BLOOD RESEARCH

Outcomes of pediatric acute myeloid leukemia patients with FLT3-ITD mutations in the pre-FLT3 inhibitor era

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Background
Acute myeloid leukemia (AML) with internal tandem duplication in FMS-like tyrosine kinase 3 (*FLT3-ITD*) is associated with poor outcomes. This study aimed to analyze the outcomes of pediatric AML patients with *FLT3-ITD* mutations in the pre-FLT3 inhibitor era.

Methods
We retrospectively reviewed and identified 18 patients diagnosed with non-M3 AML with *FLT3-ITD* mutations at Seoul National University Children's Hospital between May 2008 and August 2019.

Results
The median age was 13 years (range, 6–19 yr). The median follow-up time was 43 months (range, 6–157 mo). Fourteen patients received BH-AC-based (N4-Behenoy1-1-β-D-arabinofuranosy1cytosine) and 4 received cytarabine-based induction chemotherapy. Complete remission (CR) was achieved in 72.2% of the patients after the first induction chemotherapy and 80% of the patients achieved CR after salvage therapy. The overall CR rate was 94% (17/18 patients). These 17 patients underwent hematopoietic stem cell transplantation (9 matched unrelated donors, 5 matched related donors, and 3 haploidentical donors). Relapse occurred in 22% of the patients. Event free survival and overall survival rates were 53.8±12.1% and 53.6±12.1%, respectively, and they were not significantly different according to the type of induction chemotherapy ($P=0.690$) or the type of donor ($P=0.102$).

Conclusion
This study outlines the outcomes of pediatric AML patients with *FLT3-ITD*-mutations in one institution over a decade. Outcomes were significantly improved in this study compared to our previous report in 2004, where RFS and EFS were 0%. This study can provide baseline data for pediatric patients in the pre-FLT3 inhibitor era.

Key Words   *FLT3-ITD*, Acute myeloid leukemia, Pediatric, Overall survival

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy that involves the uncontrolled clonal proliferation of blasts in the bone marrow and peripheral blood. It is characterized by clonal evolution and genetic heterogeneity [1-3]. Since cytogenetic profiles have become important indicators of prognosis in AML, the World Health Organization, National Comprehensive Cancer Network, and European LeukemiaNet have incorporated certain cytogenetic and molecular abnormalities in AML classifications and risk stratifications. Internal tandem duplication in FMS-like tyrosine kinase 3 (*FLT3-ITD*) mutations have been categorized as poor prognostic factors. FLT3 gene mutations occur in about 30% of adult patients diagnosed with AML, but in pediatric patients, it is rare and occurs at a rate of 5–15% [1]. *FLT3-ITD* triggers the activation of tyrosine kinase receptors and downstream signaling, leading to increased leukemic stem and progenitor cell proliferation and their

increased survival [4]. These patients have leukocytosis, a higher percentage of blasts, a higher relapse rate, and poorer overall survival (OS) compared to patients with wild-type (wt) [5, 6]. Although there are no significant differences in complete remission (CR) rates, responses are usually short-lived and are inferior to salvage therapies. *FLT3-ITD* mutations in the pediatric AML population have also been reported to have poorer OS and event-free survival (EFS) compared to wt [6, 7]. A previous study from our institution in 2004 reported an OS and EFS rate of 0% in this population [6]. A recent study reported 5-year OS and EFS rates of 42.2% and 36.8%, respectively [8]. Due to considerable evidence of the poor prognosis associated with *FLT3-ITD* mutations, many institutions have incorporated allogeneic hematopoietic stem cell transplantation (HSCT) and newer treatment options, such as FLT3-targeted therapy, into their therapeutic regimens, especially in the adult population [5]. Nevertheless, optimal treatment has not been well-defined in the pediatric population. Thus, this retrospective study aimed to analyze the outcomes of pediatric AML patients with *FLT3-ITD* mutations receiving the current treatment regimen at a single institution over a decade. This is a follow-up study from a previous report published in 2004 and is the first to focus on pediatric AML patients with *FLT3-ITD* mutations in Korea. This study aims to provide baseline data for comparing the outcomes of newly incorporated target therapies.

MATERIALS AND METHODS

Patients

All AML patients with *FLT3-ITD* mutations aged 19 years and under, newly diagnosed between May 2008 and August 2019 at Seoul National University Children's Hospital, were included in this study. Patients with acute promyelocytic leukemia were excluded. A total of 18 patients were identified for this study by a retrospective medical record review and followed until June 1, 2020. This was a retrospective, single-center study carried out after approval and waived informed consent was received from the Institutional Review Board of Seoul National University Hospital (IRB H-2004-146-1118).

Data collection and method

Demographic characteristics, laboratory findings, treatments, toxicities, and outcome data were collected by reviewing medical records. AML classification was determined according to the French-American-British (FAB) criteria. Cytogenetic and genetic analyses were conducted using the trypsin-Giemsa banding technique and fluorescent in situ hybridization (FISH) on bone marrow cells at diagnosis. All patients had their *FLT3-ITD* mutation status determined by fragment analysis, polymerase chain reaction, and direct sequencing from bone marrow aspirate at diagnosis. From the initial bone marrow sample, all the patients were tested for concurrent gene mutations, including *AML1/ETO* (8;21),

MLL rearrangement, *PML-RARA*, and *CBFB* (*INV16*), using FISH.

Chemotherapy regimen

Prior to 2014, the induction chemotherapy protocol for pediatric AML patients in our institution was BH-AC-based (N4-Behenoy1-1-β-D-arabinofuranosy1cytosine) and consisted of enocitabine (300 mg/m^2/day) for 7–10 days, idarubicin (IDA, 12 mg/m^2/day) for 3 days, and intrathecal (IT) cytarabine [6]. Starting in 2014, our center began to utilize a cytarabine-based induction regimen consisting of 2 courses, the first of which involved a continuous intravenous (IV) cytarabine infusion (200 mg/m^2/day) for 7 days, IDA (12 mg/m^2/day) for 3 days, and IT cytarabine. The second course consisted of 8 doses of IV cytarabine (1,500 mg/m^2), mitoxantrone (12 mg/m^2) for 2 days, and IT cytarabine. Patients with primary refractory disease received various salvage chemotherapies, as further elucidated in the results section. After chemotherapy, patients with *FLT3-ITD* mutations received high-dose conditioning therapy and allogeneic HSCT. For donor selection, human leukocyte antigen (HLA) compatibility (HLA-A, -B, -C, -DRB1, or -DQB1) was tested. Three different conditioning regimens were used in this study. One of the earlier regimens consisted of total body irradiation (TBI) at 300–333 cGy for 3 days, cytarabine (3 g/m^2) twice a day for 2 days, fludarabine (50 mg/m^2) for 4 days with or without thymoglobulin (1.5 mg/kg) for 3 days (TBIAcFluda). Another conditioning regimen consisted of targeted busulfan for 4 days, fludarabine (40 mg/m^2) for 6 days, etoposide (20 mg/kg) for 3 days, and thymoglobulin (2.5 mg/kg) for 3 days (BuFluVPATG). Depending on the type of donor (unrelated versus related), methotrexate and tacrolimus or cyclosporine were also administered for graft-versus-host disease (GVHD) prophylaxis. Lastly, the regimen used for haploidentical donors consisted of targeted busulfan for 4 days, fludarabine (40 mg/m^2) for 5 days, and cyclophosphamide at a dose of 14.5 mg/kg for the 2 days prior to the infusion and 50 mg/kg for 2 days after the infusion (BuFludaCy). For GVHD prophylaxis, mycophenolate mofetil and tacrolimus were administered. Targeted IV busulfan was administered at a starting dose of 120 mg/m^2, and subsequent doses were analyzed using daily therapeutic drug monitoring [9-11].

Definition of outcomes

OS was defined as the duration from diagnosis to death or last follow-up, and EFS was defined as the duration from diagnosis to the first event (consisting of death or relapse). Primary refractory disease was not considered an event. Relapse free survival (RFS) was defined as the duration in months from the end of the primary treatment (HSCT) to relapse. The occurrence of death without relapse was not included as an event for RFS. Data on living patients and those who had died from any cause without relapse were recorded until the date of death or last follow-up, using a cut-off date of June 1, 2020. CR was defined as morphological remission (<5% blasts) in the bone marrow, and

primary CR (CR1) was defined as morphological remission after the first course of induction chemotherapy. Primary refractory cancer to BH-AC-based induction was defined as morphological persistence (>5% blasts) in the bone marrow after induction chemotherapy. For cytarabine-based therapy, primary refractory disease was defined as >20% blasts in the bone marrow after the first course of induction chemotherapy and/or >5% blasts in the marrow after both courses of induction chemotherapy. The neutrophil engraftment date was defined as 3 consecutive days with an absolute neutrophil count (ANC) greater than 0.5×10^9/L after infusion.

Statistical analysis

Clinical and laboratory data were analyzed using standard statistical methods. The OS, EFS, and RFS were analyzed using the Kaplan-Meier method and the difference in survival rates was determined using the log-rank test, with results expressed as percentages±standard error. Statistical significance was defined as a P-value <0.05. Data and statistical analyses were performed using STATA ver 13 (StataCorp LLC, College Station, TX, USA).

Table 1. Characteristics of patients with FLT3 mutations.

N	18
Median age at diagnosis	13 (6–19)
Gender (N) F:M	11:7
Prognostic factors (%)	
Primary refractory	5 (27.8)
High WBC count[a]	4 (22.2)
Secondary AML	2 (11.1)
FAB (N, %)	
M1	7 (38.9)
M2	4 (22.2)
M4	6 (33.3)
M5	1 (5.5)
Cytogenetic/molecular (N, %)	
Normal	9 (50)
inv (16)	0
KMT2A/MLL	1 (5.5)
PML/RARA	0
AML1/ETO	3 (16.7)
FLT/TKD	1 (5.5)
Induction chemotherapy (N, %)	
BH-AC-based[b]	14 (77.7)
Cytarabine-based[c]	4 (22.2)
HSCT (N, %)	17 (94.4)
MUD	9 (52.9)
MRD	5 (27.8)
Haploidentical	3 (16.7)

[a]WBC count of >100×10^9/L at diagnosis; [b]enocitabine, idarubicin, intrathecal cytarabine; [c]cytarabine, idarubicin, mitoxantrone.
Abbreviations: AML, acute myeloid leukemia; CR, complete remission; DOD, died of disease; FAB, French-American-British classification; HSCT, hematopoietic stem cell transplant; MRD, matched related donor; MUD, matched unrelated donor; N, number; TRM, treatment-related mortality.

Characteristics of patients

A total of 18 patients were newly diagnosed with non-M3 AML with *FLT3-ITD* mutations between 2008 and 2019. The demographics are shown in Table 1. The median age at diagnosis was 13 years of age (range, 6–19 yr). Eleven were female and 7 were male. The median follow-up time was 43 months (range, 6–157 mo). For the FAB classification, M1 was the most common type (N=7, 39%), followed by M4 (N=6, 33%). Of the 18 patients, 16 had primary AML and 2 (11%) had secondary AML with the primary cancers being Wilms tumor and myelodysplastic syndrome. Four patients (22%) presented with a high white blood cell count (100×10^9/L) at diagnosis. Nine patients (50%) had normal cytogenetics at diagnosis. All patients were tested for *FLT3/TKD* mutations, *inv(16)*, *AML1/ETO*, *MLL*, and *PML/RARA* rearrangements. Three patients had *AML1/ETO* rearrangements, 1 had a *MLL* rearrangement, and 1 had a *FLT3/TKD* mutation. Although not all patients were tested for other concurrent mutations, 1 patient had a *NPM1* mutation and 2 patients had *DEK/NUP214* fusions.

Treatment and outcome

Fig. 1 and Table 2 outline the patients' treatments and outcomes. All 18 patients received induction chemotherapy with either a BH-AC-based or cytarabine-based regimen. Fourteen patients received BH-AC-based induction chemotherapy and the other 4 patients received cytarabine-based induction. Thirteen patients achieved primary CR (72.2%), of which 11 were in the BH-AC group (11/14, 79%) and 2 were in the cytarabine group (2/4, 50%). The CR1 rates between the 2 induction chemotherapy regimens were not significantly different (P=0.29). The remaining 5 patients (patients 7, 12, 14, 16, and 17) had primary refractory AML and underwent salvage chemotherapy. Within the BH-AC-based induction group, patient 7 underwent the 7+3 regimen (cytarabine 100 mg/m^2 for 7 days and IDA 10 mg/m^2 for 3 days), patient 12 underwent high-dose cytarabine (8 doses of cytarabine 3,000 mg/m^2), and patient 14 underwent high-dose cytarabine and IDA-FLAG [fludarabine 30 mg/m^2 for 4 days, IDA 10 mg/m^2 for 3 days, cytarabine 2 g/m^2 for 5 days, and granulocyte colony-stimulating factor (g-csf) 400 μg/m^2 for 6 days]. All 3 patients achieved CR with salvage chemotherapy. Within the cytarabine-based induction group, patient 16 achieved CR with high-dose cytarabine and patient 17 was refractory to treatment and died of disease progression despite undergoing 3 different salvage chemotherapies [high dose cytarabine, IDA-FLAG without g-csf, and Sorafenib+Ida+Ara-C (sorafenib 150–200 mg/m^2 daily for 7 days, IDA 12 mg/m^2 for 3 days, and cytarabine 1,500 mg/m^2 for 4 days)] (Fig. 1). The overall induction success rate was similar between the 2 groups, with CR achieved in 100% (14/14) of the BH-AC group and in 75% (3/4) of the cytarabine group. Overall, there was 1 death (5.9%) during induction. The OS for CR1 and primary refractory
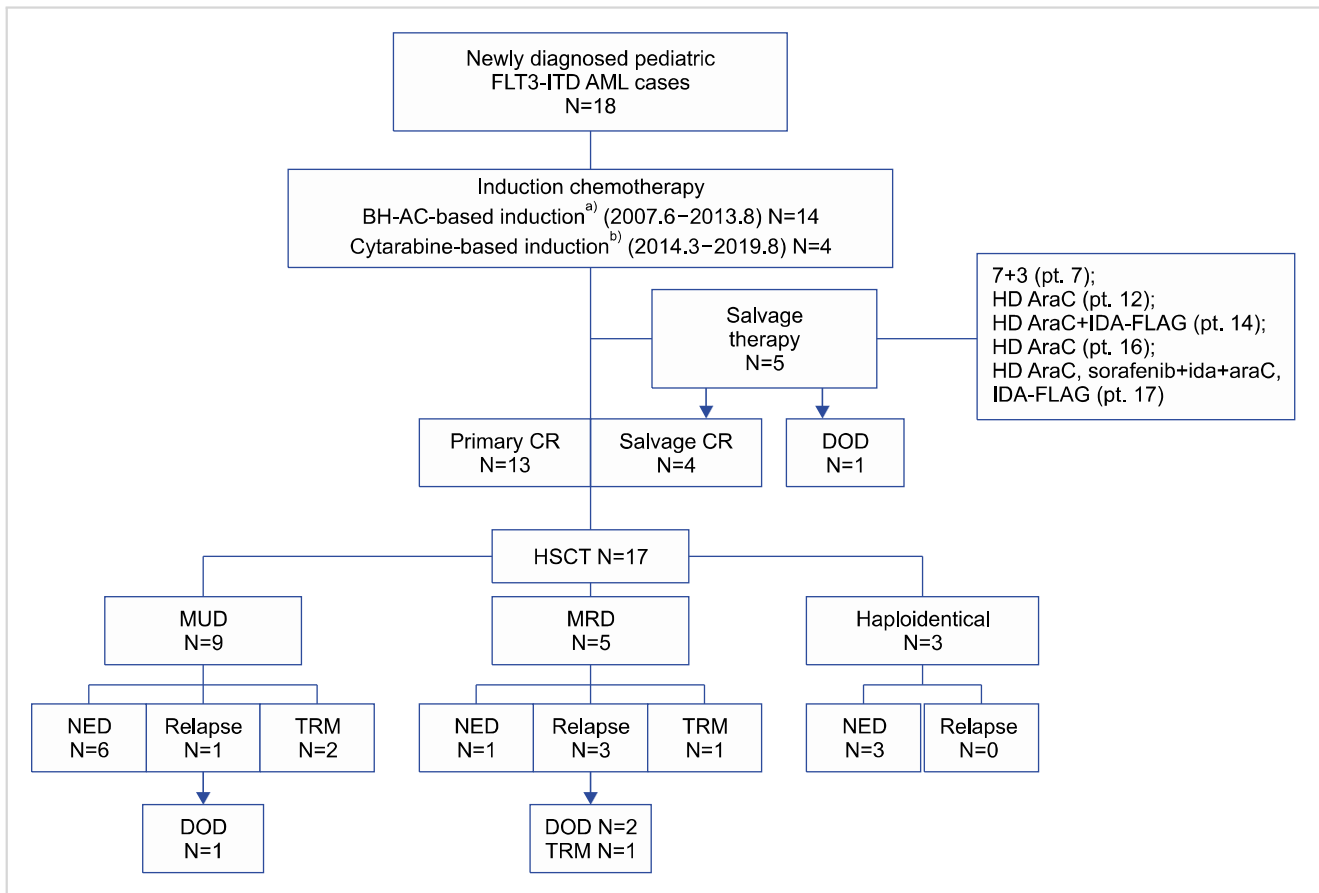
Fig. 1. Treatment and outcome of patients. [a)]Enocitabine, idarubicin, intrathecal cytarabine; [b)]cytarabine, idarubicin, mitoxantrone. Abbreviations: AML, acute myeloid leukemia; CR, complete remission; DOD, died of disease; HSCT, hematopoietic stem cell transplant; MRD, matched related donor; MUD, matched unrelated donor; N, number; NED, no evidence of disease; TRM, treatment-related mortality.

disease was 58.7±14.1% and 40.0±22.0%, respectively, and there was no statistical difference in OS between these patients (P=0.376).

Apart from the 1 patient who was refractory to treatment, the other 17 patients in CR underwent HSCT. Nine patients received grafts from matched unrelated donors (MUDs), 5 from matched related donors (MRDs), and 3 from haploidentical donors. Of the 9 MUD grafts, 6 were 10/10 HLA-compatible and 3 were 9/10 HLA-compatible. The median neutrophil engraftment dates for MRDs, MUDs, and haploidentical donors were 11 (10–12 days), 14 (11–19 days), and 18 (16–20 days), respectively. All 17 patients showed evidence of engraftment in the bone marrow 1 month after HSCT and there were no engraftment failures. GVHD was seen in 7 patients, of which 6 received MUD grafts and 1 received MRD grafts. The 2 patients with acute GVHD of the skin both received MUD grafts and were treated with short-term steroids. There were 4 cases of pulmonary chronic GVHD. These patients were treated with steroids with or without imatinib. One patient with pulmonary GVHD died during treatment related to this complication (patient 2). There were 5 patients with veno-occlusive disease (VOD), 1 of which eventually died during treatment related to this complication (patient 11) (Table 2).

One patient who received a MUD graft and 3 who received MRD grafts relapsed after HSCT. Although the relapse rate was highest among MRD recipients, RFS was not statistically different between the donor groups (haploidentical 100%, MUD 88.9%, MRD 25.0%, P=0.111). All 4 patients who relapsed died (mortality rate, 100%). Three of the relapsed patients died of disease progression and 1 was classified as treatment-related mortality (TRM) (Fig. 1, Table 2). The overall relapse rate was 24% (RFS, 72±12%) and all the patients who relapsed had received BH-AC-based induction chemotherapy; however, there was no significant difference in RFS according to chemotherapy type (BH-AC 67.3%, cytarabine 100%, P=0.38). The median time to relapse was 8 months (4–28 mo) after HSCT. The OS and EFS were 53.8±12.1% and 53.6±12.1%, respectively. The OS was not significantly different based on the type of induction therapy (P=0.690) or type of donor (P=0.102) (Fig. 2). The OS was not significantly different between the 10/10 HLA MUD and 9/10 HLA MUD donor groups (P=0.870). The OS in the normal cytogenetic group was not significantly different from the other groups (P=0.699). There was an overall mortality rate of 44.4% (8/18 patients died). Four of the deaths were relapses (22.2%, including 1 TRM; sepsis after relapse), 1 patient was refractory to treatment (5.5%), and 3 were

Table 2. Characteristics and outcome of study patients (N=18).

Pt. No.	Sex	Age (yr)	FAB	Cytogenetics at diagnosis	Combined molecular abnormality[a]	Induction	CR[d]	HSCT donor type (HLA)	Dx to HSCT (M)	CR to HSCT (M)	Conditioning regimen	Engraftment (D)	HSCT Complication	OS (entry to death, M)	Relapse (RFS, M)[e]	HSCT to last follow up (M)	Outcome
1	F	19	M4	46, XX		BH-AC[b]	Yes	MUD (10/10)	7	5	TBIAc Fluda	16	-	16	Yes (4)	9	DOD
2	M	11	M2	46,XY,t(5;21;8)(q13;q22;q22)/46,XY	AML1/ETO	BH-AC	Yes	MUD (9/10)	4	3	TBIAc Fluda	14	skin aGVHD lung cGVHD	21	No	16	TRM
3	F	9	M2	49,XX,+8,+11,+18/46,XX		BH-AC	Yes	MUD (10/10)	7	6	TBIAc Fluda	14	VOD, liver cGVHD	19	No	11	TRM
4	F	16	M4	46, XX		BH-AC	Yes	MUD (10/10)	7	6	TBIAc Fluda	19	lung cGVHD	157	No	150	Alive
5	F	14	M4	46,XX,t(6;9)(p23;q34)46,XX,inv(1)(p13q21),t(6;9)(p23;q34)	DEK/NUP214	BH-AC	Yes	MUD (9/10)	5	4	TBIAc Fluda	19	-	130	No	124	Alive
6	F	7	M5	46, XX		BH-AC	Yes	MUD (9/10)	3	2	BuFlu VPATG	17	lung cGVHD	108	No	104	Alive
7	M	9	M1	46, XY		BH-AC	Yes	MRD	6	4	TBIAc Fluda	12	-	13	Yes (4)	6	DOD
8	M	12	M1	46, XY		BH-AC	Yes	MRD	4	3	BuFlu VPATG	11	VOD	106	No	102	Alive
9	F	10	M1	46,XX,t(7;11)(p15;p15)		BH-AC	Yes	MRD	4	3	BuFlu VPATG	11	-	36	Yes (28)	32	TRM
10	F	15	M2	46,XX,t(8;21)(q22;q22)	AML1/ETO	BH-AC	Yes	MUD (10/10)	5	4	BuFlu VPATG	11	-	106	No	100	Alive
11	M	18	M1	46,XY,t(6;9)(p23;q34)/46,XY	DEK/NUP214	BH-AC	Yes	MRD	5	4	BuFlu VPATG	10	VOD, liver cGVHD	7	No	2	TRM
12	F	11	M1	46 XY		BH-AC	Yes	MRD	4	2	BuFlu VPATG	10	VOD	32	Yes (12)	27	DOD
13	M	6	M4	46, XY	FLT3/TKD	BH-AC	Yes	MUD (10/10)	4	3	BuFlu VPATG	11	VOD, lung cGVHD	101	No	96	Alive
14	F	13	M1	46,XX,16qh+		BH-AC	Yes	MUD (10/10)	4	1	BuFlu VPATG	13	skin aGVHD	50	No	46	Alive
15	M	16	M2	45,X,-Y,t(8;21)(q22;q22)	AML1/ETO	Cytarabine[c]	Yes	Haplo-identical	7	5	BuFluCy	20	-	73	No	66	Alive
16	F	13	M4	46,XX,t(11;19)(q23;p13.3)	MLL	Cytarabine	Yes	Haplo-identical	5	3	BuFluCy	18	-	70	No	65	Alive
17	M	16	M4	46, XY		Cytarabine	No	(-)	-	-	(-)	-	-	6	No	-	DOD
18	F	14	M1	46, XX	NPM1	Cytarabine	Yes	Haplo-identical	4	3	BuFluCy	16	-	13	No	9	Alive

[a]All patients were tested for *FLT3/TKD*, *CBFB inv(16)*, *AML1/ETO*, *MLL*, and *PML/RARA* rearrangements at diagnosis. Other molecular abnormalities were tested in some patients only [b]enocitabine, idarubicin, intrathecal cytarabine; [c]cytarabine, idarubicin, mitoxantrone; [d]CR including primary CR and CR after salvage therapies; [e]months from end of therapy to relapse.
Abbreviations: aGVHD, acute graft versus host disease; BuFluCy, busulfan, fludarabine, cyclophosphamide; BuFluVPATG, Busulfan, etoposide, thymoglobulin, fludarabine; cGVHD, chronic graft versus host disease; CR, complete remission; DOD, died of disease; Dx, diagnosis; FAB, French–American–British classification; HSCT, hematopoietic stem cell transplant; MRD, matched related donor; MUD, matched unrelated donor; TBIAcFluda, TBI, AraC, thymoglobulin, fludarabine; TRM, treatment related mortality; VOD, veno-occlusive disease.

classified as TRM (16.7%). TRM consisted of respiratory failure from bronchiolitis obliterans and thoracic air leak syndrome (patient 2), pneumocystis pneumonia (patient 3), and VOD (patient 11) after HSCT.

DISCUSSION

This retrospective study outlined the clinical course and evaluated the outcomes of pediatric AML patients with *FLT3-ITD* mutations in a single institution over a period of more than 20 years. However, during these 2 decades, many changes in treatment occurred. The induction regimen
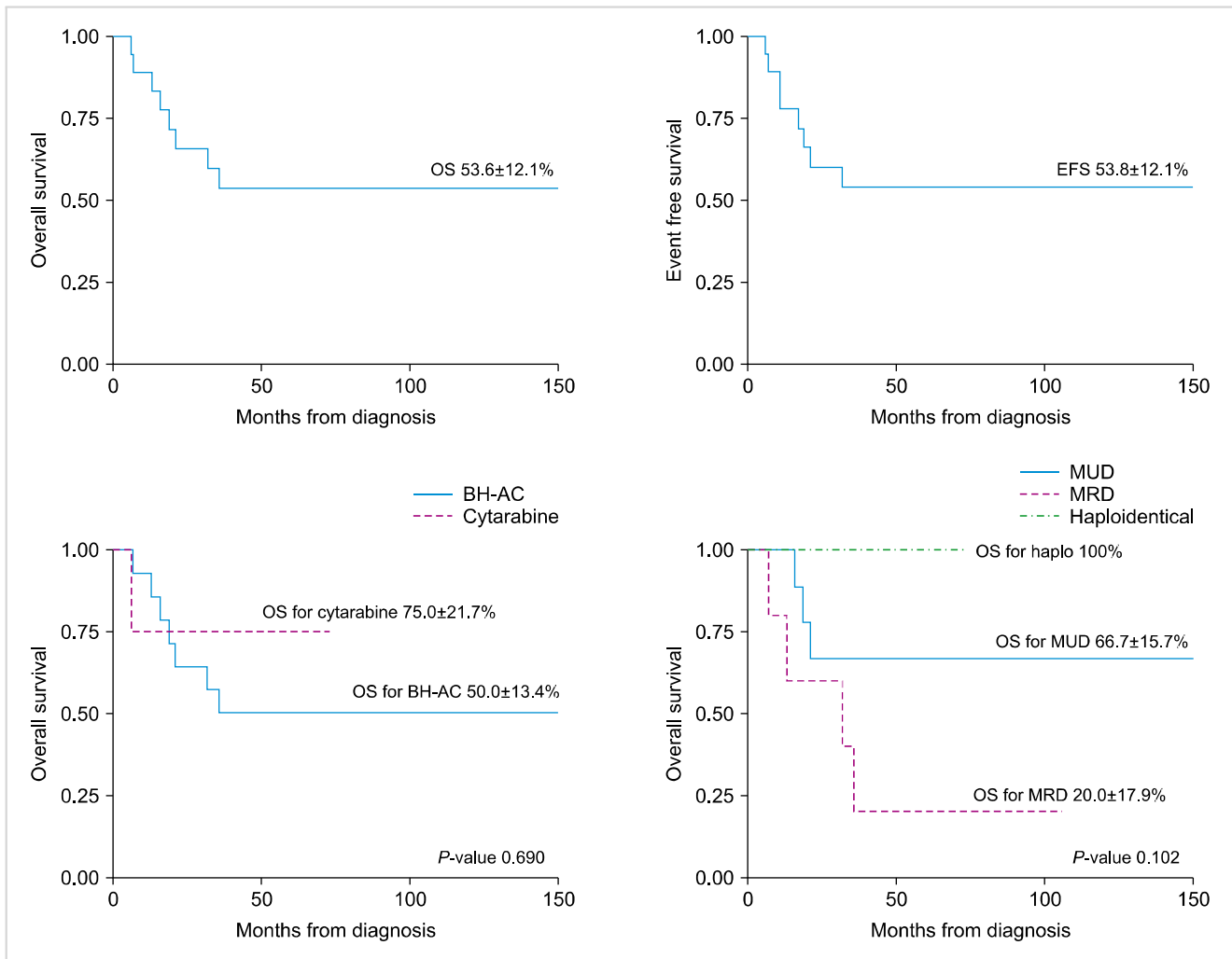
Fig. 2. Patients' overall survival (OS) and event free survival (EFS).
Abbreviations: Haplo, haploidentical; HSCT, hematopoietic stem cell transplant; MRD, matched related donor; MUD, matched unrelated donor.

changed from BH-AC-based to cytarabine-based chemotherapy, the conditioning chemotherapy regimen for HSCT was altered slightly over time, and, recently, haploidentical HSCT has become more common. However, there were no significant differences in survival outcomes based on the induction chemotherapy regimen or HSCT donor type (Fig. 2). Currently, in our institution, the two preferred donor types are the MRD and the MUD. However, to avoid delays when searching for a matched donor, haploidentical donors are becoming more common. Previously, a higher incidence of TRM caused by HLA mismatches was found associated with haploidentical HSCT. Recently, however, due to improvements in conditioning regimens and GVHD prophylaxis, reports have shown encouraging results, with TRM and OS rates similar to those of the previously preferred MRD and 10/10 MUD types [11]. According to another report, MRDs and 10/10 MUDs were preferred since the OS was higher compared to alternative donor options (9/10 MUD and haploidentical donors) [12, 13]. However, in our study, there was no significant difference in OS found between the 3 donor types (*P*=0.102) or between the 10/10 HLA

MUD and 9/10 HLA MUD groups (*P*=0.870). The haploidentical donor group also showed promising results with 0 incidences of GVHD or VOD and 0 deaths up to the end point of our study. However, studies with a larger number of patients and longer follow-up on haploidentical donor recipients are needed.

In 2004, our institution reported a study that analyzed newly diagnosed AML patients from 1996 to 2003 and found 4 patients with *FLT3-ITD* mutations. All 4 patients underwent the BH-AC-based induction regimen and achieved CR after induction. In all cases, however, relapse occurred and RFS and EFS were 0%. One patient relapsed after HSCT with an MRD, 2 relapsed before HSCT, and 1 was lost in follow-up [6]. In this study, the OS, EFS, and RFS were 53.8±12.1%, 53.6±12.1%, and 72±12%, respectively, showing a significant improvement in outcomes for pediatric AML patients with *FLT3-ITD* mutations over a period of 2 decades. This improvement in outcomes was also seen in other follow-up studies of AML patients with *FLT3-ITD* mutations owing to advances in general medical care and aggressive treatment strategies, including earlier HSCTs [3, 5, 8]. The

CR rate after first-line induction therapy was 72.2% (13/18 patients) and the CR rate after salvage therapy was 80% (4/5 patients). The overall CR rate in this study was 94.4% (17/18 patients). Both studies from our institution showed a high overall response rate of 100% and 94.4%, respectively. A study in the adult population reported that 90% of patients achieved CR after 1 or 2 induction cycles [14]. However, despite the high CR rate, more than half of the patients subsequently relapsed [14]. In our previous study, the relapse rate was 100%. However, in this study, the relapse rate was 22%, which was lower than that reported in other studies. This could be due to our high rate of HSCT (94.4%). Taylor *et al.* [14] reported a high incidence of early relapse in AML patients with *FLT3-ITD* mutations; however, when HSCT was performed at CR1, OS and remission duration significantly improved compared to when HSCT was not performed immediately. The primary reason for death in this study was relapse and disease progression (22%). All relapses in our study occurred after HSCT, with the time to relapse ranging from 4–28 months after HSCT. Our study showed relapses as late as 12 and 28 months after treatment, emphasizing the importance of off-therapy monitoring and suggesting the need for post-HSCT maintenance therapy in this population.

In this study, we detected the presence of *FLT3-ITD* mutations but did not detect the mutation level, location, or size. Studies have addressed the association between allelic ratio and prognosis, reporting that patients with *FLT3-ITD* mutations with a high allelic ratio had shorter OS, and patients with a longer inserted ITD length had a shorter RFS [15, 16]. On the other hand, other studies have reported that *FLT3-ITD* allelic ratio was not correlated with any survival outcome [14, 17, 18]. Therefore, further studies are required to establish a more solid association between the *FLT3-ITD* allelic ratio and survival rate. Cytogenetically normal AML has been regarded as a prognosis of intermediate risk, but the presence of *FLT3-ITD* mutations within this group have been recognized to reduce RFS and OS compared to the FLT3-wt [14]. However, the prognosis of *FLT3-ITD* mutations in AML patients with abnormal karyotypes is unclear. In our study, 50% of patients had a normal karyotype by conventional cytogenetic analysis at diagnosis. Consistent with other studies, there were no significant differences in the OS or EFS for normal or abnormal karyotypes in patients with *FLT3-ITD* mutations (*P*=0.699) [8, 19]. *FLT3-ITD* mutations coexisting with other specific molecular genetics and cytogenetic alterations have been frequently reported [8]. Although not all patients were tested for these in this study, known concurrent cytogenetic abnormalities were identified in 7 patients (3 with *AML1/ETO* rearrangement, 1 with *NPM1* mutation, 2 with *DEK/NUP214* fusion, and 1 with *MLL* rearrangement). The *AML1/ETO* rearrangement and *NPM1* mutation abnormalities are supposedly associated with a favorable prognosis [20]. Consistent with previous reports, all of these 4 patients achieved CR1, none had relapses, and 3 continued to have no evidence of disease (NED) (1 died of TRM). *DEK/NUP214* fusions and *MLL* rearrange-

ments are usually associated with a worse prognosis [20]; however, in our study, all 3 of these patients achieved remission, none relapsed, and 2 continued to have NED (1 died of TRM) (Table 2). Consistent with our findings, the coexistence of *NPM1* mutations has been suggested to potentially ameliorate the poor prognosis of *FLT3-ITD* in AML patients [21]. However, the presence of *NPM1* mutation has not been associated with a significant difference in survival outcomes [14, 21]. Data on the prognosis of combined *FLT3-TKD* mutations remain unclear. In our study, patient 13 had a combined *FLT3-TKD* mutation and had a good prognosis. The incorporation of routine concurrent analyses for cytogenetic abnormalities at diagnosis could provide a better understanding of potential prognostic implications in the future.

FLT3 inhibitors were not part of the treatment regimen in this study; however, these novel agents have been readily used and studied in the adult population and show promising results [2]. In vitro studies have reported that FLT3 inhibitors work synergistically with chemotherapy to induce cytotoxicity [22, 23]. Selective next generation FLT3 therapies (gliteritinib, crenolanib, quizartinib) have greater specificity for FLT3 and higher potency than multitargeted TKIs (midostaurin and sorafenib), and clinical trials have shown an improvement in OS even in relapsed/refractory patients [2, 19]. This is promising because relapse is common in these patients, and relapsed patients usually present with higher *FLT3-ITD* mutation burdens and have a poorer response to treatment [24]. Sorafenib has been extensively studied as a potential post-HSCT maintenance therapy medication, with reports suggesting it was both well tolerated and effective in significantly reducing the incidence of relapse while improving survival after HSCT [2, 25].

In summary, our study demonstrated a relapse rate of 22% after HSCT and OS and EFS rates of 53.8±12.1% and 53.6±12.1% in pediatric AML patients with *FLT3-ITD* mutations. This study was the first to focus on pediatric AML patients with *FLT3-ITD* mutations in Korea and outline their clinical course in a single institution over a decade. There were no significant differences in survival outcomes based on the induction chemotherapy regimens or the HSCT donor types in this study. Compared to our previous report from 2004, significant improvements in outcomes were seen, owing to advances in general medical care and aggressive treatment strategies, including earlier HSCTs. There are growing reports that FLT3 inhibitors are effective when added to induction, consolidation, salvage, and post-HSCT maintenance therapies. The incorporation of targeted FLT3 inhibitor therapies into current management strategies will hopefully improve the prognosis of this disease in the pediatric population. Thus, the significance of this study is that it can serve as a reference for future diagnostic (allelic ratio, molecular genetic testing) and therapeutic (FLT3 inhibitors) regimens yet to be incorporated into this high-risk pediatric population.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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