Int J Hematol (2016) 104:139–143
DOI 10.1007/s12185-016-1971-9

CASE REPORT

Hematopoietic stem cell transplantation for pediatric mature B-cell acute lymphoblastic leukemia with non-L3 morphology and *MLL-AF9* gene fusion: three case reports and review of the literature

Takeo Sarashina[1,3] · Haruko Iwabuchi[2] · Naoyuki Miyagawa[1] · Masahiro Sekimizu[1] · Tomoko Yokosuka[1] · Kunio Fukuda[1] · Satoshi Hamanoue[1] · Fuminori Iwasaki[1] · Shoko Goto[1] · Masae Shiomi[1] · Chihaya Imai[2] · Hiroaki Goto[1]

Received: 26 August 2015 / Revised: 1 March 2016 / Accepted: 2 March 2016 / Published online: 18 March 2016

Abstract Mature B-cell acute lymphoblastic leukemia (B-ALL) is typically associated with French-American-British (FAB)-L3 morphology and *MYC* gene rearrangement. However, rare cases of mature B-ALL with non-L3 morphology and *MLL-AF9* fusion have been reported, and such cases are characterized by a rapid and aggressive clinical course. We here report three such cases of pediatric mature B-ALL in female patients respectively aged 15 months, 4 years, and 4 months. Bone marrow smears at diagnosis showed FAB-L1 morphology in all patients. Immunophenotypically, they were positive for cluster of differentiation (CD)10, CD19, CD20 (or CD22), Human Leukocyte Antigen-DR, and surface immunoglobulin λ. No evidence of *MYC* rearrangement was detected in any of the cases by fluorescent in situ hybridization (FISH) analysis. However, *MLL* rearrangement was detected by FISH, and *MLL-AF9* fusion was confirmed by reverse transcriptase-polymerase chain reaction. All patients achieved complete remission after conventional chemotherapy and subsequently underwent hematopoietic stem cell transplantation as high-risk ALL; patient 3 for infantile ALL with *MLL* rearrangement and the others for ALL with *MLL* rearrangement and hyperleukocytosis (white blood cell count at diagnosis >50 × 10^9/L). At the latest follow-up for each case (12–98 months post-transplantation), complete remission was maintained. Moreover, we discuss the clinical, genetic, and immunophenotypic features of this rare disease.

Keywords *MLL-AF9* · Non-L3 morphology · Stem cell transplantation · Mature B-ALL

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous group of hematopoietic neoplasms. B-cell precursor ALL, which is characterized by French-American-British (FAB)-L1 or L2 morphology and a surface immunoglobulin (sIg) negative (−), terminal deoxynucleotidyl transferase (TdT) positive (+), immature B-cell phenotype, comprises the majority of ALL cases [1, 2]. On the other hand, mature B-cell acute lymphoblastic leukemia (B-ALL) is a rare entity, accounting for only 1–2 % of all pediatric ALL cases. Mature B-ALL, characterized by sIg(+), is typically associated with rearrangement of the v-myc avian myelocytomatosis viral oncogene homolog (*MYC*) gene and FAB-L3 morphology in both adult and pediatric patients [3, 4]. Patients with mature B-ALL have a poor response to and shortened survival with standard ALL therapeutic regimens; however, newer dose-intensive regimens used for Burkitt lymphoma have led to improved cure rates [5].

Chromosomal rearrangements involving the human mixed lineage leukemia or myeloid/lymphoid leukemia (*MLL*) gene at 11q23 are associated with the development of acute leukemias, and are commonly detected in infantile, as well as in therapy-induced, leukemias [6, 7]. Despite recent technological advances having gradually clarified the molecular mechanisms of *MLL* fusion-dependent leukemogenesis [8], the presence of certain *MLL* rearrangements

✉ Takeo Sarashina
sara5p@asahikawa-med.ac.jp

[1] Division of Hemato-Oncology/Regenerative Medicine, Kanagawa Children's Medical Center, Yokohama, Japan

[2] Department of Homeostatic Regulation and Development, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

[3] Department of Pediatrics, Asahikawa Medical University, Midorigaoka-Higashi 2-1-1-1, Asahikawa 078-8510, Japan

is still an independent dismal prognostic factor, and such patients are usually treated according to high-risk protocols.

Among the reported cases of mature B-ALL with non-L3 morphology and no *MYC* rearrangement in children, *MLL-AF9* chimeric gene positive cases are rare, and this entity is associated with especially poor clinical outcomes [6, 9, 10]. Additionally, the optimal treatment, including the indication of transplantation for ALL with such features, has not yet been established. Herein, we describe three additional cases of childhood ALL with non-FAB-L3 morphology expressing a mature B-cell immunophenotype and showing *MLL-AF9* chimeric gene expression without *MYC* rearrangement. All patients achieved complete remission after conventional chemotherapy and subsequently underwent hematopoietic stem cell transplantation (HSCT). All patients have maintained complete remission. Further, we also discuss the clinical, genetic, and immunophenotypic features of this rare entity.

Case reports

Patient 1 was a 15-month-old female who presented with hepatomegaly and multiple petechiae on the skin covering the trunk. The complete blood count revealed a white blood cell (WBC) count of 54,000/μL with 94 % blasts, a hemoglobin level of 7.8 g/dL, and a platelet count of 41,000/μL. At admission, central nervous system involvement was noted. Radiographic evaluation revealed abdominal paraaortic lymphadenopathy and bilateral renal enlargement. Bone marrow aspirate consisted of 97 % blast cells with FAB-L1 morphology and no myeloperoxidase reaction. However, the blast cells exhibited a mature B-ALL phenotype, with λ sIg(+), cluster of differentiation (CD)10(+), CD19(+), CD20(+), human leukocyte antigen (HLA)-DR(+), and CD34(−). The karyotyping was unable to be confirmed due to no metaphase cell being obtained. Multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) screening detected *MLL-AF9* gene fusion, and *MLL* gene rearrangement was confirmed by fluorescence in situ hybridization (FISH) analysis. The patient was treated according to the Tokyo Children's Cancer Study Group NHL0105 protocol, which is a Berlin-Frankfurt-Münster (BFM)-derived protocol for non-Hodgkin lymphoma (NHL) or mature B-ALL. Complete remission (CR) was achieved after pre-phase and induction therapy, as demonstrated using bone marrow smears, radiographic evaluation, and lumbar puncture. At this time, the *MLL-AF9* fusion gene transcript was negative. After three additional courses of chemotherapy, the patient received bone marrow transplantation using a total body irradiation (TBI)-containing regimen [12 Gy TBI, fractionated in six doses, 60 mg/kg (once daily i.v.) of etoposide, and 180 mg/m^2 (90 mg/m^2 once daily i.v. for 2 days) of melphalan] from an HLA-matched sibling donor. At the latest follow-up (over 9 years from diagnosis), the patient remained in CR.

Patient 2 was a 4-year-old female who presented with hepatomegaly and scattered petechiae on the surfaces of the extremities. The complete blood count revealed a WBC count of 209,000/μL with 93 % blasts, and a platelet count of 21,000/μL. Other than the bone marrow, no organ involvement was present at admission. Radiographic evaluation revealed no thoracic or abdominal masses. Bone marrow smears showed numerous blasts with FAB-L1, similar to for Patient 1. Flow cytometric immunophenotypic and genetic analyzes of the bone marrow were performed, and revealed that the blast cells were positive for λ sIg, CD10, CD19, CD22, and HLA-DR, and negative for CD34. RT-PCR detected *MLL-AF9* gene fusion. The patient was treated according to the group D protocol of BFM-NHL95 [11]. She achieved CR after induction therapy, and the *MLL-AF9* fusion gene transcript was negative by RT-PCR. Four months after the initial diagnosis, cord blood transplantation was performed using the same 12-Gy TBI-containing regimen as for patient 1, from an HLA 8/8 matched unrelated donor. At the latest follow-up (1 year from diagnosis), she was disease-free and without adverse events, including chronic graft-versus-host disease.

Patient 3 was a 4-month-old female who presented with purplish skin lesions on her back and hepatosplenomegaly. The complete blood count revealed a WBC count of 295,000/μL, with blasts. At admission, the patient had anemia (hemoglobin: 2.9 g/dL) and thrombocytopenia (platelets: 45,000/μL). Although bone marrow aspiration indicated ALL with FAB-L1 morphology, blast cell typing by flow cytometry revealed a mature B-ALL immunophenotype; the blast cells expressed λ sIg, CD10, CD19, CD20, and CD22. She had a normal karyotype by conventional G-banding. RT-PCR detected *MLL-AF9* gene fusion, and *MLL* rearrangement was detected by FISH, whereas there was no evidence of *MYC* rearrangement. The patient received the Japanese Pediatric Leukemia/Lymphoma Study Group MLL03 treatment protocol [12], and complete remission was documented after the end of the induction phase upon bone marrow smears. At this time, no *MLL-AF9* fusion gene transcripts were detected by RT-PCR. After two additional courses of chemotherapy, allogeneic SCT was performed using 5/6 HLA-matched unrelated cord blood with a busulfan-containing regimen comprising 9.6 mg/kg (0.6 mg/kg four times a day i.v. for 4 days) of busulfan, 60 mg/kg (once daily i.v.) of etoposide, and 120 mg/kg (60 mg/kg once a day i.v. for 2 days) of cyclophosphamide. At the latest follow-up (6 years from diagnosis), the patient remained disease-free.

Table 1 Phenotypic and cytogenetic findings at diagnosis of all reported cases of mature B-cell acute lymphoblastic leukemia with non-L3 morphology and *MLL-AF9*

	CD19	CD20	CD22	CD10	CD34	TdT	cIg	sIg	Cytogenetic abnormality	*MLL-AF9* RT-PCR (#1/#2)	Refs.
Case 1	+	NA	+	−	−	−	+	+	t(9;11)(p22;q23)	+/−	Ref. [9]
Case 2	+	+	+	−	−	−	+	+	t(9;11)(p21-p22;q23)	NA/−	Ref. [9]
Case 3	+	−	+	−	−	−	+	+	t(9;11)(p22;q23)	+/−	Ref. [9]
Case 4	+	+	+	+	−	−	+	+	t(9;11)(p22;q23)	+/−	Ref. [9]
Case 5	+	−	+	−	−	−	+	+	t(9;11)(p22;q23), 11q23FISH	NA/NA	Ref. [6]
Case 6	+	+	+	−	−	−	+	+	t(9;11)(p22;q23), 11q23FISH	NA/NA	Ref. [6]
Case 7	+	+	NA	−	NA	+	+	+	t(9;11)(p22;q23)	NA/NA	Ref. [10]
Case 8	+	+	NA	+	−	NA	NA	+	11q23 FISH	+/−	Patient 1
Case 9	+	−	+	+	−	NA	NA	+	11q23 FISH	+/−	Patient 2
Case 10	+	+	+	+	−	−	NA	+	11q23 FISH	+/−	Patient 3

TdT terminal deoxynucleotidyl transfelase, *cIg* cytoplasmic immunoglobulin, *sIg* surface immunoglobulin, *RT-PCR* reverse transcriptase-polymelase chain reaction, *ref.* reference, *NA* not available, *11q23 FISH* 11q23 rearrangement fluorescence in situ hybridization

#1: at diagnosis

#2: at the end of induction phase

Table 2 Characteristic clinical features of all reported cases of mature B-cell acute lymphoblastic leukemia with non-L3 and *MLL-AF9*

	Age	Gender	WBC (/μL)	Extramedullary involvement	Morphology	Clinical course	Refs.
Case 1	1 year 1-month	F	24×10^3	CLN, hepatosplenomegaly	Non-L3	FRALLE2000 → CR (on Tx)	Ref. [9]
Case 2	1 year 11 months	F	87×10^3	Hepatosplenomegaly, CNS, kidney	Non-L3	LMB89 → Re (DFS 2mo) → 2nd CR → BMT → died of BMT related toxicity	Ref. [9]
Case 3	8 months	M	15×10^3	Splenomegaly	Non-L3	INTERFANT99 → Re (DFS ?) → 2nd CR → uCBT → died of SCT related toxicity	Ref. [9]
Case 4	1 year 4 months	F	93×10^3	Splenomegaly, CNS	Non-L3	EORTC02 → Re (on Tx) → 2nd CR → waiting for BMT	Ref. [9]
Case 5	4 months	M	33×10^3	Hepatosplenomegaly, skin, testis	Non-L3	INTERFANT99 → Re (on Tx) → died of disease	Ref. [6]
Case 6	8 months	F	161×10^3	Hepatosplenomegaly, skin	Non-L3	INTERFANT99 → uCBT → Re (day + 65) → died of disease	Ref. [6]
Case 7	8 years	F	44×10^3	Splenomegaly	L1	Tx unknown (based on VPL) → died of disease	Ref. [10]
Case 8	1 year 3 months	F	54×10^3	CLN, hepatomegaly, CNS, kidney	L1	TCCSG-NHL → CR → MSD-BMT → CR (DFS > 6 years)	Patient 1
Case 9	4 years 2 months	F	210×10^3	Hepatosplenomegaly	L1	NHL-BFM95 → CR → uCBT → CR (DFS > 1 year)	Patient 2
Case 10	4 months	F	295×10^3	Skin	L1	JPLSG MLL03 → CR → uCBT → CR (DFS > 6 years)	Patient 3

WBC white blood cell, *ref.* reference, *CLN* cervical lymph node, *CR* complete remission, *DFS* disease-free survival, *CNS* central nervous system, *Re* relapse, *BMT* bone marrow transplantation, *uCBT* unrelated cord blood transplantation, *Tx* therapy, *VPL* vincristine, prednisone and L-asparaginase, *MSD-BMT* bone marrow transplantation from matched sibling donor

Discussion

We here described three rare cases of mature B-ALL with non-L3 morphology and *MLL-AF9* chimeric gene positive status. The immunophenotype data of our patients and previous cases are summarized in Table 1. As shown in this table, most leukemia cells with these features were negative for CD34 and TdT expression (0/9 and 1/8 positive cases, respectively). Iwamoto et al. demonstrated that mature B-ALL cells frequently show negative expressions

of TdT and CD34 [13], whereas both of these immaturity markers, especially TdT, are often positive in *MLL*-rearranged B-lineage ALL [14]. On the other hand, although CD10, known to be a common ALL antigen, was positive in four of 10 cases, *MLL*-rearranged B-lineage ALL cases have been reported to usually lack significant CD10 expression [14, 15]. These findings may indicate a characteristic of mature B-ALL rather than childhood B-lineage ALL with *MLL* rearrangement. Additionally, Jansen et al. reported that *MLL-AF9*-positive patients have more mature immunoglobulin gene rearrangements than other *MLL*-rearranged subtypes [15]. Hence, further analysis is necessary to clarify the relation between *MLL-AF9* gene fusion and B-lineage ALL.

Table 2 summarizes the clinical characteristic of pediatric patients with mature B-cell ALL with non-L3 and *MLL-AF9* fusion. In the previous reports, the outcomes of ALL patients with a mature B immunophenotype, non-L3 morphology, and *MLL-AF9* were poor, especially after relapse [6, 9, 10], and Pui et al. similarly reported that B-lineage ALL with *MLL-AF9* gene fusion was associated with a poor prognosis [16]. Additionally, in one previous study, three of four patients (cases 1-4) who achieved molecular CR, as determined by RT-PCR for *MLL-AF9* fusion transcripts, after induction therapy (shown in Table 1), relapsed after or during chemotherapy. Today, the detection of minimal residual disease after induction is a useful predictor of outcome and is used to guide the decision to perform a transplant after the first CR. Although it is necessary to consider the publication bias for past reports on such unique cases of ALL showing a poor prognosis, this finding may suggest that patients belonging to this rare subgroup of ALL require HSCT after the first CR regardless of the minimal residual disease levels.

A recent review of HSCT in pediatric ALL patients did not support allogeneic SCT when *MLL*-rearranged ALL is the sole adverse risk factor, and described that the presence of *MLL*-positive cells along with other risk factors (age, WBC, prednisone response, and other cytogenetic abnormalities) could be used to define very high-risk groups, for which allogeneic SCT may be recommended [17]. From this review, our cases do not necessarily satisfy the transplant indications, and late effects, such as growth failure, endocrine abnormalities, and secondary cancer caused by the HSCT, are major problem for pediatric patients. Accumulation of cases with similar features and further biological studies of ALL will help clarify the clinical significance of this unique phenotype in childhood ALL.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interests.

References

1. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, et al. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9:1783–6.
2. Rubnitz JE, Behm FG, Downing JR. 11q23 rearrangements in acute leukemia. Leukemia. 1996;10:74–82.
3. Brunning RD, Borowitz M, Matutes E, Head D, Flandrin G, Swerdlow SH, et al. Precursor B lymphoblastic leukaemia/lymphoblastic lymphoma. Pathology and Genetics of Tumors of Hematopoietic an Lymphoid tissues. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, editors. World Health Organization Classification of Tumours. Lyon: IARC Press; 2001. p. 111–4.
4. Frater JL, Batanianet JR, O'Connor DM, Grosso LE. Lymphoblastic leukemia with mature B-cell phenotype in infancy. J Pediatr Hematol Oncol. 2004;26:672–7.
5. Lee GR, Foerster J, Lukens J, Paraskevas I, Greer JP, Rodgers GM, editors. Wintrobe's Clinical Hematology. 10th ed. Baltimore: Williams & Wilkins; 1999. p. 2246.
6. Tsao L, Draoua HY, Osunkwo I, Nandula SV, Murty VV, Mansukhani M, et al. Mature B-cell acute lymphoblastic leukaemia with t(9;11) translocation: a distinct subset of B-cell acute lymphoblastic leukemia. Mod Pathol. 2004;17:832–9.
7. Meyer C, Schneider B, Jakob S, Strehl S, Attarbaschi A, Schnittger S, et al. The MLL recombinome of acute leukemias. Leukemia. 2006;20:777–84.
8. Yokoyama A. Molecular mechanisms of MLL-associated leukemia. Int J Hematol. 2015;101:352–61.
9. Blin N, Mëchinaud F, Talmant P, Garand R, Boutard P, Dastugue N, et al. Mature B-cell lymphoblastic leukemia with MLL rearrangement: an uncommon and distinct subset of childhood acute leukemia. Leukemia. 2008;22:1056–9.
10. Lorenzana AN, et al. Immunoglobulin gene rearrangements in acute lymphoblastic leukemia with the 9;11 translocation. Genes Chromosom Cancer. 1991;3:74–7.
11. Woessmann W, Seidemann K, Mann G, Zimmermann M, Burkhardt B, Oschlies I, et al. The impact of the methotrexate administration schedule and dose in the treatment of children and adolescents with B-cell neoplasms: a report of the BFM group study NHL-BFM95. Blood. 2005;105:948–58.
12. Koh K, Tomizawa D, Moriya Saito A, Watanabe T, Miyamura T, Hirayama M, et al. Early use of allogeneic hematopoietic stem cell transplantation for infants with MLL gene-rearrangement-positive acute lymphoblastic leukemia. Leukemia. 2015;29:290–6.
13. Iwamoto S, Deguchi T, Ohta H, Kiyokawa N, Tsurusawa M, Yamada T, et al. Flow cytometric analysis of de novo acute lymphoblastic leukemia in childhood: report from the Japanese Pediatric Leukemia/Lymphoma Study Group. Int J Hematol. 2011;94:185–92.
14. Attarbaschi A, Mann G, König M, Steiner M, Strehl S, Schreiberhuber A, et al. Mixed lineage leukemia-rearranged childhood pro-B and CD10-negative pre-B acute lymphoblastic leukemia constitute a distinct clinical entity. Clin Cancer Res. 2006;12:2988–94.
15. Jansen MW, Corral L, van der Velden VH, Panzer-Grümayer R, Schrappe M, Schrauder A, et al. Immunobiological diversity in infant acute lymphoblastic leukemia is related to the occurrence and type of MLL gene rearrangement. Leukemia. 2007;21:633–41.
16. Pui CH, Gaynon PS, Boyett JM, Chessells JM, Baruchel A, Kamps W, et al. Outcome of treatment in childhood acute

lymphoblastic leukemia with rearrangements of the 11q23 chromosomal region. Lancet. 2002;359:1909–15.
17. Oliansky DM, Camitta B, Gaynon P, Nieder ML, Parsons SK, Pulsipher MA, et al. Role of cytotoxic therapy with hematopoietic stem cell transplantation in the treatment of pediatric acute lymphoblastic leukemia: update of the 2005 evidence-based review. Biol Blood Marrow Transplant. 2012;18:505–22.