Acta Med. Okayama, 2016
Vol. 70, No. 6, pp. 503–506


Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

*Case Report*

# Two Relapsed Stage III Childhood Anaplastic Large Cell Lymphoma Patients with *NPM-ALK* Fusion in Bone Marrow from Initial Diagnosis

**Yui Kanazawa[a], Yuka Yamashita[b], Mitsuhiro Fujiwara[c], Michiko Muraoka[a], Kana Washio[a], Kiichiro Kanamitsu[a], Hisashi Ishida[a], Takae Nakano[a], Miho Yamada[b], Keizo Horibe[b], Takehiro Tanaka[d], Tadashi Yoshino[d], and Akira Shimada[a]***

*Departments of [a]Pediatrics, and [d]Pathology, Okayama University Hospital, Okayama 700–8558, Japan,*
*[b]Clinical Research Center, Nagoya Medical Center, Nagoya 460–0001, Japan,*
*[c]Department of Pediatrics, Kurashiki Central Hospital, Kurashiki, Okayama 710–8602, Japan*

**Childhood anaplastic large cell lymphoma (ALCL) accounts for approx. 10–30% of cases of non-Hodgkin lymphoma, and the ALCL99 study reported 60–75% disease-free survival; however, a relatively high relapse rate was observed (25–30%). We report 2 patients with Stage III ALCL who relapsed 6–18 months after the end of ALCL99 chemotherapy. A retrospective molecular analysis identified the nucleophosmin (NPM)-anaplastic lymphoma kinase (ALK) fusion gene in the first diagnostic bone marrow samples taken from both patients. However, antibodies against the ALK protein appeared to be relatively low in the serum of both patients (×100 and ×750). An increase in chemotherapy intensity may be beneficial if Stage III ALCL patients are shown to be *NPM-ALK* chimera-positive in the first diagnostic bone marrow sample.**

**Key words:** ALCL, *NPM-ALK* fusion, lymphoma

Anaplastic large cell lymphoma (ALCL) is a T-cell or null-phenotype lymphoma. Most cases have t(2;5)(p23;q25), which results in a fusion between the anaplastic lymphoma kinase (ALK) gene at chromosome band 2 and the nucleophosmin (NPM) gene at chromosome band 5. This fusion increases the activity of the ALK protein, which results in the proliferation of ALCL cells.

Childhood ALCL accounts for 10–20% of childhood non-Hodgkin lymphoma cases, and the advanced stages (stage III and IV) of childhood ALCL were reported to have a 2-year event-free survival (EFS) rate of 70–80%. Standard therapy for patients with stage I or II ALCL who have undergone a total resection of the tumor is only three courses of intensive combined chemotherapy, whereas patients at other advanced stages of cancer often receive 6 courses of chemotherapy. Hematopoietic stem cell transplantation (HSCT) is not recommended as a first-line therapy for ALCL [1–3]. The BFM, SIOP, and UKCCSG retrospective studies demonstrated that invasion to the mediastinum, skin, liver, and lung were risk factors for a poor prognosis of ALCL [4].

Here, we report 2 patients who relapsed 6 to 18 months after the end of ALCL99 therapy [5]. Our analysis of stored samples taken at the initial diagnosis identified the *NPM-ALK* fusion gene in both patients.

Received January 4, 2016; accepted May 13, 2016.
*Corresponding author. Phone:+81–86–235–7249; Fax:+81–86–221–4745
E-mail:pajj236e@okayama-u.ac.jp (A. Shimada)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

The prognostic impact of monitoring the *NPM-ALK* chimera will also be discussed.

## Case Report

***Patient 1.*** In September 2010, a 15-year-old Japanese female presented with fever and swelling of the cervical lymph nodes, cervix, axillae, supraclavicular fissure, mediastinum, hilus, paravertebrae, and the upper abdomen (Fig. 1A). Because she was in critical condition on admission, positron emission tomography (PET) images could not be obtained. The diagnosis of Stage III ALCL was made by lymph node biopsy. Horseshoe-shaped large cells were positive for both CD30 and ALK in the nucleus and cytoplasm (Fig. 2A, B). Bone marrow (BM) aspirate was normal, and no malignancy was observed in the cerebrospinal fluid. She had achieved complete remission after undergoing the ALCL99 regimen; but 8 months after the end of chemotherapy, relapse was confirmed in the right inguinal lymph node. She was treated by with the Berlin-Frankfurt-Munster (BFM) regimen and achieved a second complete remission [1].

The patient eventually underwent an unrelated bone marrow transplantation (BMT) with a conditioning regimen of etoposide (VP16), endoxan (CY), and total body irradiation (TBI). Graft-versus-host disease (GVHD) prophylaxis of tacrolimus and short-term methotrexate was administered. She transiently suffered from pure red cell aplasia from an unknown cause, but she has maintained complete remission for 38 months since the BMT.

***Patient 2.*** A 14-year and 5-month-old Japanese female presented with fever and lymph node swelling throughout her entire body in May of 2011 (Fig. 1B). Computed tomography (CT) and PET CT showed enlarged lymph nodes both above and below the diaphragm, and invasion into the left adrenal grand. The diagnosis of ALCL was made based on the lymph node biopsy. Large cells positive for CD30 and ALK in both the nucleus and cytoplasm were observed (Fig. 2C, D). BM aspirate demonstrated normocellular marrow, and no malignant evidence found in the cerebrospinal fluid.

She achieved a first complete remission by ALCL99; however, relapse occurred in multiple lymph nodes, observed by CT 18 months after the end of the chemotherapy. She underwent a BMT with a conditioning regimen of VP16, CY, and TBI. GVHD prophylaxis of cyclosporine A and short-term methotrexate was administered. She has maintained complete remission for 33 months since the BMT.

***The Histopathological Examinations.*** Biopsy samples were stained with hematoxylin and eosin. The immunohistochemical analysis was performed using an automatic immunostainer (Ventana Medical Systems, Tucson, AZ, USA) with CD30 (Ber-H2) (Dako, Carpenteria, CA, USA), ALK, CD2, CD3 (polyclonal), CD5, CD43, CD15, CD20, CD79a, CD45RO(UCHL-1), CD45(LCA), S-100, TIA-1, Granzyme B, Ki-67 and epithelial membrane antigen (EMA; Dako) antibodies.

***Quantification of NPM-ALK Chimera by Real-time PCR and ALK antibody.*** We investigated the presence of the *NPM-ALK* chimera by conducting a real-time polymerase chain reaction (PCR), and we measured the antibody response to ALK as described [6,7]. The copy number of the *NPM-ALK* fusion transcript was normalized by $10^4$ copies of ABL, and the antibody dilutions used were 1/50, 1/100, 1/250, 1/750, 1/6,750, 1/20,250, and 1/60,750. Informed consent was obtained from the

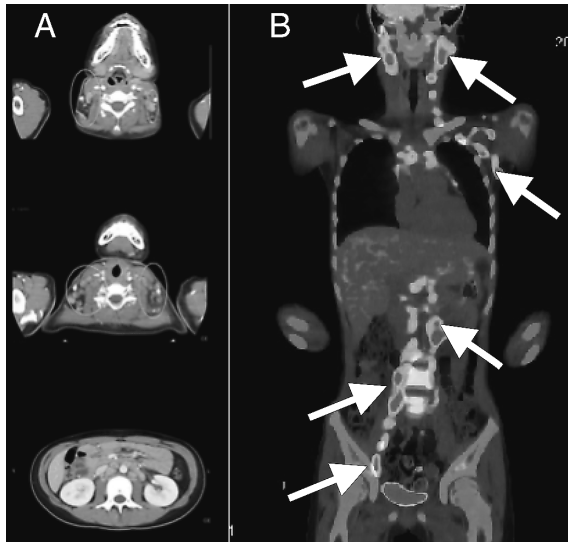


Fig. 1 **A**, Swelling of the lymph nodes in the postcervical space, supraclavicular fissure, axillae, lesser curvature of stomach, mesenteries, and around the para-aortic area of the internal and external iliac artery in Patient 1. No malignancy was observed in extranodal sites of the brain, lung, liver, spleen, or bone; **B**, Lymph nodes were enlarged above and below the diaphragm. Invasion was observed in the left adrenal grand in Patient 2. No malignancy was observed in the liver, spleen, or bone.

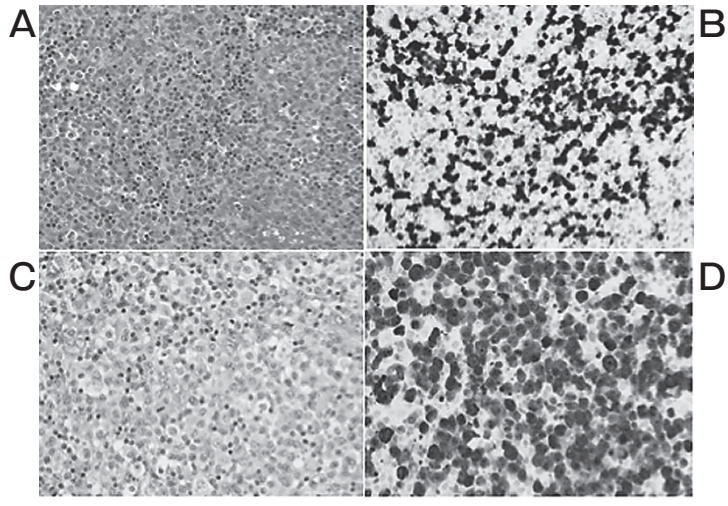


**Fig. 2** In Patient 1, the nucleus of the large, atypical cells had a "horseshoe", "fetal" shape (**A**). In the immunohistochemical study, the cells were CD20(−), CD3(−), CD30(+), S100(−), and ALK-positive in both the nucleus and cytoplasm (**B**). In Patient 2, growing cells showed an enlarged cytoplasm, creating a clear boundary between the large nucleus and cytoplasm, and were observed around (interfollicular and sinus) normal lymph follicles (**C**). In the immunohistochemical study, the cells were CD20(−), CD3(−), CD30(+), and ALK-positive in both the nucleus and cytoplasm. (**D**) Cells were TIA-1(+), Granz B(+), and highly Ki-67 (+).

patients' parents, and approval was obtained for this study from the Ethics Committee of our institution.

In Patient 1, the copy number of *NPM-ALK* was 90 copies/10,000 copies of ABL in the BM, and 1,278 copies/10,000 copies of ABL in the peripheral blood (PB) at the initial diagnosis. On the other hand, the antibody titer of ALK was ×100 times at diagnosis. *NPM-ALK* in the BM was shown to be positive by reverse transcribed (RT)-PCR (the titer was too low to measure by real-time PCR) at relapse, and *NPM-ALK* in the BM was negative before BMT.

In Patient 2, the copy number of *NPM-ALK* was 92 copies/10,000 copies of ABL in the BM; however, *NPM-ALK* was not detected in the PB at initial diagnosis. The antibody titer of ALK was ×750 times at diagnosis, and ×2,250 times at relapse. Due to the lack of a sample, *NPM-ALK* could not be examined in the relapsed BM of Patient 2.

## Discussion

We report 2 cases of relapsed stage III ALCL. Although both patients achieved complete remission by the ALCL99 regimen, they relapsed after the end of chemotherapy. Both patients were successfully treated with HSCT, and they survived without disease. As described above, we consider allogeneic HSCT for relapsed ALCL patients based on the results of a retrospective study of Japanese childhood ALCL [8]. The possibility of performing autologous HSCT remained when the minimal residual disease (MRD) was negative, but it was reported that a relatively high relapse rate was observed in ALCL patients with autologous HSCT compared to those of allogeneic HSCT, and we therefore chose allogeneic HSCT in the present 2 cases.

The *NPM-ALK* chimera was identified in BM samples from the initial diagnosis in both of our patients, although BM invasion was negative by usual examination. In addition, ALK antibodies were relatively low in samples from the initial diagnosis in both patients.

Mussolin *et al.* reported that in ALCL patients with *NPM-ALK* chimera and minimal disseminated disease (MDD) shown by RT-PCR, the 5-year progression-free survival was 41±11% for patients with positive BM versus 100% for patients with negative BM [9]. Damm Welk *et al.* reported that ALCL patients whose BM was *NPM-ALK*-positive by RT-PCR showed a higher cumulative incidence of relapse than those who were *NPM-ALK* negative [6,10]. Mussolin *et al.* proposed that a low ALK antibody titer was correlated with relapse, and patients were classified in a high-risk group when they were MDD-positive and had an antibody titer ≤1/750 [11]. The autologous

anti-ALK antibodies were produced by NPM-ALK oncogenic fusion protein, and the strength of the ALK autoantibody response was inversely correlated with stage and tumor dissemination, as well as with the risk of relapse [7].

MDD in childhood ALCL detected by qualitative RT-PCR was reported to be a strong risk factor for a poor prognosis, and it was also observed to be correlated with minimal residual disease (MRD) and the ALK antibody titer [12–14]. In our Patient 1, NPM-ALK was negative in the MRD, prior to BMT.

Although it remains to be clarified what percentage of patients with Stage III ALCL have MDD-positive BM by real-time PCR, the EFS rate in advanced ALCL patients was reported to be approx. 25%[15]. Although we did not monitor MDD in the present two cases, the survival rate for MDD-positive patients might improve in the future with the use of combined treatment, such as TKI targeting ALK and/or HSCT.

In conclusion, we report the cases of 2 patients with relapsed stage III ALCL who were successfully treated by HSCT. Both showed the presence of the *NPM-ALK* chimera and low ALK antibody at diagnosis.

Acknowledgments. Supported in part by a Grant-in-Aid for Cancer Research and a grant for Clinical Cancer Research and Research on Children and Families from the Ministry of Health, Labor and Welfare of Japan. We thank all of the medical staff who participated in the patients' care and contributed to the collection of the biological samples and clinical information.

## References

1. Seidemann K, Tiemann M, Schrappe M, Yakisan E, Simonitsch I, Janka-Schaub G, Dörffel W, Zimmermann M, Mann G, Gadner H, Riehm H and Reiter A: Short-pulse B-non-Hodgkin lymphoma-type chemotherapy is efficacious treatment for pediatric anaplastic large cell lymphoma: a report of the Berlin- Frankfurt-Münster Group Trial NHL-BFM 90. Blood (2001) 97: 3699–3706.
2. Le Deley MC, Reiter A, Williams D, Delsol G, Oschlies I, McCarthy K, Zimmermann M and Brugieres L on behalf of the European Intergroup for Childhood Non-Hodkin Lymphoma: Prognostic factors in childhood anaplastic large cell lymphoma: results of a large European intergroup study. Blood (2008) 111: 1560–1566.
3. Brugières L, Le Dely MC, Rosolen A, Williams D, Horibe K, Wrobel G, Mann G, Zsiros J, Uyttebroeck A, Marky I, Lamant L, and Reiter A: Impact of the methotrexate administration dose on the need for intrathecal treatment in children and adolescents with anaplastic large-cell lymphoma: results of a randomized trial of the EICNHL Group. J Clin Oncol (2009) 27: 897–903.
4. Le Deley MC, Reiter A, Williams D, Delsol G, Oschlies I, McCarthy K, Zimmermann M and Brugières L: Prognostic factors in childhood anaplastic large cell lymphoma: results of a large European intergroup study. Blood (2008) 111: 1560–1566.
5. Le Deley MC, Rosolen A, Williams DM, Horibe K, Wrobel G, Attarbaschi A, Zsiros J, Uyttebroeck A, Marky IM, Lamant L, Woessmann W, Pillon M, Hobson R, Mauguen A, Reiter A and Brugi`eres L: Vinblastine in children and adolescents with High-risk anaplastic large-cell lymphoma: Results of a randomized ALCL99-Vinblastine trial. J Clin Oncol (2010) 28: 3987–3993.
6. Damm-Welk C, Busch K, Burkhardt B, Schieferstein J, Viehmann S, Oschlies I, Klapper W, Zimmermann M, Harbott J, Reiter A and Woessmann W: Prognostic significance of circulating tumor cells in bone marrow or peripheral blood as detected by qualitative and quantitative PCR in pediatric NPM-ALK–positive anaplastic large-cell lymphoma. Blood (2007) 110: 670–677.
7. Ait-Tahar K, Damm-Welk C, Burkhardt B, Zimmermann M, Klapper W, Reiter A, Pulford K and Woessmann W: Correlation of the autoantibody response to the ALK oncoantigen in pediatric anaplastic lymphoma kinase–positive anaplastic large cell lymphoma with tumor dissemination and relapse risk. Blood (2010) 115: 3314–3319.
8. Fukano R, Mori T, Kobayashi R, Mitsui T, Fujita N, Iwasaki F, Suzumiya J, Chin M, Goto H, Takahashi Y, Hara J, Park YD, Inoue M, Koga Y, Inagaki J, Sakamaki H, Adachi S, Kawa K, Kato K, Suzuki R. Haematopoietic stem cell transplantation for relapsed or refractory anaplastic large cell lymphoma: a study of children and adolescents in Japan. Br J Haematol. (2015) 168: 557–563.
9. Mussolin L, Pillon M, d'Amore ES, Santoro N, Lombardi A, Fagioli F, Zanesco L and Rosolen A: Prevalence and clinical implications if bone marrow involvement in pediatric anaplastic large cell lymphoma. Leukemia (2005) 19: 1643–1647.
10. Damm-Welk C, Mussolin L, Zimmermann M, Pillon M, Klapper W, Oschlies I, d'Amore ES, Reiter A, Woessmann W and Rosolen A: Early assessment of minimal residual disease identifies patients at very high relapse risk in NPM-ALK-positive anaplastic large-cell lymphoma. Blood (2014) 123: 334–337.
11. Mussolin L, Damm-Welk C, Pillon M, Zimmermann M, Franceschetto G, Pulford K, Reiter A, Rosolen A and Woessmann W: Use of minimal disseminated disease and immunity to NPM-ALK antigen to stratify ALK-positive ALCL patients with different prognosis. Leukemia (2013) 27: 416–422.
12. Damm-Welk C, Pillon M and Mussolon L: Prognostic factors in pediatric large cell lymphoma: role of ALK. Front Biosci (Schol Ed) (2015) 7: 205–216.
13. Mussolin L, Bonvini P, Ait-Tahar K, Pillon M, Tridello G, Buffardi S, Lombardi A, Pulford K and Rosolen A: Kinetics of humoral response to ALK and its relationship with minimal residual disease in pediatric ALCL. Leukemia (2009) 23: 400–402.
14. Kalinova M, Krskova L, Brizova H, Kabickova E, Kepak T and Kodet R: Quantitative PCR detection of NPM-ALK fusion gene and CD30 gene expression in patients with anaplastic large cell lymphoma--residual disease monitoring and a correlation with the disease status. Leuk Res (2008) 32: 25–32.
15. Alexander S, Kraveka JM, Weitzman S, Lowe E, Smith L, Lynch JC, Chang M, Kinney MC, Perkins SL, Laver J, Gross TG and Weinstein H: Advanced stage anaplastic large cell lymphoma in children and adolescents: results of ANHL0131, a randomized phase III trial of APO versus a modified regimen with vinblastine: a report from the children's oncology group. Pediatr Blood Cancer (2014) 61: 2236–2242.