Treatment Outcomes in Children with Very High Risk Acute Lymphoblastic Leukemia Intended to Treat with Allogeneic Hematopoietic Stem Cell Transplantation

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Ⅰ. Introduction

Unlike in adult population, chemotherapy is relatively effective in treatment of ALL in children[1]. However, chemotherapy alone is noteffective in children with ALL who are classified as the very high risk group at the time of diagnosis. The VHR ALL has subgroups consist of those with Philadelphia chromosome (Ph+), infant ALL with MLL gene rearrangement, white blood cell (WBC) count more than 200,000/μL at the time of diagnosis or those who are not able to achieve complete remission even after intensive induction chemotherapy [1-3]. For these patients of the very high risk group, Allo-HSCT is recommended after the first remission [1]. In this study, we studiedthe clinical course and outcomes of children with VHR ALL who were intended to treat with Allo-HSCT after intensive chemotherapy and tried to find the clinical characteristics of each subgroup.

Ⅱ. Materials and Methods

A. Patients

Sixteen children who were diagnosed according to our institutional definition within 1 week after initiation of induction chemotherapy as VHR ALL were selected at the Department of Pediatrics, Ajou University Hospital from June 2001 to June 2010. At our institution, the VHR ALL is defined as the survival is less than 45% event-free survival(EFS) rate as the group has been reported in previous studies[1-3], which includes those with Ph+ chromosome, hyperdiploidy, failure of remission, WBC count more than 200,000/μL and infant ALL with MLL gene rearrangement. They all were waiting for Allo-HSCT receiving following induction and/or consolidation chemotherapy and searching for matched related sibling donor firstly, if not, search alternative donors who were included unrelated matched donor or unrelated cord blood. HLA-A, -B, and DRB1 by serological method between 2001 and 2004 and_HLA-A, -B, -C and DRB1 between 2005 and 2010 were done for searching matched donors.

The diagnosis of ALL was based on FAB (French-American-British) classification, and the cytochemical examinations were done in both peripheral blood and bone marrow. We used flow cytometry for immunophenotype and examined genetic rearrangements such as Ph+ or MLL gene rearrangement. No blast cells in peripheral blood and less than 5% blasts in full recovery bone marrow were defined as complete remission (CR). Among children who have acquired CR, those who had HLA-matched donors underwent Allo-HSCT from sibling or unrelated donor, while those without matched donors continued chemotherapy.

B. Treatment for Allo-HSCT

i . Conditioning

From eight to five days prior to transplantation, Total Body Irradiation (TBI) of 150 cGY twice a day was done, and cyclophosphamade 60 mg/kg was given from three days to two days prior to transplantation. In addition, etoposide 60 mg/kg, cytarabine 3000 mg/m^2/day or antithymocyte globulin 2.5 mg/kg/day was given according to general condition, source of hematopoietic stem cell and HLA correspondence. For infant ALL with MLL gene rearrangement, bulsulfan (0.8-1 mg/kg four times a day)was given from eight days to five days prior to transplantation.

ii. Prevention and Treatment of Graft Versus Host Disease (GVHD)

Combination of cyclosporine (CyA) and methotrexate (MTX) was used for prevention of GVHD, while combination of tacrolimus and MTX was used in one case. CyA 5 mg/kg/day was given one day before transplantation and intravenous injection of CyA 1.5 mg/kg/dose twice a day was given from the day of transplantation so that serum level of CyA maintained within the range of 150-300 μg/ml. Tacrolimus was injected at a dose of 0.03 mg/kg/day from one day prior to transplantation, so that serum level maintained within the range of 5-15 ng/ml. When subjects were able to take medication per oral, CyA and tacrolimus were given as oral agents once or twice a day, at dosages equivalent to three to four times of that when given as intravenous agents. 15 mg/m^2/day of MTX was injected on the day of transplantation, while 10 mg/m^2/day was given on the third, sixth and tenth day of transplantation. In one case where the transplantation was from cord blood, combination of CyA and mycophenolate mofetile (MMF, 15-20 mg/kg).

iii. Conservative care

Fluconazole, itraconazole or micafungin was given from 10 days prior to transplantation until 30 days after transplantation for prevention of fungal infections. Acyclovir of 250 mg/m^2/day was given from five days prior until 30 days after transplantation, either by intravenous agents for the whole period or by oral agents from the 14^{th} day after transplantation. Immunogloblin was given at a dosage of 500 mg/kg/day from 7 days prior to transplantation. Trimethoprim- sulfamethoxazole (8 mg/kg/day of trimethoprim) twice a day was given from nine days to two days prior to transplantation for prevention of Pneumocystis Carinii pneumonia and it was resumed when the absolute neutrophil count (ANC) was below 1,000/μLfor two consecutive days. To stimulate the hematopoietic function after transplantation, 5 μg/kg/day of granulocyte-colony stimulating factor (G-CSF) was given from the 5^{th} day after transplantation until the ANC exceeded 3,000/μL.It was resumed when the ANC decreased to level below 1,000/ul. Ursodeoxycholic acid and liposomal prostaglandin E1 (1 μg/kg/day) were given from the 1^{st} day to 30^{th} day after transplantation for the prevention of veno-occlusive disease (VOD).

C. Statistics

EFS was defined as the time from induction chemotherapy to recurrence of disease, or occurrence of severe infection which would be the cause of death. OS was defined from the time of diagnosis until death of subjects. SPSS 12.0 was used for the statistical analysis of all data, and Kaplan-Meier survival curve was used for five-year EFS. The analysis was done on 28 February, 2011.

Ⅲ. RESULTS

A. Patients characteristics at diagnosis

Among 148 children who were diagnosed as ALL at the Department of Pediatrics of Ajou University Hospital from June 2001 to June 2010, those who were classified as the very high risk group were 16 (11 boys and 5 girls), occupying 10.8%. The age at diagnosis was less than one year old in four cases, between one to ten years old in six cases and more than ten years old in six cases. (Table 1).

At diagnosis, WBC count was less than 5,000/μL in 4 cases, between 5,000 to 200,000/μL in 5 cases and higher than 200,000/μL in 7 cases. The median value was 189,200/μl (range of 2,000-477,600/μL). The subjects were generally anemic with the median value of hemoglobin 9.2 g/dL (range of 4.9-15.9 g/dL). The platelet count was between 6,000/μL and 279,000/μL with the median value of 71,000/μL. The invasion of CNS was identified by the presence of blast cells in cerebrospinal fluid. One out of 16 (6.2%) subjects was found to have CNS involvement.

Eight subjects were early precursor B cell type, three subjects were precursor B cell type and other three subjects were T cell type. CD34 was presentedin 14 subjects. Simultaneous presentation of myeloid cell markers such as CD 13 and CD 33 was found in seven subjects, occupying 43.7% (Table 2). Cytogenetic abnormality was found in 13 cases, 8 subjects with Ph+, 4 subjects with MLL

rearrangement and 1 subject with both of them (Fig. 1).

Table 1. Characteristic of Patientsat the Time of Diagnosis

No. Case	Sex/ Age at Dx (year)	WBC ($10^3/\mu L$)	Factor for VHL ALL	CNS involve	Respons e to Tx D7/D28	HSCT
1	M/2.8	21.6	Ph+	-	M1/CR	Y
2	M/6.2	195.6	Ph+	-	M1/CR	Y
3	F/2.8	159.9	Ph+	-	M1/CR	Y
4	F/12.5	2	Ph+	-	M1/CR	Y
5	M/8	233.7	Ph+ Hyperleukocytosis	-	M1/CR	Y
6	M/10.1	128.5	Ph+	-	M1/CR	Y
7	M/14.3	48.7	Ph+	-	M1/CR	Y
8	M/7.9	102.5	Ph+	-	M1/CR	Y
9	M/0.6	301.8	MLL hyperleukocytosis	-	M2/CR	Y
10	M/0.8	25.8	MLL	-	M1/CR	Y
11	F/0.3	276.0	MLL hyperleukocytosis	-	M1/CR	N
12	F/0.8	149.6	MLL	-	M2/CR	Y
13	M/16.8	344.8	hyperleukocytosis	+	M1/CR	N
14	F/14.6	353.0	Hyperleukocytosis	-	M2/CR	Y
15	M/12.8	477.6	Hyperleukocytosis	-	M1/CR	Y
16	M/4	207.4	hyperleukocytosis	-	M1/CR	

Abbreviation: Dx, diagnosis; WBC, white blood cell Tx, initial - induction chemotherapy; HSCT, hematopoietic stem cell transplantation MLL, MLL gene rearrangement on fluorescence in situ hybridization BM, bone marrow M1, BM blast <5%; M2, BM blast 5-25%;M3, BM blast >25% or bulky residual mass in lymphoma-leukemia patients; CR, complete remission

Table 2. Molecular and cytogenetic analysis of patients with VHR ALL

Case No.	FAB Classification	Immuno-phenotype	Myeloid Co-expression	Chromosome study	Molecular genetics
1	L1	EPre-B (CD10,19,22,34, HLA-DR, TdT)	CD13	46,XY, t(9;22) 58,XX,YY.t(9;22)	
2	L1	EPre-B (CD10,19,20,22,34,cCD79a, HLA-DR,TdT)	-	46,XY, t(9;22)	
3	L2	EPre-B (CD5,7,10,19,22,34, HLA-DR,Tdt)	CD33	46,XX, t(9;22)	
4	L2	Pre-B (CD10,19,20,22,34, HLA-DR,TdT,cyIgM)	-	46,XX, t(9;22)	
5	L1	Pre-B(CD10,19,20,34,45, HLA-DR,TdT,sIgM)	-	46,XY, t(9;22) 45,XY,der(7;9)	
6	L1	EPre-B (CD10,19,22,34,HLA-DR)	CD13,CD33	46,XY, t(9;22),	dupMLL+
7	L1	EPre-B(CD10,19,22,34,HLA-DR,TdT)	CD13,CD33	46,XY, t(9;22) 47,XY,t(9;22) +der(22)t(9;22)	
8	L2	EPre-B(CD14,19,22,34,HLA-DR)	CD13,CD33	46,XY, t(9;22)	
9	L1	Epre-B (CD7,19,22,34,cCD79a,HLA-DR)	-	46,XY, t(4;11)(q21;q23)	MLLex8/AFF1(1546) MLLex6/AFF1(1459)
10	L1	EPre-B(CD10,19,22,34,HLA-DR)	CD13	46,XY, t(16;21)(p11;q22)	MLL+ Nuc ish(MLLX2) (5'MLLSep 3'MLLX1) , TLS(ex7)/ERG(ex9)
11	L1	Pre-B(CD19,22,34,45,HLA-DR)	-	46,XX,	MLL+t(4;11)(q21;q23) MLL1(11q23)AF4(4q21)
12	L2	Pre-B(CD19,22,34,HLA-DR,TdT)	-	46,XX,	MLL+t(4;11)(q21;q23)
13	L1	T(cCD3,CD5,CD7,TdT),CD4-,CD8-	CD13	46, XY	
14	L1	Pre-B(CD10,19,22,34,HLA-DR,TdT)	-	46,XX	
15	L1	T(CD4,5,7,8,34)	-	46,XY,del(1p34)	
16	L1	T(CD5,7,TdT)	-	46,XY	

Abbreviation: Pre-B, precursor B cell; EPre-B, early precursor B

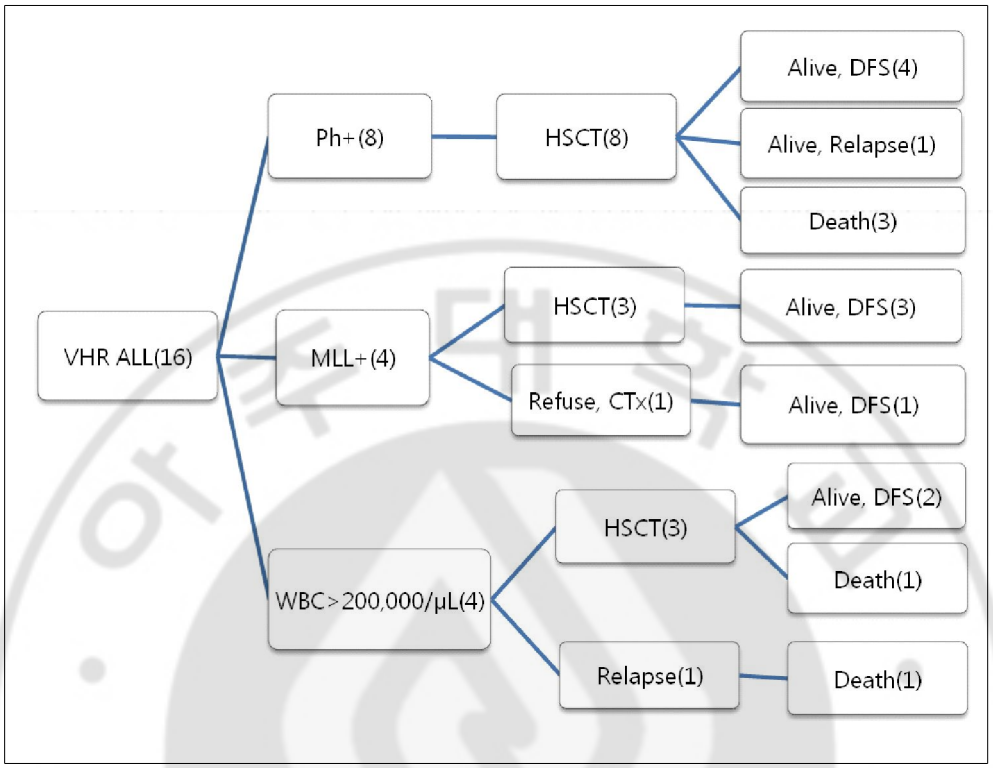

Figure 1. Outcome of treatment in children with very high risk ALL

B. Clinical Course and Treatment

i. Patients who received Allo-HSCT

All 16 patients diagnosed with VHR ALL achieved CR after the first induction chemotherapy for four weeks (Table 1), and 14 of them received Allo-HSCT while 2 did not undergo transplantation. Among patients who have received Allo-HSCT, 9 subjects are alive without relapse, 1 subject experienced relapse but survived, while 4 subjects expired. Among patients who did not undergo transplantation, 1 subject continued chemotherapy alone because parents rejected Allo-HSCT but she has survived without relapse of disease. The other subject was unable to receive Allo-HSCT because of recurrent infections after the first induction chemotherapy, and she expired. EFS of VHR ALL patients was 64.7% and OS was 57.7% (Fig. 2).

Among patients diagnosed with VHR ALL, the number of subjects who received All-HSCT was 14 (87%). All of 8 patients with Ph+ (100%), 3 out of 4 Infant ALL with MLL gene rearrangement (75%) and 2 out of 4 patients with hyperleukocytosis (50%) received Allo-HSCT. Twelve subjects received All-HSCT at status of the first complete remission (CR 1), while two subjects received at the status of the second complete remission (CR 2). The average time from diagnosis to transplantation was 6 months (range of 4-10 months). Among subjects who received Allo-HSCT, 2 werefrom related donors and 12 from unrelated donors. Thirteen cases were HLA-matched

transplantation while one case was HLA-unmatched transplantation. The source of the hematopoietic stem cell was bone marrow in 11 cases and cord blood in 3 cases. The time of engraftment was defined as the first day of three consecutive days with the absolute neutrophil count above 500/μL. The average time to engraftment was 15.5 days (range of 9-32 days). The average time taken until platelet count was maintained above 20,000/μLwas 20.5 days (range of 10-40 days). Three subjects who received Allo-HSCT needed donor lymphocyte infusion (DLI) because of relapse.

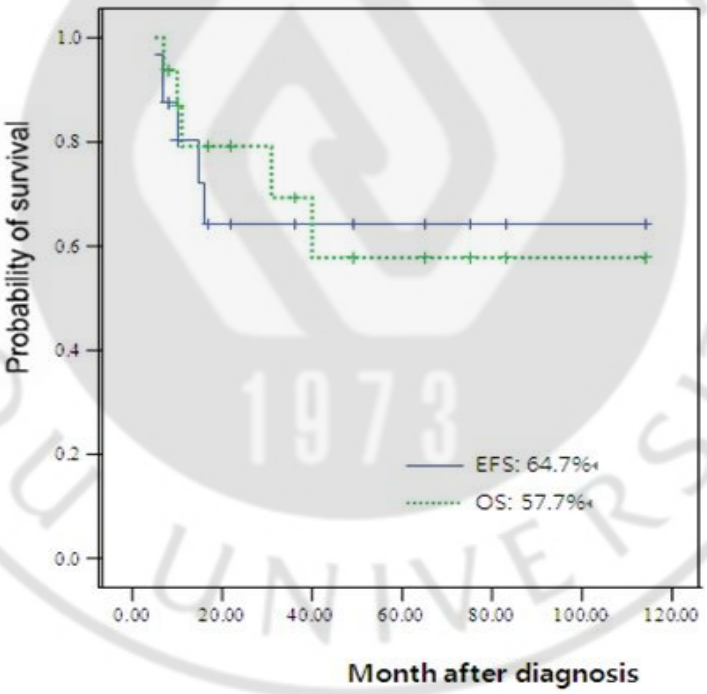

Figure 2. Kaplan-Meier probability of event free survival (EFS) and overall survival (OS) in all patients (n= 16) since diagnosed as ALL. EFS and OS were 64.7% and 57.7%, respectively.

ii. Patients with Philadelphia chromosome

All 8 patients with Ph+ received Allo-HSCT after chemotherapy. All of them achieved M1 state (bone marrow blast < 5%) on the 7^{th} day of induction chemotherapy and received Allo-HSCT at CR 1 status. After Allo-HSCT, 5 subjects (62.5%) survived without relapse, 1 subject (12.5%) experienced relapse but survived and 2 subjects (25%) expired after relapse (Fig. 1). DLI was done for all 2 subjects but they expired before achieving the CR 2. The patient who experienced relapse but survived, received the first DLI and is on continuous medication of imatinib mesylate (340 mg/m^2) while waiting for the CR 2. Among subjects who survived without relapse, one experienced leukoencephalopathy and hypothyroidism due to MTX, and another subject who was originally diagnosed with congenital adrenal hyperplasia experienced avascular necrosis of femur head and hypothyroidism while taking exogenous steroids. Among patients who underwent Allo-HSCT, four patients took imatinib mesylate before Allo-HSCT. The five-yearEFS of subjects who took imatinib mesylate was 75%, while that of subjects who did not take imatinib mesylate was 50%. The five-year EFS of Ph+ ALL patients was 58.3% while the OS was 45.7% (Fig. 3).

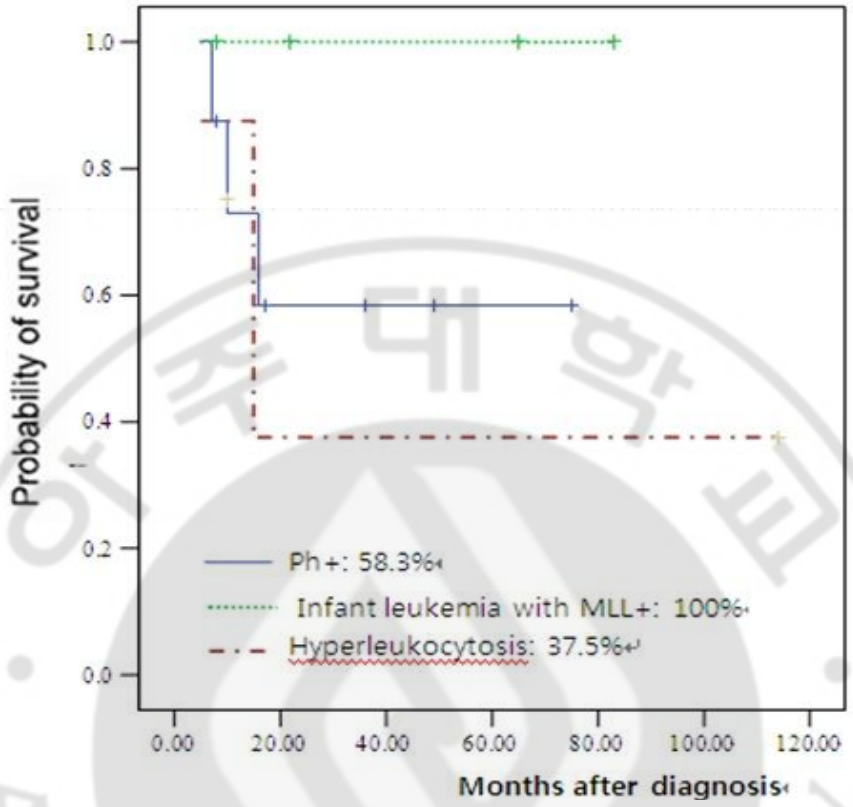

Figure 3. Kaplan-Meier probability of event free survival (EFS) according to cause of very high risk group . EFS in Ph+(n=8), MLL+(n=4) and hyperleukocytosis (n=4) were 58.3%, 100% and 37.5%, respectively.

iii. Infant ALL patients with MLL gene rearrangement

Four cases were found to be infant ALL with MLL generearrangement and the median age at diagnosis was 6 months old (range of 3-8 months). Among the four, three subjects received Allo-HSCT from unrelated donors while the other one subject received chemotherapy (POG 9407) alone because parents rejected Allo-HSCT. All four patients with infant ALL has survived without relapse. Taking development of CNS into consideration, the conditioning of infant ALL patients was done with bulsulfan instead of TBI. Two subjects were found to have no known brain lesion while one patient is on regular follow up with brain MRI due to mild ventriculomegaly. In one case of infantALL, bilateral renal enlargement was the only physical sign at the time of diagnosis, without hypertrophy of any other organs. The both EFS and OS of patients with MLL rearrangement were 100%.

ⅳ. Patients with hyperleukocytosis at diagnosis

Seven subjects were found to have hyperleukocytosis at the time of diagnosis, one with Ph+ and two subjects with MLL generearrangement. The other four cases had hyperleukocytosis without cytogenetic abnormality. The median value of WBC count at diagnosis was 345,700/μL (range of 207,400-477,600/μL). The average age was 12 years old (range of 4-16.8 years) and three of them was above 10 years old. T cell type, which was most common, was found in 3 cases. There was no patients who were found to have widened mediastinum. Leukopheresis was performed in two cases to lower the risk of stasis of WBC. Three out of four subjects who had hyperleukocytosis without cytogenetic abnormality received Allo-HSCT at status of CR 1. One of them received Allo-HSCT from a HLA-matched related donor and survived without complication, and another patient experienced facial nerve palsy due to CNS relapse before Allo-HSCT. This patient received cord blood transplantation after the second induction chemotherapy and survived without relapse. Facial nerve palsy, though, is still present. The other one patient received Allo-HSCT from a HLA-matched unrelated donor but expired due to CNS relapse. The one subject who did not receive Allo-HSCT was unable to continue chemotherapy after the first complete remission because of recurrent bacterial infections, experienced relapse and expired due to multi-organ failure and sepsis. The EFS and OS of subjects with hyperleukocytosis at diagnosis who received Allo-HSCT were 37.5% and 50% respectively.

v. Complications and mortality

Two patients (14%) experienced veno-occlusive disease (VOD). Out of two, one subject who was diagnosed as infant ALL with MLL gene rearrangement was not confirmed as VOD with doppler sonography and liver biopsy, but had increased abdominal circumference, increased amount of ascites, indirect bilirubin level of 9.2 mg/dL and direct bilirubin level of 6.7 mg/dL. This patient recovered without complication after treatment with fluid restriction, defibriotide and liposomal prostaglandin E1 (1 ug/kg/day). The other subject with VOD was a patient with Ph+ and recovered after fluid restriction only. Hemorrhagic cystitis occurred in 5 cases, 2 due to BK virus, 2 due to JC virus and virus study was not done in the other one case. Acute GVHD of grade at least II was found in 9 cases (64%). Chronic GVHD of limited stage was seen in 6 cases (43%) while chronic GVHD of extended stage was found in 4 cases (28%) (Table 3).

Among 16 patients who were diagnosed as VHR ALL, five expired. Three subjects with Ph+ experienced bone marrow relapse after Allo-HSCT and the average time to relapse was 8 months (range of 3-12 months). One of them experienced bone marrow relapse three months after transplantation. DLI was performed but she expired before the CR 2 due to GVHD in lung. Another patient was found to have bone marrow and CNS relapse nine months after transplantation. Two cycles of DLIwere done but the patient continued to have seizures due to generalized brain atrophy and expired because of

fungal infection. The last one patient experienced relapse twelve months after transplantation and expired because of lung GVHD, acute respiratory failure and multi-organ failure. Other than three patients with Ph+, the other two subjects had hyperleukocytosis. One of them was unable to continue chemotherapy because of recurrent infections after the first induction chemotherapy, and expired due to bone marrow relapse and multi-organ failure. The other subject underwent Allo-HSCT from a unrelated donor but expired due to CNS and bone marrow relapse two months after transplantation.

Table 3. Patient's Characteristic of Hematopoietic Stem cell transplantation

Case No.	Pre-HSCT status	Time to HSCT	Cell sources	Conditioning regimen	GVHD prophylaxis	Engraftment(day)		VOD	Hemo-cystitis	CMV	Acute GVHD	Chronic GVHD	Follow upc) and outcome
						ANCa)	Plateletb)						
1	CR1	6m	UBM	TBI/Cy/Eto	CSA,MTX	D+13	D+10	-	-	-	I	L	D+12m, Alive(DFS)
2	CR1	5m	UBM	TBI/Cy/Eto	CSA,MTX	D+15	D+19	-	+(BKV)	+	II	E	D+8m, Alive(relapse:5m,DLI)
3	CR1	5m	UBM	TBI/Cy/Eto	CSA,MTX	D+17	D+18	-	-	+	II	E	Dead at 5m(relapse:3m,DLI)
4	CR1	4m	UCBd)	TBI/Cy/Flu	CSA,MTX	D+32	D+40	+	+(BKV)	+	III	L	D+36m,Alive(DFS)
5	CR1	6m	UBM	TBI/Cy/Eto	CSA,MTX	D+14	D+15	-	+(JCV)	+	II	L	D+44m, Alive(DFS, MTX-induced LEP)
6	CR1	7m	UCB	TBI/Cy/ Ara-C/TG	CSA,MTX	D+25	D+36	-	-	+	III	L	D+88m, Alive(DFS,CAH, AVN, Hypothyroidism)
7	CR1	7m	UBM	TBI/Cy	CSA,MTX	D+15	D+17	-	-	-	IV	No	Dead at 30m(relapse:9m,DLI)
8	CR1	7m	PMRBM d)	TBI/Cy	CSA,MTX	D+12	D+25	-	-	+	II	E	Dead at 36m(relapse:12m)
9	CR1	5m	UBM	Bu/Cy	CSA,MTX	D+11	D+13	-	-	+	I	No	D+4m, Alive(DFS)
10	CR1	4m	UBM	Bu/Cy	Tac, MTX	D+10	D+19	+	-	+	IV	E	D+20m,Alive(DFS,)
11	CR1	5m	UBM	Bu/Cy	CSA,MTX	D+9	D+17	-	-	+	I	L	D+60m, Alive(DFS)
13	CR2	7m	UCB	TBI/Cy/Eto/TG	CSA,MMF	D+14	D+25	-	-	+	II	No	D+4m, Alive(relapse:3m,FC)
15	CR2	10m	UBM	TBI/Ara-C/Cy	CSA,MTX	D+20	D+21	-	+(JCV)		I	No	Dead at 9m(CNS relapse:2m)
16	CR1	6m	MRBM	Bu/Cy	CSA,MTX	D+11	D+12	-	-	-	I	L	D+120m,Alive(DFS)

a) Absolute Neutrophil Count > 500/㎕; b) Platelet Engraft time PLT> 20,000/㎕ c) Follow up from HSCT d) 1-mismatch

Abbreviation: UBM, unrelated bone marrow; MRBM, matched related bone marrow; PMRBM, partiallymatched related bone marrow; UCB, Unrelated cord blood; TBI, Total body irradiation; Cy, cyclophosphamide; Eto, Etoposide; Bu, Bulsulfan; Ara-C, Cytarabine; TG, thymoglobulin; MNC, mononuclear cell; CSA, cyclosporine MTX; methotraxate; MMF, MycophenolateMofetil; Neu, Neutrophil; VOD, veno-occlusive disease; DFS, disease free status; BKV, member of thepolyomavirus family JCV, member of thepolyomavirus family LEP, leukoencephalopathy; CAH, congenital adrenal hyperplasia; AVN, avascular necrosis of hip; L, limited; E, extensive ; DLI, donor lymphocyte infusion; FP. Facial palsy

Ⅳ. Discussion

In this study, we have studied the patients the clinical course and outcomes of children with VHR ALL who intended to undergone Allo-HSCT following induction and/or consolidation chemotherapy.

Generally, Allo-HSCT is considered in a restricted portion of patients with ALL because children with ALL shows very good response rate to chemotherapy.

However, there has been no agreement for indications of Allo-HSCT at the status of CR 1. There are some differences in defining VHR ALL , which includes those with Ph+, MLL gene rearrangement, failure ofremission after four weeks of induction chemotherapy, or WBC count more than 200,000/ μL at the time of diagnosis are considered as VHR ALL. For this group of patients, early Allo-HSCT should be considered because the remission rate with intensive chemotherapy alone is relatively low.

However, there is no consensus about superiority among chemotherapy and Allo-HSCT because there are few prospective studies comparing the outcomes of two treatment modalities and even retrospective studies show considerable limitations due to multiple factors.

Therefore, in this study we have analyzed the outcomes of patients who were expected to have high relapse rate and poor prognosis because they had either of Ph+, MLL gene rearrangement or hyperleukocytosis at the time of diagnosis.

ALL with Ph+occupies 2-5% of ALL in children, and is known to have poorer prognosis with remission rate of only 10% and thus requires Allo-HSCT to achieve CR [4].

In this study, all patients with Ph+ showed good response to induction chemotherapy, achieving M1 state on the 7thday and CR 1 on the 28thday of induction chemotherapy.

There have been a previous report stating that Allo-HSCT within 6 months from diagnosis resulted in a better prognosis [5] and the average time taken from diagnosis to transplantation in this study was 6 months (range of 4-10 months). This was possible because a prompt Allo-HSCT was planned and proposed once diagnosed as VHR ALL.

Previous reports have mentioned that the use of imatinib mesylate together with induction chemotherapy before Allo-HSCT in patients of ALL with Ph+resulted in better outcomes[5].

In this study as well, the EFS of subjects treated with imatinib mesylate was 75%, which was higher than that (50%) of subjects who did not take mesylate . Therefore, use of mesylate should be considered for patients waiting for Allo-HSCT.

MLL gene is located at 11q23 of the chromosome 11 and rearrangement of this gene is found in patients with ALL. There are various types of cytogenetic abnormalities such as t(4;11)(q21;q23), t(11;19)(q23;p13.3), t(9;11)(p21-22;q23) and age at the time of diagnosis is a crucial factor in predicting the outcome. InfantALL occurs in patients younger than one year old and they are 2-4% of ALL in children. Compared to ALL in children older than one year old, they have significantly poorer prognosis. Patients diagnosed with infant ALL sometimes show hyperleukocytosis at the time of diagnosis, experience hepatosplenomegaly and CNS invasion or relapse, and are found to be related with negativity for CD 10, simultaneous presentation of myeloid cell markers and MLL gene rearrangement [2, 7, 8]. Among four cases of infant ALL with MLL gene rearrangement in

this study, the average WBC count at the time of diagnosis was high with the average value of 188,000/μL (range of 25,000-301,800/μL), and hepatospelenomegaly was seen in 50% of patients.

There was no case with CNS involvement or CNS relapse, three cases (75%) were proved to be negative for CD 10 and one case showed simultaneous presentation of myeloid cell markers.

In our analysis, infant ALL without MLL gene rearrangement were excluded. Allo-HSCT is not considered in the early treatment plan for patients of infant ALL without MLL gene rearrangement, because they are known to have higher (50-75%) EFS rates than those with MLL gene rearrangement [8]. ALL Patients with MLL gene rearrangement but older than one year old is known to have better outcomes than those younger than one year old [9], thus only infant ALL with MLL rearrangement, who should be considered for Allo-HSCT, were included in this study.

Recently the remission rate of infant ALL has increased as high as 90-95% due to advancement in chemotherapy, but 30-35% of patients experience relapse mostly within one year.

The five-year EFS rate and OS rate of ALL patients with MLL gene rearrangement are 22-54% and 30-50% respectively, showing high relapse rate and mortality rate related with treatment.

Hence early Allo-HSCT after CR is essential in this group of patients. Among all four patients who achieved CR in our study, 3 subjects received Allo-HSCT after the average time of 4.6 months (range of 4-5 months) and they are in disease-free state, while the other one subject who rejected Allo-HSCT and continued chemotherapy, is also in disease-free state. According to studies done by International Bone Marrow Transplantation Registry (IBMTR), the EFS rates of patients who underwent conditioning

with bulsulfan and cyclophsphamide (Bu/Cy) and that of patients who received conditioning of whole body irradiation and cyclophosphamide (TBI/Cy) were 35% and 50% respectively [10].
Between the two groups, the relapse rates showed no significant difference but the mortality rate related with treatment was higher in Bu/Cy group.
In this study we used conditioning with Bu/Cy for all three cases of infantALL who received Allo-HSCT after discussion with their parents.
Except mild ventriculomegaly in one case, all three patients did not experience relapse or significant complications related to treatment, suggesting conditioning with Bu/Cy in infant ALL with MLL gene rearrangement may improve survival rates while lowering relapse rates and mortality rate related with treatment.

The hyperleukocytosis in childhood ALL is generally defined as peripheral WBC count higher than 100,000/ul and is found in 5-20% of newly diagnosed childhood ALL patients [11].
However in this study we considered Allo-HSCT in patients with WBC count higher than 200,000/ul because Equiguren at al [11] reported that the EFS rate of patients with WBC count higher than 200,000/ul was significantly lower (34%) than that (64%) of patients with WBC count between 100.000-200,000/ul.
In addition, bone marrow or CNS relapse was significantly higher in patients with WBC count more than 200,000/μL.
Therefore in this study, only patients with WBC higher than 200,000/μL, who were expected to have significantly poorer prognosis, were considered for Allo-HSCT after induction chemotherapy.
Seven patients in this study were presented with hyperleukocytosis and four of them were included in the analysis, excluding the three

who had cytogenetic abnormalities.

Three subjects received Allo-HSCT and only one of them has survived without relapse. One expired due to CNS relapse and the other experienced CNS relapse before Allo-HSCT. One who was not able to receive Allo-HSCT, could not continue chemotherapy because of recurrent infections after the first remission and eventually expired due to multi-organ failure and sepsis.

Lowe at al [12] previously reported that about 2% of childhood ALL patients with hyperleukocytosis expired because of cerebral hemorrhage, but none of the 7 patients in this study experienced cerebral hemorrhage. CNS irradiation, exchange transfusion, steroids and leukopheresis were reported as effective to reduce the risk of stasis of WBC [11].

In this study, leukopheresis was used for two subjects (Case No. 13, 15) who had WBC count greater than 300,000/μL.

Comparison of the EFS rates of the group with WBC count between 100,000/μL and 200,000/μL, and the group with WBC count higher than 200,000/μL may show a meaningful difference between two groups, but it was not done in this study.

In addition to hyperleukocytosis, further analyses about patients with cytogenetic abnormalities are required because they are generally known to have higher relapse rate and mortality rate.

The condition of patients before transplantation is an essential factor when evaluating the result of Allo-HSCT.

Patients receiving Allo-HSCT at CR 2 status are known to have poorer outcomes than patients receiving Allo-HSCT at CR 1 status [13].

In this study, the EFS rate of subjects who received Allo-HSCT at CR 1 was 71.3% while that at CR 2 was 0%.

Regarding the source of hematopoietic stem cell, HLA-matched

siblings are generallyknown as the best donors but the engraftment rate from unrelated donors is improving nowadays due to the advancement in supportive treatment against GHVD and other transplantation-related diseases.

Therefore even if children diagnosed with VHR ALL do not have HLA-matched related donors, they should look for unrelated donors while undergoing induction and consolidation chemotherapy. Unlike adults, children who receive Allo-HSCT from unrelated donors are more tolerable against transplantation-related toxicities and show better outcomes if Allo-HSCT is done before relapse.

Hence when appropriate supportive cares are accompanied, a better prognosis may be anticipated in children group.

In this study, we used Bu/Cy instead of TBI before Allo-HSCT in infant ALL patients with MLL gene rearrangement, minimizing the CNS injury while improving outcomes without relapse or treatment-related complications. However the overall survival rate remains to be 57.7%, suggesting the need for other treatment modalities other than Allo-HSCT. Especially, early Allo-HSCT and additional aggressive treatment modalities are required in patients with hyperleukocytosis because the EFS rate in this group is as low as 37.5%.

One of the limitations in this study is the small number of subjects, making the multilateral analysis difficult.

Also, comparison of treatment outcomes between the group with hyperleukocytosis and cytogenetic abnormality and the group without cytogenetic abnormality was not done in this study. Further investigations should be done in this area. In addition, factors other than Ph+, MLL gene rearrangement and hyperleukocytosis must be evaluated to diagnose a patient as VHR ALL, so that early Allo-HSCT could be planned before the general

condition of patients deteriorates.

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