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【 CASE REPORT 】

# Acute Megakaryoblastic Leukemia Developing as Donor Cell Leukemia after Umbilical Cord Blood Transplantation

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**Abstract:**
A 64-year-old man with acute myeloid leukemia underwent umbilical cord blood transplantation (UCBT). After 11 months of complete remission (CR) following UCBT, the bone marrow showed 7.5% myeloblasts. CR was obtained after a single course of azacitidine monotherapy, but the myeloblasts gradually increased in the blood. We made a diagnosis of acute megakaryoblastic leukemia derived from donor cell with a fluorescence *in situ* hybridization (FISH) analysis of the sex chromosomes and an immunophenotypic analysis. Azacitidine was administered again and produced a therapeutic effect of stable disease. This case suggests that azacitidine may be a useful therapy for patients with acute megakaryoblastic leukemia in situations in which intensive chemotherapy and transplantation are not indicated.

**Key words:** acute megakaryoblastic leukemia, umbilical cord blood transplantation, donor cell leukemia, azacitidine

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## Introduction

Acute megakaryoblastic leukemia has been described as a subtype of acute myelogenous leukemia (AML) (1) and was incorporated into the French-American-British (FAB) classification of AML as M7 (2). The associated bone marrow characteristics include the proliferation of abnormal megakaryoblasts identified by the presence of platelet-specific surface glycoprotein and frequently extensive myelofibrosis. Histopathologic evidence of M7 AML itself is an independent poor prognostic factor for the overall survival (3).

Azacitidine, a demethylation agent, has demonstrated a significant survival benefit in patients with high-risk myelodysplastic syndrome (MDS) relative to conventional care and has also been proved effective in older patients with newly diagnosed AML with >30% blasts (4, 5). However, whether or not azacitidine is effective for acute megakaryoblastic leukemia is unclear, as it is rare in adults.

No reports are available on acute megakaryoblastic leukemia developing as donor cell leukemia (DCL) after allogeneic hematopoietic stem cell transplantation (HSCT) and treatment with hypomethylating agents (azacitidine or decitabine). We herein report a case of acute megakaryoblastic leukemia developing as DCL after umbilical cord blood transplantation (UCBT) that was treated with azacitidine.

## Case Report

A 64-year-old man had pancytopenia and was referred to our hospital (Table 1). An analysis of bone marrow aspirate revealed that myeloblasts comprised 49.5% of nucleated cells with multilineage dysplasia, so we made a diagnosis of AML with myelodysplasia-related changes. He achieved hematological complete remission (CR) after induction chemotherapy consisting of idarubicin and cytarabine and underwent UCBT with a reduced-intensity conditioning regimen from an unrelated female donor in first CR 8 months after the initial diagnosis.

On day 28 of UCBT, a fluorescence *in situ* hybridization (FISH) analysis of the sex chromosomes and a chimerism

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**Table 1. The Laboratory Data at the Onset of Original Leukemia.**

| | | | |
|---|---|---|---|
| Blood biochemistry | | Fe | 201 μg/dL |
| Total protein | 7.2 g/dL | UIBC | 184 μg/dL |
| Albumin | 4.4 g/dL | Ferritin | 144 ng/mL |
| BUN | 12.8 mg/dL | IgG | 1,197 mg/dL |
| Creatinine | 0.94 mg/dL | IgA | 400 mg/dL |
| AST | 20 IU/L | IgM | 54 mg/dL |
| ALT | 28 IU/L | TSH | 0.63 μIU/mL |
| LDH | 175 IU/L | Free-T3 | 2.63 pg/mL |
| ALP | 210 IU/L | Free-T4 | 1.22 ng/dL |
| UA | 5.3 mg/dL | WT-1 mRNA | $9.3\times10^3$ copies/μgRNA |
| CPK | 65 IU/L | | |
| Na | 142 mEq/L | Hematology | |
| K | 3.9 mEq/L | WBC | 1,100 /μL |
| Cl | 108 mEq/L | Neutrophil | 12 % |
| Ca | 9.6 mg/dL | Lymphocyte | 86 % |
| P | 2.6 mg/dL | Monocyte | 2 % |
| Glucose | 129 mg/dL | Myeloblast | 0 % |
| CRP | 0.09 mg/dL | RBC | $275\times10^4$ /μL |
| PT-INR | 0.97 | Hemoglobin | 9.6 g/dL |
| APTT | 27.4 sec | MCV | 102.9 fL |
| Fibrinogen | 300 mg/dL | Platelet | $5.3\times10^4$ /μL |
| FDP | <5.0 μg/mL | Reticulocyte | 11 ‰ |
| D-dimer | 0.9 μg/mL | | |

CRP: C-reactive protein, UIBC: unsaturated iron-binging capacity, TSH: thyroid stimulating hormone, WT-1: Wilm's tumor 1, WBC: white blood cell, RBC: red blood cell, MCV: mean corpuscular volume

evaluation by a short tandem repeat (STR) analysis revealed the complete donor type of the bone marrow cells and the peripheral blood cells. On day 56 of UCBT, however, a FISH analysis of the sex chromosomes revealed that 2% of all nucleated cells (ANC) in the bone marrow presented with XY signals, and a STR analysis revealed that the donor chimerism of T cells in the peripheral blood had decreased to 83.5%. We made a diagnosis of molecular relapse of the original disease and reduced the dose of the immunosuppressive agent.

Thereafter, complete donor chimerism was achieved, and hematological remission was sustained for 11 months. Progressive thrombocytopenia appeared, and the amount of Wilm's tumor 1 (WT-1) mRNA in the peripheral blood was $2.0\times10^4$ copies/μgRNA as determined by a kit using quantitative reverse transcription-polymerase chain reaction (RT-PCR) (Otsuka Pharmaceutical Company, Tokyo, Japan; normally <50 copies/μgRNA) 11 months after UCBT (Table 2). A bone marrow examination showed normocellular marrow with 7.5% abnormal blasts, suggesting a relapse of the original disease. However, an immunophenotypic analysis by flow cytometry (FCM) was unable to differentiate the leukemic blasts in the bone marrow sample.

The subsequent clinical course is shown in Fig. 1. The patient was treated with azacitidine at a dose of 75 mg/m$^2$ on 7 consecutive days because we initially assumed a relapse of the original disease. However, a FISH analysis before azacitidine monotherapy showed that 100% of the bone marrow cells had XX signals; therefore, we considered this to be a case of donor-derived hematologic malignancy. On day 27 of azacitidine monotherapy, a bone marrow examination revealed hematological CR. He was followed up as an outpatient without additional treatment; however, the blasts eventually reappeared and gradually increased in the peripheral blood with the elevation of lactate dehydrogenase (LDH) levels. On day 97 of azacitidine therapy, we conducted a bone marrow biopsy because of a dry tap in the bone marrow aspirate and found that myeloperoxidase-negative blasts had invaded the bone marrow (Fig. 2). An immunophenotypic analysis by FCM showed the expression of CD7, CD21, CD33, CD34, CD38, CD56 and human leukocyte antigen (HLA)-DR on the leukemic blasts. The blasts showed a normal female karyotype in a G-banding chromosomal examination. Another FISH analysis showed that 100% of the bone marrow cells had XX signals again. We therefore made a diagnosis of myeloid/natural killer cell precursor acute leukemia (M/NKPAL) derived from donor cells. Whole-body computed tomography (CT) revealed no extramedullary lesions.

Because it was not clear whether allogeneic HSCT could significantly improve the outcome of patients with DCL and because the patient refused to undergo any transplantation, we did not plan to give him a second HSCT.

He underwent chemotherapy consisting of idarubicin (10 mg/m$^2$ on days 1-3) and cytarabine (100 mg/m$^2$ on days 1-7). He demonstrated prolonged neutropenia, which caused a

**Table 2. The Laboratory Data at the Onset of Donor Cell Leukemia.**

| | | | |
|---|---|---|---|
| Blood biochemistry | | Fe | 140 μg/dL |
| Total protein | 7.0 g/dL | UIBC | 111 μg/dL |
| Albumin | 4.3 g/dL | Ferritin | 417 ng/mL |
| BUN | 21.4 mg/dL | IgG | 1,359 mg/dL |
| Creatinine | 1.47 mg/dL | IgA | 249 mg/dL |
| AST | 20 IU/L | IgM | 41 mg/dL |
| ALT | 14 IU/L | | |
| LDH | 233 IU/L | Hematology | |
| ALP | 266 IU/L | WBC | 6,100 /μL |
| UA | 6.8 mg/dL | Neutrophil | 49 % |
| CPK | 54 IU/L | Lymphocyte | 39 % |
| Na | 145 mEq/L | Monocyte | 9 % |
| K | 3.9 mEq/L | Eosinophil | 3 % |
| Cl | 110 mEq/L | RBC | $331\times10^4$ /μL |
| Ca | 9.2 mg/dL | Hemoglobin | 10.8 g/dL |
| P | 3.6 mg/dL | MCV | 96.7 fL |
| Glucose | 115 mg/dL | Platelet | $6.9\times10^4$ /μL |
| CRP | 0.09 mg/dL | Reticulocyte | 14 ‰ |
| WT-1 mRNA | $2.0\times10^4$ copies/μgRNA | | |

CRP: C-reactive protein, WT-1: Wilm's tumor, UIBC: unsaturated iron-binging capacity, WBC: white blood cell, RBC: red blood cell, MCV: mean corpuscular volume

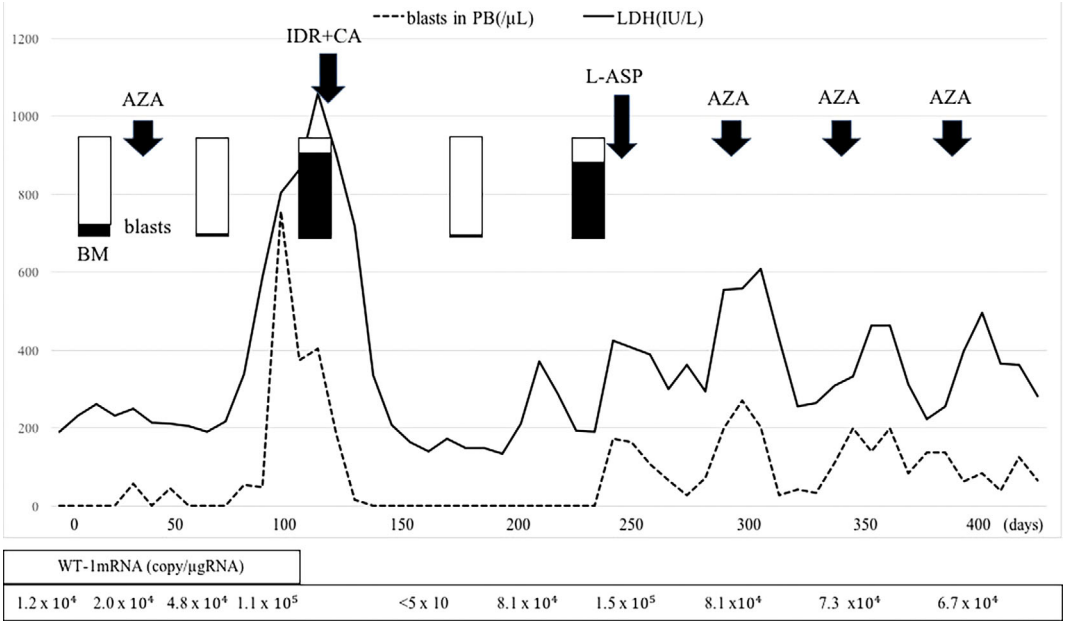


**Figure 1. The clinical course of the present patient. AZA: azacitidine, BM: bone marrow, CA: cytarabine, IDR: idarubicin, L-ASP: L-asparaginase, LDH: lactate dehydrogenase, PB: peripheral blood, WT-1: Wilm's tumor 1**

severe infection and acute kidney injury. After a single course of intensive chemotherapy, a bone marrow examination revealed CR, and the amount of WT-1 mRNA in the peripheral blood dropped below 50 copies/μgRNA.

The patient experienced a second relapse of DCL with WT-1 mRNA $8.1\times10^4$ copies/μgRNA and the appearance of blasts in the peripheral blood 2 months after achieving CR. He underwent L-asparaginase (L-ASP) monotherapy (6,000 IU/$m^2$ on days 1-7), which caused febrile neutropenia and severe liver damage. After L-ASP monotherapy, we re-examined the surface markers of the leukemic cells by FCM, which showed positivity for CD41, as well as CD7, CD21, CD33, CD34, CD38, CD56 and HLA-DR (Fig. 3). This result led us to diagnose his DCL as acute megakaryoblastic leukemia rather than M/NKPAL.

The L-ASP monotherapy was unfortunately ineffective; therefore, we started azacitidine monotherapy (75 mg/$m^2$ on days 1-5, 8, 9) again. Following a single course of azacitidine, the number of abnormal blasts and amount of WT-1 mRNA in the peripheral blood were almost unchanged, but

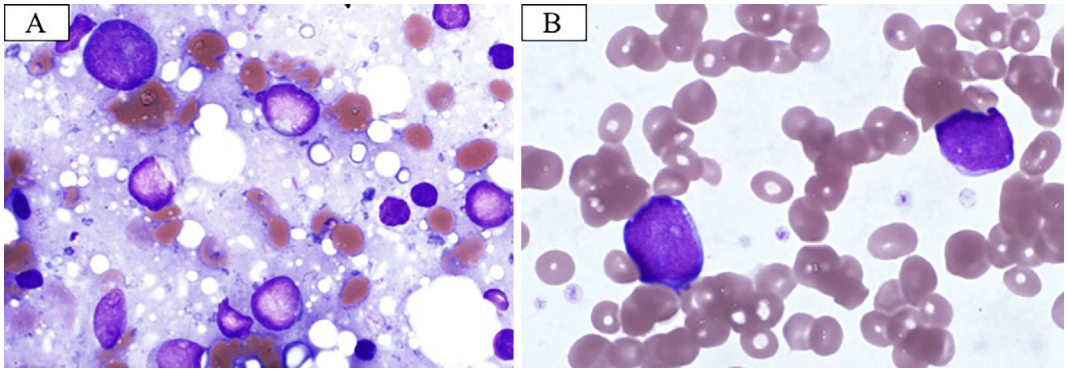


**Figure 2.** **May-Grünwald-Giemsa-stained (Wright-Giemsa-stained) bone marrow cells (A) and peripheral blood cells (B) at the first relapse of DCL. The bone marrow was filled with myeloperoxidase-negative blast cells and a few blast cells were found in the peripheral blood. (×1,000, Wright-Giemsa staining). DCL: donor cell leukemia**

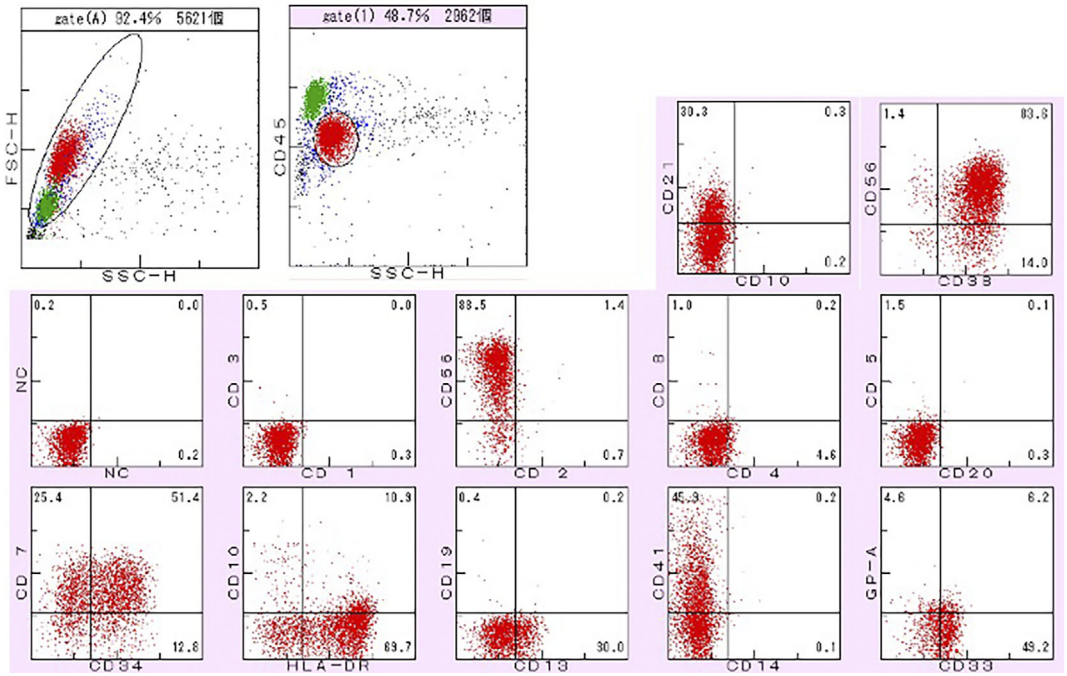


**Figure 3.** **A flow cytometric analysis of DCL at the diagnosis of acute megakaryocytic leukemia. Blast cells were positive for CD7, CD21, CD33, CD34, CD38, CD41, CD56 and HLA-DR. DCL: donor cell leukemia**

**Table 3.** **Main Characteristics of the Original Leukemia and Donor Cell Leukemia.**

| | Original leukemia | Donor cell leukemia |
|---|---|---|
| Myeloperoxidase stain | Positive | Negative |
| Cell surface markers | Positive: CD13, CD34, HLA-DR<br>Negative: CD7, CD33, CD41, CD56 | Positive: CD7, CD13, CD21, CD34, CD33, CD38, CD41, CD56, HLA-DR |
| G-banding stain | 47,XY,+1,der(1;7)(q10;p10),+8[13]/46,XY[7] | 46,XX[20] |
| WHO classification | AML with myelodysplasia-related changes | Acute megakaryoblastic leukemia |

WHO: World Health Organization, AML: acute myelogenous leukemia

the patient's overall condition improved with a mild decrease in LDH levels; he continued to receive treatment with azacitidine alone as an outpatient. He underwent red blood cell transfusions regularly, but no severe adverse events such as febrile neutropenia were observed during azacitidine monotherapy. He demonstrated disease progression four months after azacitidine monotherapy. He underwent low-dose combination chemotherapy and gemtuzumab ozogami-

cin monotherapy, but they were ineffective and caused severe myelosuppression and sepsis. He ultimately died of brain hemorrhaging 28 months after UCBT.

## Discussion

Acute megakaryoblastic leukemia can develop as DCL after allogeneic HSCT. Table 3 shows the main characteristics of the original leukemia and donor cell leukemia in our report. HSCT is an effective treatment for many hematologic malignancies, but disease relapse remains a major cause of post-transplant mortality (6). DCL is *de novo* leukemia developing in donor-derived hematopoietic precursor cells and is a rare but severe complication after allogeneic HSCT. The incidence of DCL may have been underestimated because clinicians do not perform thorough examinations to distinguish between recipient-derived cells and donor-derived cells in cases of leukemia developing after HSCT. DCL has become well known, and a number of reports of DCL have been published in recent years (7-9). It is important to distinguish DCL from relapse of original diseases when patients develop leukemia mimicking the original diseases after allogeneic HSCT, especially in cases where a decision regarding treatment for the leukemia must be made.

In the present report, we diagnosed DCL through a FISH analysis of the sex chromosomes, although we initially suspected relapse of the original disease. Compared to relapsed disease, which almost always occurs within 2 years post-transplant, DCL is commonly diagnosed later, with a median time to DCL of 31 months (range: 2-312 months) (10). Shiozaki et al. reported that the characteristics of DCL occurring after UCBT were different from those occurring after bone marrow transplantation (BMT), including the duration of time between transplantation and the occurrence of DCL, and the types of abnormal karyotypes (11). DCL occurred within a significantly shorter period after UCBT than after BMT, with a median duration of 14.5 months for UCBT and 36 months for BMT. The frequency of monosomy 7 observed in DCL after UCBT was significantly higher than that in DCL after BMT. Greaves reported that up to 5% of CB samples might harbor potentially preleukemic clones, raising the possibility that DCL might be disproportionately common after UCBT (12). It has been reported that risk factors for DCL include the use of cells obtained from elderly donors and cells obtained from cord blood (13). In our case, the use of cord blood for the cell source was a risk factor for developing DCL as well as for the early occurrence of DCL.

Azacitidine may be effective for treating acute megakaryoblastic leukemia. Acute megakaryoblastic leukemia is a rare form of adult AML, occurring in about 1% of all AML cases (14). Because of its low incidence, clinical data on acute megakaryoblastic leukemia are limited.

We treated the present patient with anthracycline-based chemotherapy, which led to CR. However, such an intensive chemotherapy regimen caused severe complications, including prolonged neutropenia, severe infection, acute kidney injury and severe liver damage. We next treated the patient with L-ASP alone because of our initial diagnosis of M/NKPAL, with reference to the reports of two pediatric cases of M/NKPAL successfully treated with L-ASP-based therapy (15, 16). However, we reached a correct diagnosis of acute megakaryoblastic leukemia by re-assessing surface markers of his DCL including CD41, after unsuccessful treatment with L-ASP. M/NKPAL is negative for the cytochemical myeloperoxidase (MPO) reaction, suggesting that this leukemia is incorporated into the FAB classification of AML as M0. It has been shown that some cases of $CD7^+$ $CD56^+$ AML with MPO-negative were categorized as M7 because of CD41 positivity and infrequently showed extramedullary involvement (17). We need to discriminate between these types of leukemia by examining surface markers, including CD41, when a patient is diagnosed with $CD7^+$ $CD56^+$ AML. We subsequently treated the patients with azacitidine, which is effective in patients with high-risk MDS or AML who are unfit for intensive chemotherapy (4, 15, 18). Azacitidine actually showed some degree of efficacy in our patient.

In conclusion, DCL should be considered in all cases of acute leukemia developing after allogeneic HSCT and should be differentiated from relapse of the original disease by a thorough examination with a chimerism analysis using molecular or cytogenetic technique in cases with leukemia mimicking the original disease. Azacitidine monotherapy may be an effective alternative therapy for patients with DCL of myeloid lineage who are unsuited for intensive chemotherapy because of various complications or organ damage after HSCT. Further reports should be accumulated to determine whether or not azacitidine is as effective against *de novo* acute megakaryoblastic leukemia as it is for the treatment of other types of AML.

**The authors state that they have no Conflict of Interest (COI).**

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