Acta Med. Okayama, 2017
Vol. 71, No. 1, pp. 79–83


Acta Medica Okayama
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*Case Report*

# Positive Minimal Residual Disease of *FLT3*-ITD before Hematopoietic Stem Cell Transplantation Resulted in a Poor Prognosis of an Acute Myeloid Leukemia

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**Acute myeloid leukemia (AML) patients with *fms-related tyrosine kinase 3* (*FLT3*)-internal tandem duplication (ITD) often have a poor prognosis, even after hematopoietic stem cell transplantation (HSCT). We report a case of AML with *FLT3*-ITD identified upon initial diagnosis, who received HSCT at complete remission after 3 consecutive chemotherapies. However, the patient relapsed when the same *FLT3*-ITD clone emerged, and finally died. Retrospective analysis revealed an allelic ratio of FLT3-ITD/wild type of 1.1 and 0.0096 upon initial diagnosis and before HSCT, respectively. The detection of any minimal residual *FLT3*-ITD clone before HSCT is useful in the treatment of AML with *FLT3*-ITD.**

**Key words:** acute myeloid leukemia, relapse, *FLT3*-ITD, HSCT, minimal residual disease

*Fms-related tyrosine kinase 3* (*FLT3*)-internal tandem duplication (ITD) is observed in approximately 30% of adult and 15% of pediatric AML patients [1], and both groups generally show poor prognosis, even after hematopoietic stem cell transplantation (HSCT) [2]. A recent study report the heterogeneity of prognosis in AML patients with *FLT3*-ITD, but further details remain to be clarified [3]. We encountered a 12-year-old female AML patient with *FLT3*-ITD. Successful complete remission was achieved after 2 consecutive induction chemotherapies, ECM (etoposide, cytarabine and mitoxantrone) and HCEI (high-dose cytarabine, etoposide and idarubicine), following the Japan Pediatric Leukemia Study Group (JPLSG) AML05 protocol [4]. After 1 more course of intensified chemotherapy, the patient received HSCT from her one-locus mismatched sister after undergoing a busulfex (BU) and melphalan (L-PAM) conditioning regimen. Relapse occured 4 months after HSCT, and the same *FLT3*-ITD clone was observed. A retrospective study revealed an allelic FLT3-ITD/wild type (WT) ratio of 0.0096 in the bone marrow sample taken prior to HSCT. Furthermore, the *WT1* mRNA level before HSCT did not fall below the cutoff point. A second HSCT was performed from the patient's human leukocyte antigen (HLA)-matched brother, but the presence of *FLT3*-ITD remained detectable. The patient died 5 months after the second HSCT. In this report, we discuss the prognostic usefulness of minimal residual disease (MRD) of *FLT3*-ITD in AML patients.

## Case Report

The patient was a 12-year-old girl who had suffered general fatigue for a month, and presented with pallor

Received May 24, 2016; accepted September 5, 2016.
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Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

and petechiae. Blood tests showed a white blood cell count of $8.2 \times 10^4$/μL, with 94% myeloblasts, a hemoglobin level of 5.3 g/dL, and a platelet count of $1.2 \times 10^4$/μL. Bone marrow examination revealed a nucleated cell count of $49.9 \times 10^4$/μL, with 74% myeloblasts, classifying the patient as subgroup M2 according to the French-American-British (FAB) classification. Blasts were positive for CD13, CD33, and HLA-DR, and negative for CD2, CD19, CD34, and CD41 by flow cytometric analysis. Chromosomal analysis by the G-banding method of bone marrow cells showed no abnormalities, but molecular analysis revealed a 24 bp *FLT3*-ITD. Induction chemotherapy was administered according to the JPLSG-AML05 protocol (Fig. 1) [4], and complete remission was achieved after the second course of induction therapy. According to the protocol, this patient was classified into the high risk group and underwent allogeneic bone marrow transplantation from her HLA 7/8-matched sister. Myeloablative conditioning was carried out with BU and L-PAM, and graft versus host disease (GVHD) prophylaxis was done with tacrolimus and methotrexate. Engraftment occurred on Day + 19. No acute GVHD developed. The patient was discharged on Day + 71, and tacrolimus was discontinued on Day + 79. On Day + 114, blood tests revealed an elevated white blood cell count of 9,770 /μL with 21% myeloblasts. On Day + 119, bone marrow examination revealed 83.2% myeloblasts, and relapse of AML was confirmed. The patient subsequently underwent donor lymphocyte infusion (DLI) and high-dose cytarabine treatment, but did not achieve remission. She proceeded to undergo a second allogeneic peripheral blood stem cell transplantation (SCT) from her HLA-matched brother using non-myeloablative conditioning with gemtuzumab ozogamicin, cytarabine, idarubicin, fludarabine, and total body irradiation

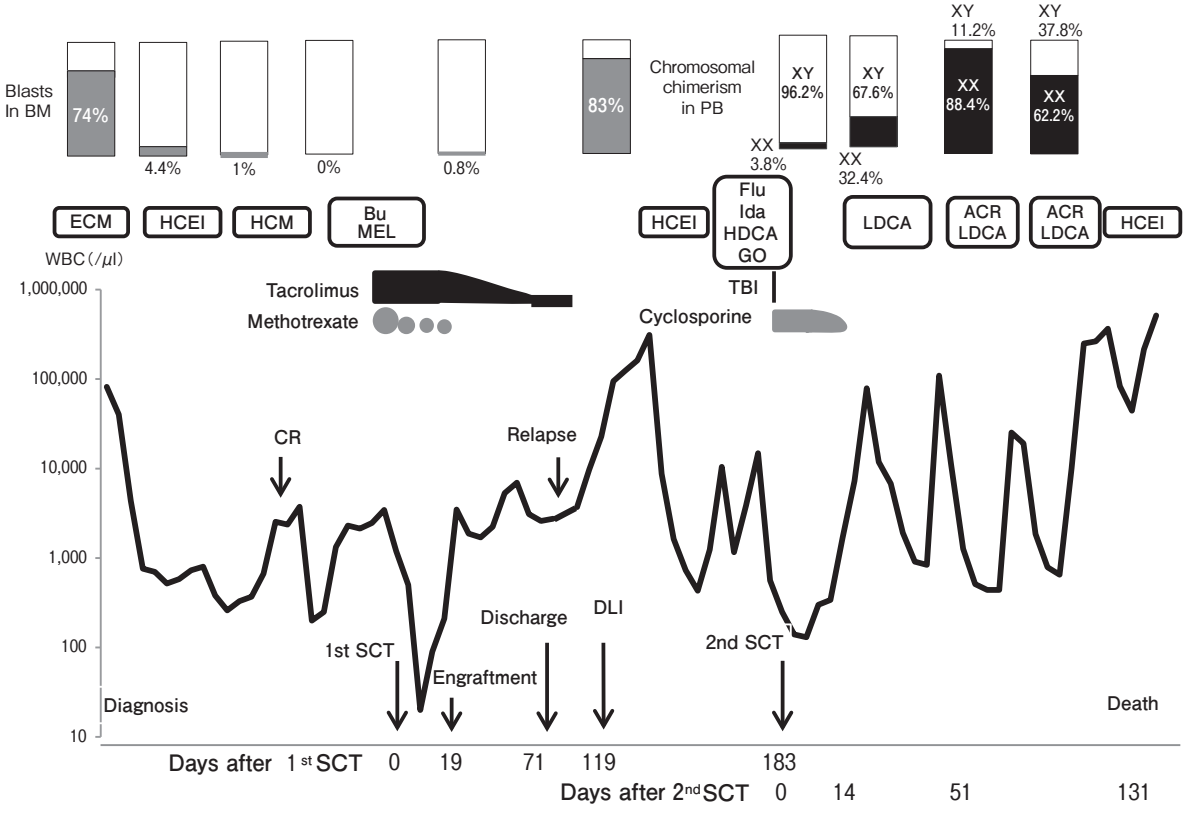


**Fig. 1** Clinical course of the patient. ECM, etoposide, cytarabine, mitoxantrone; HCEI, high-dose cytarabine, etoposide, idarubicine; HCM, high dose cytarabine, mitoxantrone; BU, busulfex; L-PAM, melphalan; Flu, fludarabine; IDA, idarubicine; HDCA, high-dose cytarabine; GO, gemtuzumab ozogamicin; LDCA, low-dose cytarabine; ACR, aclarubicin; CR, complete remission; DLI, donor lymphocyte infusion; SCT, stem cell transplantation; TBI, total body irradiation.

(TBI, 2Gy). GVHD prophylaxis was carried out with cyclosporine. Donor chimerism in the peripheral blood according to Y-chromosome Fluorescence in situ hybridization (FISH) analysis was 96.2% on Day + 14 after the second SCT, suggesting potential engraftment. However, blasts in the peripheral blood increased rapidly thereafter. After four courses of palliative chemotherapy, the patient died on Day + 131 after the second SCT.

## Materials and Methods

Informed consent was obtained from the patient and her parents. The Institutional Ethics Committee of Okayama University approved the genetic analysis (No. 2282).

***MRD monitoring using FLT3-ITD by PCR.*** Bone marrow DNA from samples taken at each examined time point were extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA), following the manufacturer's recommendations. Polymerase chain reaction (PCR) was performed in a 25 μL mixture containing 100 ng gDNA, 160 μM of each deoxynucleotide (dNTP), 1 × PCR buffer, 2.5 mM $MgCl_2$, 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 5% dimethyl sulfoxide, 2 pmol of forward primer 5'-[6-FAM]-GCAATTTAGGTATGAAAGCCAGC-3', and 2 pmol of reverse primer 5'-gtttcttCATCTTTGTTGCT-GTCCTTCCAC-3', which covered exons 14 and 15, on a T100™ thermal cycler (Bio-Rad Laboratories, Hercules, CA, USA). The PCR conditions were as follows: 94°C for 10 min, followed by 95°C for 15 sec, 58°C for 30 sec, and 72°C for 30 sec, for 35 cycles, and finally 72°C for 5 min. The size and positions of the peaks were analyzed using an ABI 310 genetic analyzer (Applied Biosystems). The allelic ratio of *FLT3*-ITD/wild type (AR) was calculated by GeneScan software (version 3.1.2) (Applied Biosystems).

***Direct sequencing of FLT3-ITD PCR products.*** PCR products were separated on a 3% agarose gel, and the mutant bands were isolated and directly sequenced using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and an ABI 310 genetic analyzer (Applied Biosystems). Sequences were compared to the WT sequence (Accession NG_007066. 1).

***WT1 mRNA expression by real time PCR and NUP98-NSD1 expression by RT-PCR.*** Bone marrow RNA from samples taken at each examined time point was extracted using an RNeasy Mini Kit (Qiagen), following the manufacturer's recommendations. *WT1* mRNA expression was analyzed by real time PCR using the WT1 mRNA Assay Kit "Otsuka" and the COBAS Taqman 48 Analyzer (Roche, Switzerland).

## Results

A 24 bp *FLT3*-ITD was detected in the initial bone marrow (BM) sample, and the allelic ratio (AR) was 1.1 (Figs. 2, 3). MRD was detected by *FLT3*-ITD (AR 0.0096) prior to the first HSCT. The calculated MRD by *FLT3*-ITD was estimated to be less than $10^{-2}$ (0.0096/1.1 = 0.008727). No *FLT3*-ITD was detected in the BM samples on Day + 33 or Day + 62 after the first HSCT; however, *FLT3*-ITD was detected at relapse on Day + 119 after the first HSCT. The size of the ITD was the same as at initial presentation, and the AR was 1.1. *WT1* mRNA levels at each time point showed comparable changes. (Fig. 4). Furthermore, *NUP98-NSD1* chimera or *NPM1* mutations were both negative.

## Discussion

AML patients with *FLT3*-ITD generally have a poor prognosis. The use of HSCT was recommended in the AML05 study; however, HSCT has been shown to not improve the final clinical outcome of AML patients with *FLT3*-ITD [5]. A recent study reports the heterogeneity of prognosis in AML patients with *FLT3*-ITD [3]. Patients with a high AR of *FLT3*-ITD/WT or *NUP98-NSD1* chimera have been shown to have a poor prognosis, but patients with both *FLT3*-ITD and *NPM1* gene mutation have been shown to have a relatively good prognosis [3, 5]. In the present case, the patient's poor prognosis may have been indicated by the high AR (1.1).

On the other hand, after hematological remission, MRD before HSCT is reported to be an important predictor of prognosis [6]. Many AML patients with *FLT3*-ITD have normal karyotypes, and chimera such as *MLL-AF4* are not observed; in such cases, the assessment of MRD by flow cytometry is recommended [7]. The assessment of MRD using *WT1* mRNA expression has also been reported to be useful [8]. Araki *et al.* examined the MRD of *FLT3*-ITD and report a significant difference in the final outcome of patients with

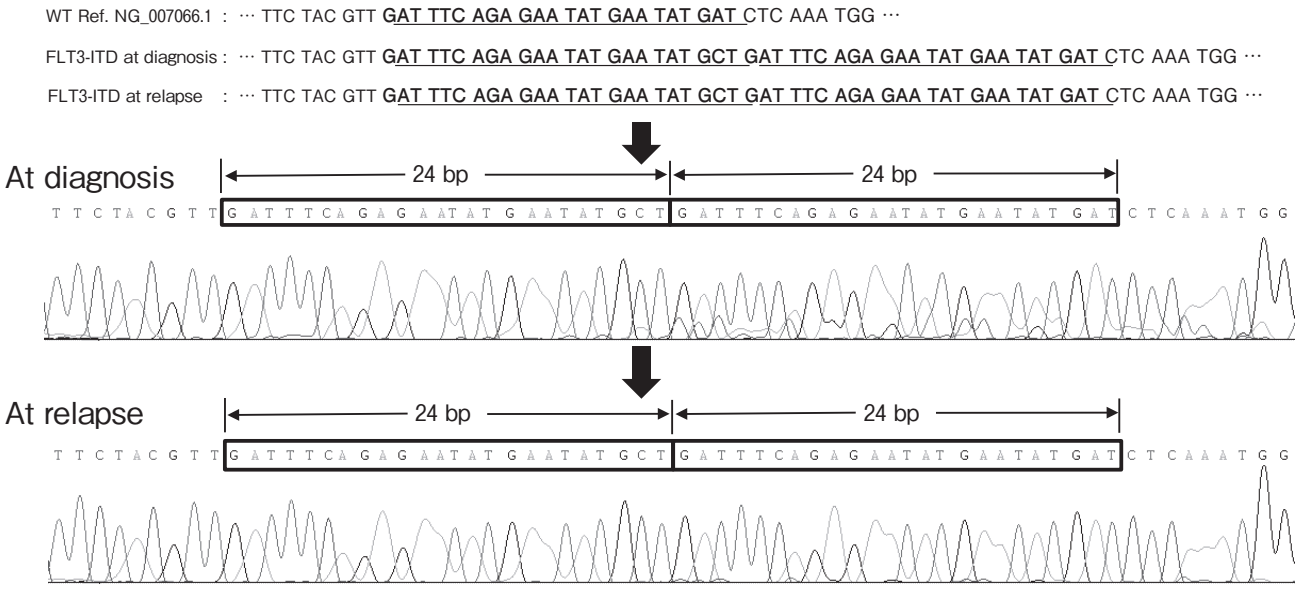


**Fig. 2** A 24 bp FLT3-ITD was detected in both the patient's initial diagnostic and relapse samples. Furthermore, an amino acid change was found only in the first ITD region (GCT>GAT, p.Ala600Asp).

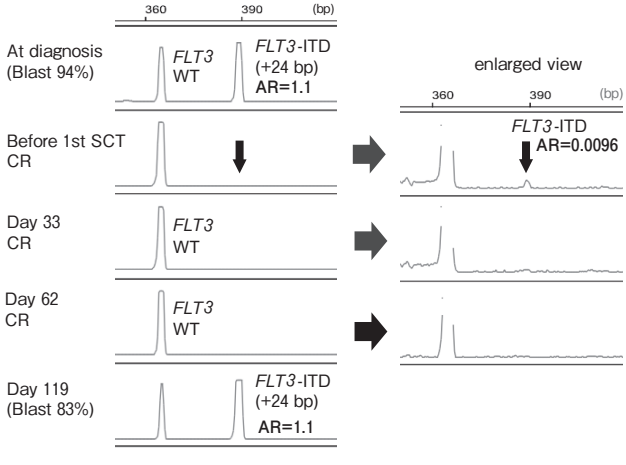


**Fig. 3** The *FLT3*-ITD/WT allelic ratio (AR) over time. The AR was 1.1 in the samples taken both at diagnosis and at relapse. The AR before SCT was 0.0096.

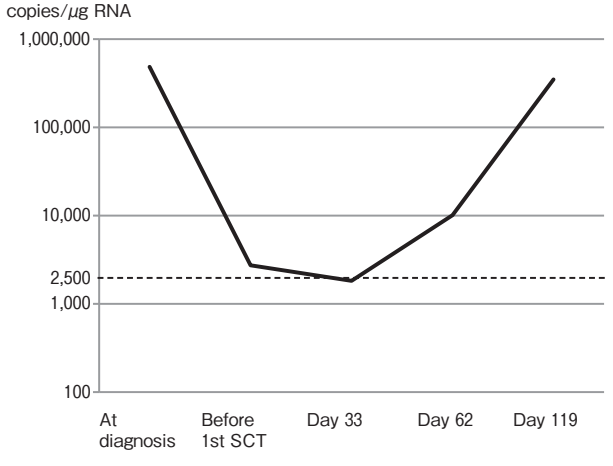


**Fig. 4** *WT1* mRNA expression level in the bone marrow samples over time. The *WT1* mRNA expression was higher than the cut-off level prior to 1st SCT.

MRD-positive remission (n=76) vs. those with MRD-negative remission (n=235) in 3-year relapse rate (67% vs. 22%) and 3-year overall survival rate (26% vs. 73%) [9]. The present case also showed MRD-positive remission detected by *FLT3*-ITD and WT1mRNA, both, before HSCT and resulted in a poor prognostic outcome. Thus, MRD monitoring by specific *FLT3*-ITD clone was shown to be helpful in these patients, and may allow treatment improvement in patients with MRD after HSCT, including the consideration of early withdrawal of immune-suppressive drugs or DLI. On the other hand, the first *FLT3*-ITD clone observed at

diagnosis may disappear, or a new clone may emerge during the relapse phase in the some cases. Therefore, careful and serial assessment for the presence of *FLT3*-ITD clones is important.

In addition, the preferred method for MRD detection based on the presence of *FLT3*-ITD has recently been shifting from PCR to next-generation sequencing [10, 11]. Thus, the assessment of *FLT3*-ITD detection method sensitivity is warranted in future studies.

In conclusion, MRD of *FLT3*-ITD before HSCT seemed to be a predictor of poor prognosis in this AML patient. Further larger-scale studies to assess the usefulness of this mutation as a predictor will be needed.

Acknowledgment. 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.

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