) and progression-free survival (PFS) rates of 49% and 36%, respectively, compared with the more favorable 5-year OS and PFS rates of 70% and 60% for ALK-positive disease (ALK+ALCL).[2] Although ALCL cases with extensive bone marrow and peripheral blood involvement manifested as "leukemic phase" are extremely rare, most patient survive for less than a year.[3] Most ALK-ALCL patients are treated with CHOP-like regimens, but the treatment strategies for ALK-ALCL are still controversial and an optimal therapy has not yet been established.

Brentuximab vedotin (BV) is an antibody-drug conjugate composed of an anti-CD30 chimeric antibody conjugated with the microtubule-disrupting agent, monomethyl auristatin E (MMAE). BV efficacy in relapsed or refractory ALCL has been recently reported to have favorable outcomes.[4] Herein is reported the case of a refractory ALK-ALCL patient in leukemic phase. In this case, the patient achieved complete remission (CR) with single agent BV therapy, and could undergo umbilical cord blood transplantation. This case indicates that BV monotherapy could act as an effective bridge to allogeneic hematopoietic stem cell transplantation (allo-HSCT), despite refractoriness to the three preceding regimens.

Case Report

A 54-year-old Japanese woman without a previous medical history was referred to our hospital with complaints of right supraclavicular lymph node enlargement. Physical examination revealed a 38.1°C body temperature and right cervical and supraclavicular lymphadenopathy. Laboratory findings showed leukocytosis (white blood cell count, 74.4x10^9/L) with 72% atypical lymphocytes and increased aspartate transaminase (AST; 695 IU/L), alanine transaminase (ALT; 422 IU/L), lactate dehydrogenase (LDH; 8,013 IU/L), and soluble interleukin-2 receptor (29,946 U/mL). The patient was also negative for anti-human T-cell lymphotropic virus type I antibody and anti-human immunodeficiency virus antibody in her sera. Laboratory results are shown in Table 1.

Bone marrow aspiration revealed a hypercellular marrow (nuclear cell counts of 305,000//μL and myeloid/erythroid ratio of 2.3) with large atypical lymphocytes accounting for 42%. Moreover, flow cytometry analysis of bone marrow cells revealed that atypical cells expressed CD2, CD3, CD4, CD7, CD25, CD30, CD56, HLA-DR, and TCR-αβ without TdT. Almost all atypical cells strongly expressed CD30. Additionally, clonal T-cell receptor (TCR) gene rearrangement was detected by Southern blotting. Biopsy of the right supraclavicular lymph node showed diffuse growth of medium-sized atypical lymphocytes (Figure 1A). Immunohistochemistry revealed neoplastic cell positivity for CD3, CD4, CD30, CD56, MUM1, Granzyme B, and TIA, and negativity for CD5, CD8, CD20, PAX-5, ALK, and EMA (Figure 1B, data not shown). Epstein-Barr virus (EBV)-encoded RNA was also negative by *in situ* hybridization. ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography (^{18}F-FDG PET/CT) scan showed lymphadenopathy of several lymph

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nodes, with the liver, spleen, and bone marrow showing intense ^{18}F-FDG PET/CT accumulation (Figure 1C). Our patient was diagnosed with Stage IVB ALK-ALCL in leukemic phase. Subsequent cytogenetic analysis revealed a complex karyotype, including an additional chromosomal aberration of 1p36.1 in all 20 metaphase cells (Figure 1E). Interestingly, RUNX3, which is mapped to human chromosome 1p36.1, has an oncogenic role in natural killer/T-cell lymphoma and is transcriptionally regulated by MYC.[5] Western blot analysis showed that the RUNX3 protein was highly expressed in this patient's peripheral blood mononuclear cells (PBMCs) including lymphoma cells (Figure 1F). The patient received a CHOP regimen in three-week cycle; cyclophosphamide (CPA) 750 mg/m^2, vincristine (VCR) 1.4 mg/m^2, and doxorubicin (DXR) 50 mg/m^2 on day 1, and prednisolone (PSL) 100 mg/body on days 1-5. Although the tumor burden decreased, the patient suddenly developed pain in the whole body after two CHOP cycles (Figure 2). A lumbar puncture was immediately performed due to suspicion of central nervous system (CNS) involvement. The protein level in the cerebrospinal fluid (CSF) was 731 mg/dL and the glucose level were 15 mg/dL; 499 atypical cells/μL were detected. Bacterial, tuberculosis, and fungal cultures were

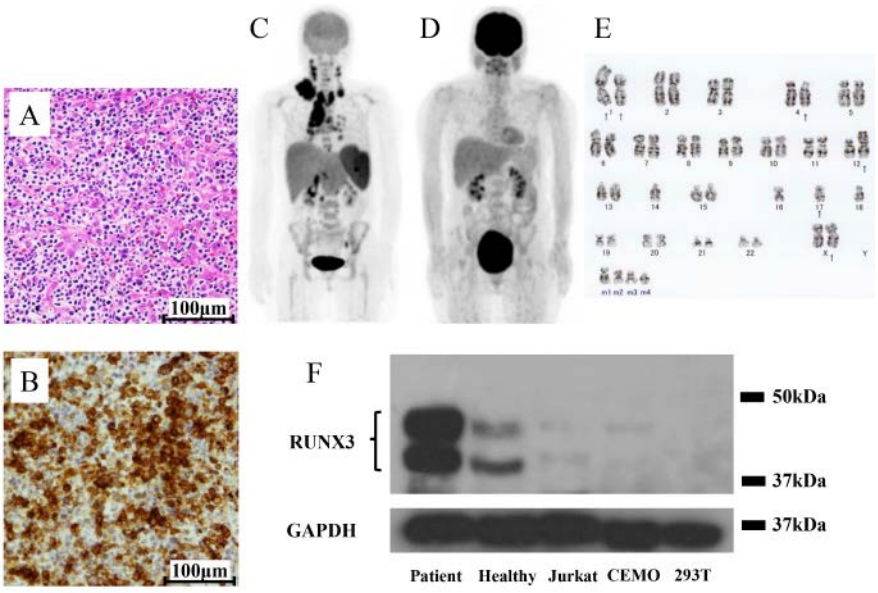


Figure 1. Pathological images obtained from the right supraclavicular lymph node of our patient. Hematoxylin and eosin staining (A, x400). Anti-CD30 immunostaining (B, x400). 18F-FDG PET/CT at initial diagnosis (C) and just before allo-HSCT (D). The G-banding chromosomes in the patient's bone marrow revealed 46, X, add(X)(p11.2), add(1)(p36.1), del(1)(p?), add(4)(p11), add(12)(p11.2), -14, -16, -17, add(17)(q21), -18, +mar1, +mar2, +mar3, and +mar4 in all 20 metaphase cells (E). Western blotting analysis of protein extracts from the patient's peripheral blood mononuclear cells (PBMCs), healthy donor's PBMCs, Jurkat cell line (derived from acute T cell leukemia), CEMO cell line (derived from acute B cell leukemia), and HEK293T cell line (derived from human embryonic kidney) using mouse monoclonal anti-RUNX3 antibodies (R3-5G4; Santa Cruz, CA, USA) (F).

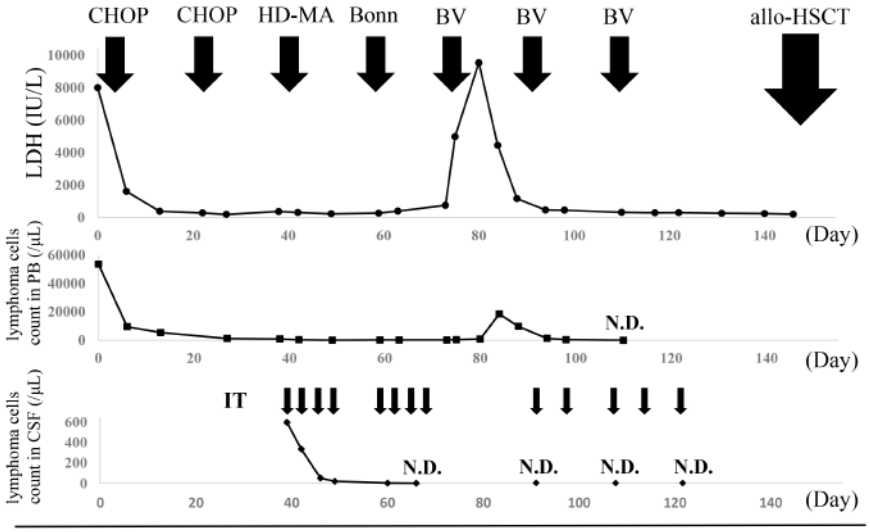
Table 1. Laboratory data of the patient at the first visit to our hospital

Complete blood count	Coagulation system	Chemistry
White Blood Cells 74.4×10^9/L	Prothrombin time 13.2 s	Creatinine 0.51 mg/dL
Metamyelocyte 1.0%	APTT 32.0 s	Blood Urea Nitrogen 28.0 mg/dL
Neutrophil 13.0%	Fibrinogen 230 mg/dL	Sodium 128 mEq/L
Lymphocyte 12.0%	FDP 13.9 mg/L	Potassium 4.1 mEq/L
Monocyte 2.0%		Chloride 94 mEq/L
Atypical lymphocyte 72.0%		Calcium 8.9 mg/dL
Red Blood Cells 4.85×10^{12}/L		Creatine Kinase 56 IU/L
Hemoglobin 14.2 g/dL		Aspartate Transaminase 695 IU/L
Hematocrit 40.5%		Alanine Transaminase 422 IU/L
Reticulocytes 71.0×10^9/L		Total Bilirubin 1.5 mg/dL
MCV 83.5 fL		Lactate Dehydrogenase 8,013 IU/L
Platelets 96×10^9/L		Total Protein 7.0 g/dL
		Albumin 3.5 g/dL
		C-reactive Protein 2.86 mg/dL
		IgG 15.6 g/L
		IgA 2.87 g/L
		IgM 2.36 g/L
		Ferritin 6,182ng/mL
		sIL-2R 29,946 U/mL
		anti-HTLV-1 antibody negative
		anti-HIV antibody negative

MCV: mean corpuscular volume; APTT: Activated partial thromboplastin time; FDP: Fibrin degradation products; Ig: Immunoglobulin; sIL-2R: soluble interleukin-2 receptor; HTLV-1:Human T-cell leukemia virus type 1 HIV: Human immunodeficiency virus.

negative. Cytology revealed lymphoma cells. Flow cytometry analysis of CSF lymphoma cells showed expression of CD2, CD3, CD4, CD7, CD25, CD30, CD56, and TCR-αβ. These results were consistent with peripheral blood and bone marrow.

The patient's subsequent treatments were as follows: one cycle of high-dose methotrexate (MTX) and cytarabine (Ara-C) (MTX 1 g/m^2 on day 1, Ara-C 4 g/m^2 on days 2 and 3); one cycle of modified Bonn protocol cycle A (MTX 3 g/m^2 on day 1, VCR 2 mg/body on day 2, ifosfamide 800 mg/m^2 on days 2-5, and dexamethasone 16.5 mg/body on days 2-5),[6] with eight administrations of twice weekly intrathecal chemotherapy (IT) consisting of MTX 15 mg, Ara-C 40 mg, and PSL 20 mg. However, lymphoma cells in peripheral blood did not disappear; abnormal cells of 203/μL. Furthermore, her left supraclavicular lymph node had regrown and her serum LDH level was increased rapidly. But, lymphoma cells in CSF was not detected. Therefore, the patient received BV 1.8 mg/kg every 21 days with weekly IT. After three BV cycles and five IT administrations, bone marrow aspiration and PET/CT revealed complete remission (CR) (Figure 1D). BV worsened grade 2 vincristine-induced peripheral neuropathy (PN). Due to grade 3 PN, the patient's BV therapy was discontinued, and we scheduled the patient to undergo allo-HSCT. However, as a suitable human leukocyte antigen (HLA) identical donor was not found, umbilical cord blood from two of six HLA antigen mismatched female donor was selected as the stem cell source (patient HLA: A0201/3303, B3501/4403, Cw0303/1403, DR0403/0803; donor HLA: A0201/2603, B3501/5901, Cw0102/0303, DR0405/0802). The amounts of nucleated cells and CD34-positive cells in this cord blood were 3.07x10^7 /kg and 1.43x10^5 /kg, respectively. Due to the high rate of early relapse for refractory leukemic phase ALK-ALCL, we selected to use allo-HSCT with a myeloablative conditioning (MAC) regimen consisting of intravenous busulfan 12.8 mg/kg (3.2 mg/kg on days -7 to -4) and CPA 120 mg/kg (60 mg/kg on days -3 and -2). For graft-versus-host-disease (GvHD) prophylaxis, tacrolimus was started from day -1, and mycophenolate mofetil 1,500 mg/body was started from day 0. Neutrophil engraftment was observed on day 26 and bone marrow aspiration on day 97 showed no malignancy and detected complete donor chimerism. CT scan after the transplantation revealed that no lymphoma lesion was detected. Due to regimen-related toxicity, including pre-engraftment immune reaction, febrile neutropenia, and acute GvHD (grade I with skin stage 2), the patient's performance status deteriorated to 4. Hemorrhagic cystitis due to BK virus and unidentified pneumonia further aggravated her overall status. Unfortunately, she died on day 129 due to progression of respiratory failure, with no response to antibiotic and steroid treatments. Autopsy findings revealed that her respiratory failure was compatible with idiopathic pneumonia syndrome and showed no lymphoma cells in multiple organs including the CNS.



LDH: lactate dehydrogenase; PB: peripheral blood; CSF: cerebrospinal fluid; CHOP (cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2 and doxorubicin 50 mg/m^2 on day 1 and prednisolone 100 mg/body on day 1-5); HD-MA: High dose-MTX/AraC (methotrexate 1 g/m^2 on day 1 and cytarabine 4 g/m^2 on day 2, 3); Bonn: Modified Bonn protocol cycle A (methotrexate 3 g/m^2 on day 1, vincristine 2 mg/body on day 2, ifosfamide 800 mg/m^2 on day2-5 and dexamethasone 16.5 mg/body on day2-5); BV: Brentuximab Vedotin 1.8 mg/kg; allo-HSCT: allogeneic hematopoietic stem cell transplantation; IT: intrathecal chemotherapies (methotrexate 15 mg/body, cytarabine 40mg/body and prednisolone 20 mg/body); N.D.: Not detected

Figure 2. The patient's clinical course before the allo-HSCT.

Discussion and Conclusions

Leukemic phase ALCL, characterized by extensive bone marrow and peripheral blood involvement, is rare. Such cases have been occasionally reported, most of which are childhood ALK+ALCL cases. ALCL patients in leukemic phase have a poor prognosis, regardless of ALK expression.[3] Like the surface marker in our patient, the expression of CD56 in ALK-positive and ALK-negative ALCL is associated with a higher incidence of bone marrow involvement and is also considered a poor prognostic factor for OS.[7] Moreover, CNS involvement is an uncommon event in peripheral T cell lymphoma (PTCL), with incidence rates ranging from 2.1 to 4.5%, and the median OS after CNS relapse reported to be 1.1 to 1.5 months.[8,9] Therefore, management of PTCL with CNS involvement is particularly challenging. To the best of our knowledge, only six cases of ALK-ALCL in leukemic phase have been reported, and only one case has been reported with CNS involvement.[3,10] These cases, including our patient, provide a valuable source of information for treatment strategies for this extremely rare ALK-ALCL in leukemic phase.

Autologous HSCT (auto-HSCT) for ALK-ALCL had a superior OS and PFS compared with only conventional chemotherapy.[11] Although ALCL in leukemic phase or with CNS recurrence has a dismal prognosis, some studies also have reported long-term survival outcomes with auto-HSCT.[3,12] According to a report by Smith *et al.*, auto-HSCT has a better prognosis than allo-HSCT for ALCL (3y-OS; 68% vs 41%. non-relapse mortality; 5% vs 32%).[13] However, allo-HSCT for ALCL may be effective depending on the patient's condition. Undoubtedly, the efficacy of allo-HSCT in refractory ALCL has been demonstrated in recent years, given the potential for graft-versus-lymphoma effects and reports of durable remissions and few late relapses.[13,14] Meanwhile, in non-Hodgkin's lymphoma, one concern is the higher relapse rate associated with reduced intensity conditioning (RIC) regimen compared to MAC regimen.[15] Nevertheless, our patient should have been selected RIC regimen when considering her clinical outcome after the transplantation.

The efficacy of BV in patients with ALCL has been reported in recent years. Pro

et al. reported favorable treatment outcomes in patients with relapsed or refractory systemic ALCL treated with single-agent BV (CR ratio of 66%, 5-year OS of 60%, 5-year PFS of 39%).[4] In the present case, the patient achieved CR with single-agent BV combined with IT despite chemoresistance and CNS involvement. It has previously been reported that a patient with ALCL with CNS involvement responded to BV-based regimens,[16] and the present case suggests BV efficacy for ALCL patients with CNS involvement. Although the patient received BV as the fourth treatment regimen, CR was achieved for the first time. It can be speculated that BV monotherapy as a first-line regimen might have been even more effective in this patient.

Recent reports indicate that, for patients with CD30-positive PTCL, first-line combination therapy with BV can significantly improve the patient's outcome. Horwitz et al. reported the efficacy of A+CHP regimens (BV, CPA, DXR, and PSL) for CD30-positive PTCL, including ALCL. According to those authors, front-line treatment with A+CHP is superior to CHOP, as shown by the significant PFS and OS improvement with a manageable safety profile observed in a global, double blind randomized, phase 3 trial (median PFS of 48.2 months in the A+CHP group and 20.8 months in the CHOP group. Median OS was not reached for either group. HR=0.66; p-value=0.0244).[17] Additionally, refractory or relapsed PTCL patients have recently been designated to several clinical trials of BV in combination with other agents, such as romidepsin and lenalidomide.[18] Combination chemotherapy, including BV as a first-line therapy, should be considered for aggressive ALK-ALCL patients, as in the present case.

The runt-related transcription factors (RUNX) family is composed of three members, RUNX1, RUNX2, and RUNX3, each of which forms a functional complex with a core binding factor β partner protein. RUNX1 and RUNX3 are expressed in various blood corpuscles and are associated with T-cell differentiation and function.[19] RUNX3, the coding gene is located on 1p36.1, acts as a tumor suppressor in various tumor types, and functions as an oncogene in NK/T lymphomas, leukemia, head and neck squamous cell carcinoma, ovarian cancer, and Ewing sarcoma.[5] In recent reports, RUNX3 was reported to be highly associated with chemoresistance in leukemic cells.[20] High RUNX3 protein expression was observed in lymphoma cells of our patient, suggesting resistance to conventional cytotoxic agents and that molecularly targeted therapy and/or monoclonal antibodies would be more effective.

In this report, ALK-ALCL in leukemic phase with CNS involvement was refractory to a variety of prior chemotherapy regimens but could be well controlled with BV monotherapy. Moreover, RUNX3 overexpression in our patient with ALK-ALCL seems to have a poorer prognostic value for the outcome. Accumulation of cases with leukemic phase ALK-ALCL is required for establishment of prognostic tools and therapeutic strategies in the future.

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