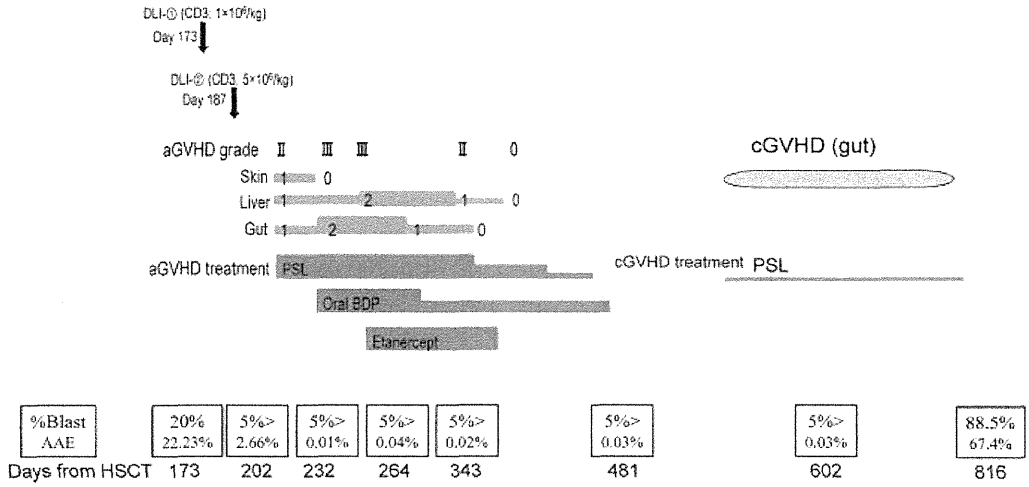

Fig. 1. Clinical course after the first DLI. T lymphocytes (CD3+) normalized and CD4+ T cell counts of more than 150/μl by 6 months after DLI. DLI, donor lymphocyte infusion; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; PSL, prednisolone; BDP, beclomethasone dipropionate; AAE, aberrant antigen expression using CD45 gating for minimal residual disease.

and GVHD prophylaxis on day +173 (Fig. 1). It took 2 weeks until there were no signs of GVHD, and the second DLI was performed. The dose of $CD3^+$ cells was increased to 5×10^6 cells/kg. BM aspirate performed 29 days after the first DLI showed a reduction in blasts to less than 5%. Thirty days after the first DLI, acute GVHD of the skin (grade I), liver (grades II–III) and gut (grades II–III) appeared, and oral beclomethasone dipropionate was administered, together with PSL (1 mg/kg/day). The patient's skin and gut GVHD reduced; however, his liver GVHD did not. Thus, we started therapy with etanercept at a dose of 0.4 mg/kg subcutaneously twice weekly for 8 weeks from day ±237. Liver GVHD progressively improved from grade III (total bilirubin level: 3.0–4.2 mg/dl) to grade I (total bilirubin level: 1.0–1.6 mg/dl) by day +278, which permitted the tapering of PSL. Mild chronic GVHD of the gut was treated with a low dose of PSL 5 mg/day while leukemia was in CR without additional therapy. However, leukemia relapsed on day ±816. HLA molecular typing was performed at relapse, and the genomic alteration resulted in the loss of HLA haplotype (Fig. 2).

DISCUSSION

MNKL is an uncommon entity and was originally proposed by Suzuki et al. [9] in 1997. In this report, seven cases were characterized by immature lymphoblastoid morphology without myeloperoxidase reactivity, $CD7^+$, $CD33^+$, $CD34^+$, and $CD56^+$ phenotype.

MNKL generally shows poor response to chemotherapy and the prognosis is poor [9–16]. Suzuki et al. [17] reported on 40 patients with neoplasms of NK-cell origin, who underwent SCT, including MNKL. The 4-year survival was better than that of patients who did not undergo SCT (39% vs. 21%, $P = 0.0003$). The probability of relapse for patients after SCT was as low as 17%. The lower incidence of relapse may indicate a GVL effect against NK-lineage tumors.

Acute leukemia relapses after allogeneic SCT has a high mortality rate. DLI can exert a GVL effect in the treatment of the molecular, cytogenetic, and chronic-phase relapses of CML with a remission induction rate as high as 80% [1–3]. When DLI is used to treat other types of leukemia, far lower response rates have been observed [1,2,4–8].

However, Huang et al. [18] reported the efficacy of DLI in patients who had a leukemic relapse after haploidentical SCT. Twenty patients received DLI at a median of 177 days after SCT. Eight patients survived in CR for a median of 1,118 days. The 2-year probability of leukemia-free survival was 40%. These data suggest that DLI is therapeutically effective in the haploidentical setting.

Two studies of patients pre- and post-SCT from an HLA-haploidentical donor describe the loss of HLA expression occurring in leukemia cells following relapse [19,20]. It was speculated in their reports and by others in the context of haploidentical transplantation that selective pressure mediated by donor T cells led to the loss of HLA haplotype in AML relapse. In our patient's case, his leukemic cells without mismatched HLA expression might have been predisposed to selective expansion through in vivo escape from immune surveillance by alloreactive T cells. Because the isolated

	A allele		B allele		Cw allele		DRB1 allele	
Patient	02:01	02:07	40:02	46:01	03:03	01:02	15:01	08:03
Donor	02:01	02:01	40:02	48:01	03:03	08:03	15:01	15:01
Blasts	02:01	-	40:02	-	03:03	-	15:01	-

Fig. 2. Loss of unshared human leukocyte antigen in enriched relapsing leukemic cells. Donors and recipient are KIR-epitope matched at the HLA-C locus.

blasts were not investigated for HLA expression at the relapse, we could not determine the time when the leukemic cells had lost their HLA. Therefore, we cannot rule out the possibility that other immune mediators such as NK cells might play a role especially in the prevention of rapid growth of the blasts that had lost the HLA.

Although our patient had a leukemic relapse with loss of mismatched HLA cell surface expression, our report demonstrates that a strong GVL effect of haploidentical SCT by directly targeting mismatched HLAs might also be expected in patients with MNKL. Novel therapeutic tools are needed for targeted or prophylaxis treatment of these peculiar variants of post-transplantation relapse.

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ORIGINAL ARTICLE

Early use of allogeneic hematopoietic stem cell transplantation for infants with *MLL* gene-rearrangement-positive acute lymphoblastic leukemia

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Sixty-two infants with *MLL* gene-rearrangement-positive acute lymphoblastic leukemia (MLL-r ALL) were treated with the MLL03 protocol of the Japanese Pediatric Leukemia/Lymphoma Study Group: short-course intensive chemotherapy followed by early allogeneic hematopoietic stem cell transplantation (HSCT) within 4 months of the initial induction. The 4-year event-free survival and overall survival rates were 43.2% (95% confidence interval (CI) = 30.7–55.1%) and 67.2% (53.8–77.4%), respectively. A univariate analysis showed younger age (<90 days at diagnosis), central nervous system disease and poor response to initial prednisolone therapy significantly associated with poor prognosis ($P<0.05$). In a multivariate analysis, younger age at diagnosis tended to be associated with poor outcome (hazard ratio = 1.969; 95% CI = 0.903–4.291; $P=0.088$). Although the strategy of early use of HSCT effectively prevented early relapse and was feasible for infants with MLL-r ALL, the fact that substantial number of patients still relapsed even though transplanted in their first remission indicates the limited efficacy of allogeneic HSCT for infants with MLL-r ALL. Considering the risk of severe late effects, indications for HSCT should be restricted to specific subgroups with poor risk factors. An alternative approach incorporating molecular-targeted drugs should be established.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) in infants younger than 1-year old accounts for 2.5–5% of childhood ALL and carries clinical and biological features distinct from those of ALL in older children.[1] Patients have a high frequency (up to 80%) of 11q23 translocations/*MLL* gene rearrangements (MLL-r) and a highly distinctive gene expression profile, and majority of infants with MLL-r ALL are characterized by a high white blood cell count and marked hepatosplenomegaly at presentation, and by a pro-B-cell phenotype of their leukemic cells, which lack CD10 expression.[2] The prognoses of these patients are very poor, as illustrated by recent published long-term event-free survival (EFS) and overall survival (OS) rates of 42–54% and 45–61%, respectively.[3–10] In a large international study, Interfant-99, the presence of MLL-r, a very high white blood cell count, age <6 months and a poor response to prednisolone prophase were associated with inferior outcomes. Notably, the 4-year EFS of MLL-r patients was only 36.9%, which is much poorer than the 74.1% EFS of *MLL*-germline patients.[3]

Between 1995 and 2001, we conducted two consecutive Japanese nationwide studies of infant ALL, designated MLL96 and MLL98, in which we stratified patients according to their *MLL* gene configurations; all MLL-r ALL infants were assigned intensive chemotherapy followed by allogeneic hematopoietic stem cell transplantation (HSCT) at their first remission.[4] Because of the high rate of early relapse before the time for HSCT was reached, which is frequently observed in infants with ALL, the overall outcomes of these MLL-r patients were far from satisfactory. However, an encouraging result in our study for 3-year posttransplantation EFS (64.4%) in patients receiving HSCT at their first remission prompted us to speculate whether an intervention with more-effective chemotherapy and HSCT in an earlier phase could prevent early relapse and produce a better outcome.[11] Therefore, we planned the MLL03 study and analyzed the outcomes of infants with MLL-r ALL.

MATERIALS AND METHODS

Patients

Between February 2004 and January 2009, 92 consecutive infants younger than 1 year with suspected newly diagnosed ALL from 126 centers and hospitals in Japan were assessed for their eligibility for MLL03. This study included more than 90% of the same patients as the national study of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG). Patients with germline *MLL* gene, mature B-cell ALL, Down syndrome or congenital ALL cases with gestational age of less than 37 weeks were excluded according to the eligibility criteria of the study (Supplementary Table 1). The diagnosis of ALL was established based on bone-marrow morphology (or peripheral

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blood morphology, if bone-marrow aspiration resulted in a dry tap), cytochemical staining and immunophenotyping, which were confirmed with a central review system. The leukemic cell karyotypes were determined with a cytogenetic analysis using a G-banding technique. The *MLL* gene configuration of each patient was determined with a Southern blotting analysis, as described previously,[12] and the chimeric genes *MLL–AF4*, *MLL–AF9*, *MLL–AF6* and *MLL–ENL* were also examined with real-time PCR. With these strategies, 30 patients were excluded because they did not meet eligible criteria of the study as follows (Supplementary Table 1): *MLL*-germline ALL ($n = 20$), mature B-cell ALL ($n = 1$), congenital ALL cases with immature gestational age ($n = 3$), death before diagnostic confirmation of ALL ($n = 1$), unable to start protocol therapy within 7 days after the registration ($n = 1$), refusal to participate by the guardian ($n = 2$), the patient was registered before the study approval by the institutional review board ($n = 1$) and the patient was transferred to a non-JPLSG member hospital ($n = 1$; Figure 1). Ultimately, 62 patients with MLL-r ALL were eligible and were enrolled in the protocol study. Written informed consent was obtained from the guardians of the patients according to the Declaration of Helsinki, and institutional review board approval was obtained for all aspects of the study.

Treatment

The details of the therapeutic regimen used in the MLL03 study are described in Table 1. The patients were non-randomly assigned to commence 7-day prednisolone monotherapy. The prednisolone response and the leukemic status of the central nervous system (CNS) were evaluated on day 8, and a prednisolone good responder was defined as an infant with a peripheral blood blast count of less than 1000/μl and a poor responder as an infant with $\geqslant$1000/μl. CNS involvement was defined as $>$5/μl mononuclear cells with a leukemic morphology. As most other study groups evaluate CNS status on day 1 before any treatment is given, CNS status in the current study might be influenced by the prednisolone prophase. In addition, day 8 evaluation of prednisolone prophase in this study is unique compared with other studies that usually assess peripheral blood blasts after 1 week of prednisolone concurrent with single intrathecal injection of chemotherapy.

The induction phase consisted of dexamethasone, vincristine, doxorubicin, cyclophosphamide and triple intrathecal chemotherapy with methotrexate, cytarabine (Ara-C) and hydrocortisone, followed by an intermediate dose of Ara-C and etoposide (VP-16). Based on the *in vitro* drug sensitivity data presented by Pieters *et al.*[13] showing that the lymphoblasts of infant ALL show high sensitivity to Ara-C, each of the two consolidation courses were intensified with high-dose Ara-C to prevent early relapse. However, L-asparaginase was not included throughout the therapy because of its low sensitivity in infants. All the patients received two initial courses (*induction* and *consolidation-1*) and their remission status was evaluated after each course. Complete remission (CR) was defined by testing bone marrow with less than 5% leukemic cells, regeneration of hematopoiesis and no evidence of leukemia cells elsewhere after either the *induction* or *consolidation-1* course.

Because the main objective of the MLL03 study was to evaluate the efficacy and safety of allogeneic HSCT in the early phase of the disease (within 4 months of the initial induction), all the patients with continuous CR were prescribed the following HSCT after *consolidation-2*. The donors were restricted to two types: human leukocyte antigen $\geqslant$4/6 serologically matched unrelated cord blood or human leukocyte antigen $\geqslant$5/6 matched related donor. The conditioning was a nonirradiation myeloablative regimen with busulfan (BU), VP-16 and cyclophosphamide. An oral formulation of BU was used until October 2006, when the intravenous formulation became available in Japan. Regardless of the drug formulation, the dose of BU was determined according to individual pharmacokinetic tests, with a targeted average steady-state concentration of 600–900 ng/ml.[14] The prophylaxis for graft-versus-host disease was either cyclosporine or tacrolimus combined with short-term methotrexate.

Statistical analyses

All the analyses were performed by the intention-to-treat approach; all the 62 eligible patients were fully analyzed even for the cases that dropped out

Table 1. Treatment for infant ALL with a rearranged *MLL* gene in MLL03 study

Phase and drug	*Delivery, duration*	*Dosage*	*Dose schedule*
PSL prophase			
PSL	IV	60 mg/m^2	Days 1–7
Induction			
DEX	IV	10 mg/m^2	Days 8–21
VCR	IV	0.05 mg/kg	Days 8, 15
CPA	IV,2 h	1 200 mg/m^2	Day 9
DXR	IV, 1 h	25 mg/m^2	Days 10, 12
TIT		age-adjusted[a]	Days 8, 22[b]
VP-16	IV, 2 h	100 mg/m^2	Days 22–25
Ara-C	IV, 4 h	500 mg/m^2	Days 22–25
Consolidation-1			
MIT	IV, 1 h	10 mg/m^2	Day 1
VP-16	IV, 2 h	100 mg/m^2	Days 1–5
Ara-C	IV, 4 h	3000 mg/m^2	Days 1–5
TIT		Age-adjusted[a]	Days 1, 8
Consolidation-2			
VCR	IV	0.05 mg/kg	Day 1
MTX	IV, 12 h	3 000 mg/m^2	Day 1
Leucovorin	IV	15 mg/m^2	36 hr after start of MTX, 7 times
Ara-C	IV, 3 h	3000 mg/m^2 × 2	Days 4, 5
TIT		Age-adjusted[b]	Days 1, 8
Conditioning regimen for hematopoietic stem cell transplantation			
BU	PO/IV	Adjusted based on PK results	Days −8, −7, −6, −5
VP-16	IV, 12 h	60 mg/kg	Day −4
CPA	IV, 2 h	60 mg/kg	Days −3, −2

Abbreviations: ALL, acute lymphoblastic leukemia; Ara-C, cytarabine; BU, busulfan; CPA, cyclophosphamide; DEX, dexamethasone; DXR, doxorubicin; IV, intravenously; MIT, mitoxantrone; MTX, methotrexate; PO, orally; PK, pharmacokinetics; PSL, prednisolone; TIT, triple intrathecal therapy; VCR, vincristine; VP-16, etoposide. The dose of each drug except VCR, PSL, and DEX were reduced by one-third in patients younger than 60 days and by one fourth in those 61–120 days of age. [a]Doses were adjusted according to the patient's age at administration as follows: 90 days old or younger, MTX 3 mg, hydrocortisone (HDC) 10 mg, Ara-C 6 mg; younger than 1 year old, MTX 6 mg, HDC 10 mg, Ara-C 15 mg; 1 year and older, MTX 8 mg, HDC 15 mg, Ara-C 20 mg. [b]Additional TITs on days 15 and 29 for patients with CNS disease.

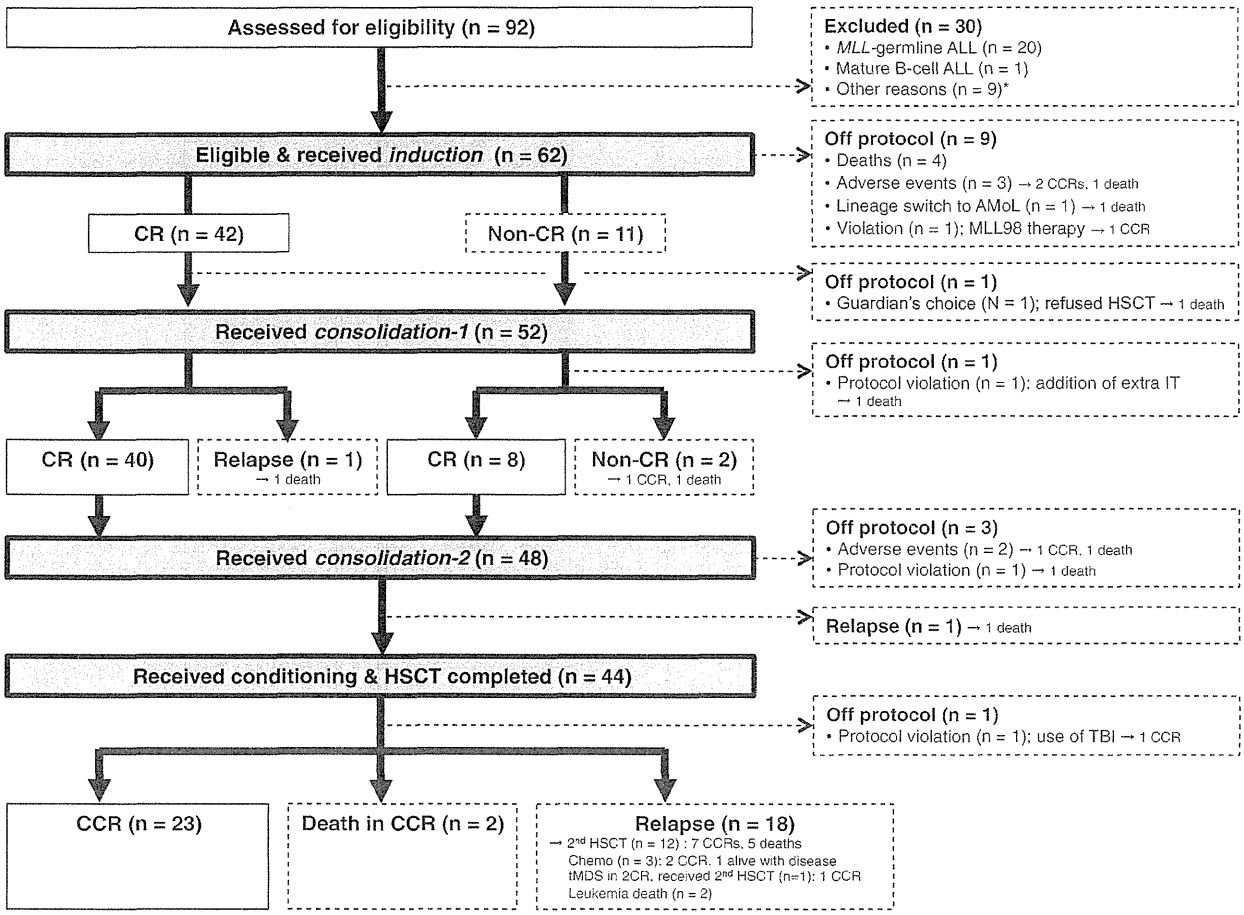

Figure 1. Patient flow chart in the MLL03 study. ALL, acute lymphoblastic leukemia; AMoL, acute monocytic leukemia; CR, complete remission; CCR, continuous CR; HSCT, hematopoietic stem cell transplantation; IT, intrathecal therapy; TBI, total-body irradiation; tMDS, therapy-related myelodysplastic syndrome; 2CR, second CR. *Reasons for exclusion of the nine patients are as follows: congenital ALL cases with immature gestational age ($n = 3$), death before diagnostic confirmation of ALL ($n = 1$), unable to start protocol therapy within 7 days after the registration ($n = 1$), refusal to participate by the guardian ($n = 2$), the patient was registered before the study approval by the institutional review board ($n = 1$) and the patient was transferred to a non-JPLSG member hospital ($n = 1$).

of the study before completing the protocol-specified therapy for various reasons (Table 1). EFS was defined as the length of time from the diagnosis of ALL to the last follow-up or first event (failure to achieve remission, relapse, secondary malignancy or death from any cause). OS was defined as the length of time from the diagnosis of ALL to death from any cause. The probabilities of EFS and OS were estimated with the Kaplan–Meier method and standard errors (s.e.) with the Greenwood formula, and were then compared with the log-rank test; 95% confidence intervals (CIs) were computed. A Cox proportional hazards regression model was used to identify the risk factors associated with the EFS rate. Variables including age at initial diagnosis (<90 days vs≥90 days), white blood cell count at initial diagnosis (≥100 000/μl vs <100 000/μl), CNS disease (positive vs negative), cytogenetics (t(4;11)(q21;q23) vs others) and response to initial prednisolone monotherapy (poor vs good responders) were considered for inclusion in the model. The significant variables associated with the EFS rate were then identified. No statistical adjustment was made for the performance of multiple tests, but two-sided *P* values greater than 0.05 were interpreted with caution. All data analyses were performed with the STATA statistical software (version 11.0; StataCorp LP, College Station, TX, USA).

RESULTS

Patient characteristics

The characteristics of the 62 enrolled infants with MLL-r ALL are shown in Table 2. Notably, the proportion of younger infants aged <180 days (6 months) at diagnosis was very high (68% (41/62)) in the present report compared with those in previous reports, in which they usually constituted as much as 50%.[3–5]

Treatment outcomes

Remission induction results. The prednisolone response was evaluated in 59 out of 62 patients (95%): 43 (69%) infants were good responders and 16 (26%) were poor responders. Forty-two patients (67.7%) achieved CR after the initial induction therapy, four patients died (because of sepsis ($n = 2$), acute respiratory distress syndrome after respiratory syncytial virus infection ($n = 1$) and liver failure ($n = 1$)) and five patients dropped out of the protocol with one of the following reasons: severe adverse events ($n = 3$: heart failure or renal failure from tumor lysis syndrome and respiratory syncytial virus bronchiolitis. The patient with heart failure eventually died of leukemia progression, and the other two are alive in continuous CR.), lineage switch to acute monocytic leukemia ($n = 1$: died of leukemia) and protocol violation ($n = 1$: alive with continuous CR after HSCT following MLL98 chemotherapy; Figure1). Notably, five of these eight patients (excluding the protocol violation case) were less than 90 days of age (six were <180 days of age) at diagnosis. In addition, one patient, although achieved CR after *induction*, dropped out of the protocol because

Table 2. Characteristics of 62 MLL-r ALL infants enrolled on study MLL03

	No. of patients (%)
Sex	
Male	27 (44)
Female	35 (56)
Age, days	
<90	22 (36)
90 to <180	20 (32)
180 to <366	20 (32)
WBC count, 10^9/L	
<100	34 (55)
100 to <300	11 (18)
⩾300	17 (27)
Immunophenotype	
Pro-B	42 (68)
Pre-B	4 (6)
Common B	9 (14)
AMLL	6 (10)
AUL	1 (2)
11q23 abnormality	
t(4;11)(q21;q23) or *MLL-AF4*	31 (50)
t(9;11)(p22;q23) or *MLL-AF9*	4 (6)
t(11;19)(q23;p13) or *MLL-ENL*	3 (5)
Other 11q23 abnormalities	5 (8)
Other abnormalities	7 (11)
Normal karyotype	9 (15)
Not evaluable	3 (5)
CNS disease	
Positive	11 (18)
Negative	48 (77)
Not evaluable	3 (5)

Abbreviations: AMLL, acute mixed-lineage leukemia; AUL, acute undifferentiated leukemia; CNS, central nervous system; MLL-r ALL, *MLL* gene-rearrangement-positive acute lymphoblastic leukemia; WBC, white blood cell.

Table 3. Comparison of results of HSCT by different donor sources in MLL03 study

	UCBT, n = 31	*RBMT, n = 11*[a]	*P value*
Infused cell dose, $x10^7$/kg			
Median (range)	10.7 (5.00–21.5)	48.0 (8.70–119)	0.01
Neutrophil engraftment			
n	31 (100%)	11 (100%)	
Median days (range)	16 (14–30)	16 (11–31)	0.65
Platelet engraftment			
n	30 (97%)	11 (100%)	
Median days (range)	40.5 (16–69)	25 (11–52)	0.04
Acute GVHD			
I–II	19	6	
III–IV	2	0	0.67
Chronic GVHD	6	1	0.75
Relapse			
Total	13	5	0.87
BM relapse	10[b]	2	
Isolated EM relapse	1	1	
Combined BM/EM relapse	2	2	
Non-relapse death	1	0	0.58
CCR	17 (54%)	6 (54%)	0.73

Abbreviations: BM, bone marrow; CCR, continuous complete remission; EM, extramedullary; GVHD, graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; RBMT, related bone marrow transplantation; UCBT, unrelated cord blood transplantation. [a]Data not available for one RBMT case, because of sudden death on day + 125. [b]One case developed therapy-related myelodysplastic syndrome 4 years after the second CR.

the guardian refused the HSCT strategy and withdrew the consent. As a result, total 52 patients received *consolidation-1* and 40/41 patients continued to be CR, 8 extra cases entered CR, 1 relapsed (died of leukemia), 2 failed to achieve CR (one is alive in continuous CR and the other died of leukemia progression) and 1 patient dropped out of the study because of protocol violation ($n = 1$: died of leukemia after the second relapse).

Thus, the overall CR rate (CR after either *induction* or *consolidation-1*) was 80.6% (50/62).

Transplantation outcome. Total 48 patients received *consolidation-2*, and another 3 patients dropped off the study because of severe adverse events ($n = 2$: one is alive in continuous CR and the other died of leukemia progression) and protocol violation ($n = 1$: died of leukemia after the second relapse), and 1 patient relapsed (died of leukemia after the second relapse; Figure1). Thus, 44 patients received HSCT in their first remission (1CR), however, one case dropped out of the study because of protocol violation using total-body irradiation as a conditioning regimen. Among the 43 patients who received HSCT per protocol, 31 patients underwent unrelated cord blood transplantation and 12 patients underwent related bone-marrow transplantation. Although the median infused cell dose was higher in the related bone-marrow transplantation group and the median days to platelet engraftment was longer in the unrelated cord blood transplantation group, there were no differences between the two groups in the incidence of acute or chronic graft-versus-host disease, relapse, non-relapsed death or the percentage of continuous CR (Table 3). Eighteen patients relapsed after HSCT: twelve in the bone marrow, two in an extramedullary site and four in both. Among these patients, 13 underwent a second HSCT: 7 are alive in continuous CR for median follow-up of 4.4 years (range = 2.5–5.4 years) post second HSCT, 1 is alive but has developed secondary myelodysplastic syndrome and 5 died (3 of disease-related and 2 of HSCT-related causes). Of the remaining five patients, two with isolated subcutaneous relapse are alive in CR after chemotherapy with or without local irradiation, one is alive but relapsed after salvage chemotherapy and two died of disease progression.

Analysis of overall outcome. The median follow-up period of all the 62 patients was 4.0 years (range = 0–7.4 years). The 18-month EFS rate, a primary end point of this study, was 53.2% (95% CI = 40.1–64.6%). The 4-year EFS and OS rates were 43.2% (95% CI = 30.7–55.1%) and 67.2% (95% CI = 53.8–77.4%), respectively (Figure 2). The 4-year EFS rates according to the different risk factors are presented in Table 4; younger age at diagnosis (<90 days), CNS disease and poor response to initial prednisolone monotherapy were significantly associated with a poor prognosis in the univariate analysis. In the multivariate analysis, only younger age at diagnosis tended to be associated with a poorer EFS rate (hazard ratio = 1.969 (95% CI = 0.903–4.291); $P = 0.088$), but the association was not statistically significant, probably because the number of infants analyzed was small. Other associations, such as with CNS disease (hazard ratio = 1.243 (95% CI = 0.421–3.655); $P = 0.694$) and poor prednisolone response (hazard ratio = 1.078 (95% CI = 0.507–2.291); $P = 0.875$), were also statistically insignificant.

Outcome by minimal residual disease (MRD). The significance of MRD was evaluated by measuring selected *MLL*-fusion transcripts in several patients using real-time quantitative PCR in an add-on study. Unfortunately, MRD could not be consistently monitored and the results were not used to guide therapy. Only 11 samples

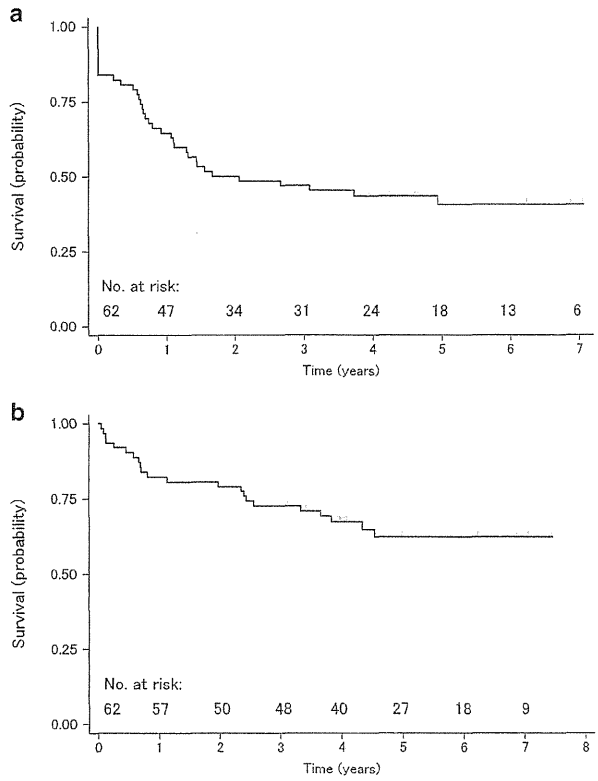

Figure 2. Outcomes of infants with ALL and *MLL*-gene rearrangements enrolled in the MLL03 study. (**a**) Event-free survival (EFS). (**b**) Overall survival.

were available at the end-of-induction time point and 10 samples at the transplantation time point because it was difficult to collect paired samples. At the end-of-induction time point, two patients were MRD positive and one eventually relapsed. The 4-year EFS rate for the nine patients negative for MRD after induction was 40.0% (95% CI = 12.2–67.0%; $P = 0.052$). At the pretransplantation time point, two patients were MRD positive and ultimately relapsed. Of the eight patients with negative MRD at HSCT, five relapsed and three cases are in continuous CR; the 4-year EFS rate was 37.5% (95% CI = 8.7–67.4%; $P = 0.081$).

Safety analysis

The grade 3 and 4 toxicities in each treatment phase, evaluated according to the second version of the National Cancer Institute Common Toxicity Criteria, are described in Table 5. Hematological toxicities and nonhematological toxicities, such as diarrhea, elevated liver transaminases and infections, were quite common throughout all treatment phases. Serious complications, such as pulmonary or neurologically related complications or hemorrhage, were predominantly observed during the *induction* phase. Notably, tumor lysis syndrome was observed in 40% of patients presumably because rasburicase, a recombinant urate oxidase, was not available in Japan during the study period.

DISCUSSION

Infant MLL-r ALL is one of the most difficult to cure of all the subtypes of childhood acute leukemia, and the EFS rates are estimated to be less than 40%, even in recently reported studies of patients treated with intensive chemotherapy with or without HSCT.[3–5] The major factor responsible for the high failure rate is the high relapse rate in the early postremission phase of treatment. In fact, more than half treatment failures occurred before HSCT in our previous MLL96 and MLL98 studies, which made it difficult to assess the true impact of allogeneic HSCT on infants with MLL-r ALL.[4] Therefore, in the present study, we intensified the pretransplantation chemotherapy with high-dose Ara-C and assigned all eligible patients to receive allogeneic HSCT in the early postremission phase, within 4 months of the initial induction therapy.

This strategy was feasible because nearly 90% (44/50) of those who achieved remission were able to undergo allogeneic HSCT in 1CR. However, the low overall CR rate, attributable to high induction toxicity, and the substantial number of patients who still relapsed after HSCT resulted in a 4-year EFS rate of 43.2%, which is no better than the previous reports including the study Interfant-99; only 12% (37/297) of the MLL-r cases in the Interfant-99 study received allo HSCT in 1CR.[3] One factor that affected the outcome of this study was an unexpectedly high proportion of younger infants, less than 180 days of age, at diagnosis. It is well known that a younger age at diagnosis is associated with a higher risk of induction toxicity and relapse, and is definitely a poor prognostic factor in MLL-r infants with ALL.[3–5,15] The 4-year EFS rate of the 42 patients <180 days old was 35.3%, whereas that of the 20 patients ⩾180 days old was 60.0%. Another potentially associated factor was the lower treatment potential of the pretransplantation chemotherapy given in this study. We completely eliminated the use of L-asparaginase because its activity against infant MLL-r ALL is low, and this strategy could have adversely affected the outcome, despite the treatment intensification with high-dose Ara-C. One should realize that *in vitro* resistance to a certain drug does not mean absolute resistance to that drug, but is a relative to that of other types of ALL. Furthermore, our strategy of minimizing the chemotherapy courses given before HSCT could have meant that they were insufficient to reduce the leukemic burden, which might have resulted in post-HSCT relapse in some cases. The correlation between MRD and treatment outcome was evaluated in a small proportion of patients in this study, and those with

Table 4. Comparison of 4-year EFS according to the risk factors in 62 MLL-r ALL in MLL03 study

	No. of patients	*4-Year EFS rate, %*	*95% CI*	P *value*
Age, days				
<90	22	22.7	8.2–41.4	0.016
⩾90	40	54.4	37.7–68.4	
<180	42	35.3	21.3–49.7	0.102
⩾180	20	60.0	35.7–77.6	
WBC count, $\times 10^9$/l				
<100	34	46.4	29.0–62.1	0.328
⩾100	28	39.2	21.6–56.5	
CNS disease				
Positive	11	27.2	6.5–53.8	0.046
Negative	48	49.7	34.9–62.8	
Karyotype				
t(4;11)(q21;q23)	31	41.9	24.6–58.3	0.613
Others	31	47.4	28.3–64.2	
Prednisolone response				
PGR	43	53.1	37.2–66.7	0.013
PPR	16	25.0	7.7–47.1	

Abbreviations: CI, confidence interval; CNS, central nervous system; EFS, event-free survival; MLL-r ALL, *MLL* gene-rearrangement-positive acute lymphoblastic leukemia; PGR, prednisolone good responder; PPR, prednisolone poor responder; WBC, white blood cell.

Table 5. Grade 3 and 4 toxic events by different treatment phases in 62 MLL-r ALL infants in MLL03 study

Toxicities *Patients assessed*	*Induction* n = *62 (%)*	*Cons-1* n = *52 (%)*	*Cons-2* n = *48 (%)*	*Conditioning* n = *44 (%)*[a]
Blood/bone marrow				
Hemoglobin	58 (94)	44 (85	41 (85)	40 (91)
Leukocytes (total WBC)	59 (95)	52 (100)	48 (100)	44 (100)
Neutrophils/granulocytes	62 (100)	52 (100)	48 (100)	44 (100)
Platelets	59 (95)	50 (96)	46 (96)	40 (91)
Gastrointestinal				
Stomatitis/pharyngitis	7 (11)	2 (4)	0 (0)	18 (41)
Vomiting	0 (0)	2 (4)	0 (0)	4 (9)
Diarrhea	13 (21)	15 (29)	6 (13)	12 (27)
Constipation	1 (2)	0 (0)	0 (0)	1 (2)
Pancreatitis	0 (0)	0 (0)	0 (0)	0 (0)
Hepatic				
Total bilirubin	7 (11)	1 (2)	2 (4)	8 (18)
AST/ALT	27 (44)	7 (13)	26 (54)	4 (9)
Metabolic/laboratory				
Amylase	0 (0)	0 (0)	0 (0)	—
Hyperglycemia	2 (3)	0 (0)	0 (0)	—
Renal/genitourinary				
Creatinine	1 (2)	0 (0)	0 (0)	0 (0)
Proteinuria	1 (2)	0 (0)	0 (0)	
Cardiovascular				
Thrombosis/embolism	1 (2)	0 (0)	0 (0)	0 (0)
Other cardiovascular	1 (2)	0 (0)	0 (0)	
Pulmonary				
Dyspnea	10 (16)	0 (0)	0 (0)	3 (7)
Hypoxia	15 (24)	1 (2)	0 (0)	5 (11)
Infection/febrile neutropenia				
Infection	45 (73)	36 (69)	17 (35)	32 (73)
Allergy/immunology				
Allergic reaction/ hypersensitivity	0 (0)	0 (0)	0 (0)	0 (0)
Syndromes				
Tumor lysis syndrome	25 (40)	0 (0)	0 (0)	—
Thrombotic microangiopathy	—	—	—	0 (0)
Veno-occlusive disease	—	—	—	6 (14)
Dermatology/skin				
Rash/desquamation	1 (2)	0 (0)	0 (0)	5 (11)
Neurology	4 (6)	0 (0)	0 (0)	1 (2)
Hemorrhage	6 (10)	0 (0)	1 (2)	1 (2)

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; *Cons-1, consolidation-1*; *Cons-2, consolidation-2*; MLL-r ALL, *MLL* gene-rearrangement-positive acute lymphoblastic leukemia; WBC, white blood cell. [a]One patient who discontinued the study because of protocol violation (use of total body irradiation) is included.

residual MRD at both the postinduction and pretransplantation time points tended to show a worse outcome. Moreover, the fact that nearly half the patients ultimately relapsed even though transplanted in 1CR indicates the limited efficacy of allogeneic HSCT itself for infants with MLL-r ALL.

Although the EFS rate of our study cohort was not satisfactory, their 4-year OS rate of 67.2% was relatively good. This can be explained by the low HSCT-related mortality rate and the relatively high salvage rate after relapse compared with those in our previous MLL96 and MLL98 studies.[16] Among the 43 patients who received allogeneic HSCT per protocol, only 2 (4.6%) died of non-HSCT toxicities (1 of transfusion-related lung injury and 1 with unknown sudden death), although this death rate was high (15.0%, 8/53) in our previous MLL96 and MLL98 studies.[4] Several reasons can be proposed to explain this observation. One is the introduction of an appropriate dose of BU in the conditioning regimen, based on individual pharmacokinetic studies. It is well recognized that the pharmacokinetics of BU vary widely among infants, which may lead to severe post-HSCT organ damage, including lung injury, hepatic veno-occlusive disease, etc.[14,17] Second, the minimum course of pre-HSCT chemotherapy might have reduced the potential organ damage that could have occurred if the patient were instead heavily treated with multiple courses of chemotherapy. Therefore, our strategy of including short-course chemotherapy and the early use of HSCT with individually tailored doses of BU could have reduced both the early-relapse rate and the transplantation-related deaths. The fact that 7 out of 13 cases of post-HSCT relapse were salvaged with a second HSCT may also reflect the low toxic potential of the early HSCT strategy, as mentioned above. Of course, late effects are yet to be evaluated in this study and must be observed especially carefully in these cases.

Because of the limited effectiveness of HSCT and the potential risk of late effects, alternative strategies with novel targeted therapies should be explored for infants with MLL-r ALL.[4,18,19] Recent research has demonstrated that the aberrant epigenetic status, induced by a reciprocal *MLL* translocation via the H3K79 methyltransferase DOT1L, has a central role in MLL-r leukemogenesis.[20–22] The clinical development of epigenetic modifiers, such as DNA methyltransferase inhibitors and/or histone deacetylase inhibitors, is currently in progress. A small-molecule inhibitor of DOT1L is also in clinical development. Meanwhile, HSCT should be restricted to patients at higher risk of relapse, who are likely to benefit from this treatment modality.[23] This stratification is currently being evaluated in our ongoing JPLSG MLL-10 study.

In conclusion, short-course chemotherapy and the early use of HSCT in our study was feasible for infants with MLL-r ALL. However, given the limited effects of HSCT and the potential risk of late effects, the indication for HSCT should be restricted to specific subgroups with poor risk factors, and an alternative approach incorporating molecular-targeted drugs should be established in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

K Koh, DT, TM, MH, Y Takahashi, AO, K Kato, KS and EI (principal investigator) participated actively in the study conception and design; K Koh, DT and EI reviewed the data analysis and interpretation and were the main authors of the manuscript; AMS and TW conducted the statistical analysis; TS was responsible for the busulfan pharmacokinetic study; TD and MT were responsible for the immunophenotyping diagnostics; YH was responsible for coordinating the molecular biology analyses; K Koh, K Kato, JT and Y Takeshita recruited patients; MT, KH and SM contributed to the financial and administrative support of the study; and all authors contributed to the conduct of the trial and were involved in the review of the results and the final approval of the manuscript.

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ORIGINAL ARTICLE

Markedly improved outcomes and acceptable toxicity in adolescents and young adults with acute lymphoblastic leukemia following treatment with a pediatric protocol: a phase II study by the Japan Adult Leukemia Study Group

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The superiority of the pediatric protocol for adolescents with acute lymphoblastic leukemia (ALL) has already been demonstrated, however, its efficacy in young adults remains unclear. The ALL202-U protocol was conducted to examine the efficacy and feasibility of a pediatric protocol in adolescents and young adults (AYAs) with *BCR–ABL*-negative ALL. Patients aged 15–24 years ($n = 139$) were treated with the same protocol used for pediatric B-ALL. The primary objective of this study was to assess the disease-free survival (DFS) rate and its secondary aims were to assess toxicity, the complete remission (CR) rate and the overall survival (OS) rate. The CR rate was 94%. The 5-year DFS and OS rates were 67% (95% confidence interval (CI) 58–75%) and 73% (95% CI 64–80%), respectively. Severe adverse events were observed at a frequency that was similar to or lower than that in children treated with the same protocol. Only insufficient maintenance therapy significantly worsened the DFS (hazard ratio 5.60, $P < 0.001$). These results indicate that this protocol may be a feasible and highly effective treatment for AYA with *BCR–ABL*-negative ALL.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) has been very successfully treated in children, however, the prognosis of patients markedly deteriorates from the onset of adolescence to adulthood. A period analysis of ALL patients between 2000 and 2004 showed 5-year relative survival rates of 80.7% in patients aged 10–14 years and 44.8% in patients aged 20–29 years.[1,2] Retrospective studies that focused on patients aged 15–21 years reported that adolescents and young adults (AYAs) treated with adult ALL protocols had poorer outcomes than similarly aged patients treated with pediatric protocols.[3–6] However, these studies compared patients treated with a pediatric protocol by pediatricians to those treated with an adult protocol by physicians; the former were adolescents with a median age of 16 or 17 and the latter were AYAs with a median age 19 or older. These findings were similar to those of recently reported prospective studies by pediatric groups.[7,8] The reason of the efficacy of pediatric protocols on young adults and how the difference in age between these patient groups is responsible for the observed differences in survival have yet to be determined, and can only be clarified by examining patients treated with pediatric protocols in an adult study group. Several prospective clinical trials using pediatric regimens for adults are currently ongoing. These studies have been divided into two types based on their regimens and patients: a pediatric-inspired protocol with dose reductions in a pediatric protocol for adults up to 60 years, and an unmodified pediatric protocol for AYA up to 30 years. A few of the former studies have already been completed, and the results obtained revealed marked improvements in the survival rates of ALL patients up to 60 years old.[9,10] However, the possibility that AYA may achieve better survival rates with the original pediatric protocol was not investigated in these studies. The feasibility and efficacy of an unmodified pediatric protocol for AYA should be examined. One previous study reported marked improvements in survival rates;[11] however, only

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standard-risk ALL were treated. Therefore, the efficacy of this protocol in young adults with high-risk ALL remains unknown.

The Japan Adult Leukemia Study Group (JALSG) conducted a phase 2 trial in which patients aged 15–24 years with *BCR–ABL*-negative ALL were treated with the same protocol developed for children with ALL by the Japan Association of Childhood Leukemia Study (JACLS). We analyzed the outcomes and prognostic factors of the 139 AYA patients treated in this trial.

PATIENTS AND METHODS

Patients and eligibility criteria

The JALSG ALL202-U (ALL202-U) study is a prospective nonrandomized phase 2 trial, a part of the JALSG ALL202 (ALL202) study conducted by JALSG and was registered at UMIN-CTR (ID: C000000064). Eligibility criteria were common with the ALL202 study.[12] The protocol was approved by the Institutional Review Board of each hospital. Written informed consent was obtained from all patients before registration in accordance with the Declaration of Helsinki. Guardians also gave written informed consent when patients were under 20 years old. The study was initiated in August 2002 and closed for patient inclusion in October 2009.

Diagnostic procedure

ALL was diagnosed according to the French–American–British classification[13] using morphology, cytochemistry and immunophenotyping studies at each institution. Mature B-cell ALL was excluded. Immunophenotyping and cytogenetic studies were performed as described previously.[14] The multiplex reverse transcription-PCR test was described previously.[12]

Study design and treatment

The study design of ALL202 has previously been described in detail.[12] Patients were treated differently according to age and the *BCR–ABL* diagnosis results. Patients aged 15–24 years and negative for *BCR–ABL* were treated with the same pediatric regimen as the ALL202-U study. The protocol was conducted for high-risk pediatric B-ALL in the ALL-02 study by the JACLS and designated as ALL-02-HR.[15] The study was initiated in April 2002, closed for patient inclusion in May 2008 and the results are awaited. The toxicity data of this study, which have been referred later, were obtained by analysis in May 2011.

The treatment schedule for ALL202-U is shown in Table 1. Patients underwent a 7-day prephase therapy with prednisolone (PSL) and a single intrathecal injection of methotrexate (MTX) after registration. Responsiveness to PSL was judged on day 8, and *BCR–ABL*-negative patients continued the protocol study. Patients who achieved complete remission (CR) after induction therapy received consolidation therapy, sanctuary therapy, reinduction therapy and reconsolidation therapy. Patients who did not achieve CR after induction therapy received consolidation therapy. If CR was not achieved with this therapy, protocol therapy was terminated as induction failure.

Allo-stem cell transplantation (SCT) was recommended for patients with t(4;11) who achieved CR during their first CR if a human leukocyte antigen-matched sibling was available, and allo-SCT from an alternative donor was allowed. An indication for SCT was decided for patients with other types according to institutional discretion. Each institution decided the preparative and post-transplant regimens for SCT according to its own discretion.

Detailed rules for treatment

Every therapy had a planned therapy duration. New therapy was started on the planned day if neutrophil and platelet counts had reached $\geqslant 0.5\times10^9$/l and $\geqslant 50\times10^9$/l, respectively, and patients had no significant infection at that time. Therapies could be started earlier if patients fulfilled the above conditions. Delays within 3 days for social reasons and 4 weeks because of complications were allowed. Folic acid rescue in sanctuary therapy was increased to every 3 h when the blood concentration of MTX was $\geqslant 1.0$ μmol/l 48 h after its administration or $\geqslant 0.2$ μmol/l after 72 h, and was continued until the MTX concentration fell to < 0.1 μmol/l. When the MTX concentration was $\geqslant 0.1$ μmol/l and < 0.2 μmol after 72 h, folic acid rescue was added only four times every 6 h. Maintenance therapy consisted of 16 courses of therapy. The dose of 6-MP was adjusted to maintain the white blood cell (WBC) count at $2–3\times10^9$/l. Central nervous system (CNS) prophylaxis included the administration of 14 courses of intrathecal therapy of MTX, cytarabine and hydrocortisone and a single intrathecal injection of MTX.

Evaluation of patients

CR was defined as the presence of all of the following: $< 5\%$ blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral blood values to neutrophil counts of at least 1.0×10^9/l and platelet counts of at least 100×10^9/l, and no evidence of extramedullary leukemia. Relapse was defined as the presence of at least one of the following: recurrence of $> 10\%$ leukemic cells in bone marrow or any leukemic cells in peripheral blood or extramedullary sites. Toxicity was evaluated based on the National Cancer Institute Common Toxicity Criteria (NCI-CTC) Version 2.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf). Corticosteroid sensitivity was defined as a peripheral blood blast cell count $< 1.5\times10^9$/l after the 7-day corticosteroid prephase.

Sample size estimation and statistical analysis

This study was designed as phase 2 and the sample size was determined before the study. We set an expected 5-year disease-free survival (DFS) rate of 50%, and estimated that 96 patients were required to achieve a 95% confidence interval (CI) of narrower than ±10%. Considering potential dropout because of ineligibility or loss to follow-up, we finally used 120 as the required number of patients.

The primary objective of this study was to assess DFS rate, and the secondary aims were to assess toxicity, the CR rate and the overall survival (OS) rate. An exploratory evaluation of potential prognostic factors was also conducted. We defined DFS as the time from the date of achieving CR to relapse, death or the last visit, and OS as the time from the first day of therapy to death or the last visit. Patients undergoing SCT were not censored at the time of transplantation and were evaluated with the inclusion of a post-transplantation period. The results for Ph-negative ALL patients under 25 years old in the JALSG ALL97 study (ALL97-U) were used as a reference. The treatment schedule for ALL97 has been reported previously[14] and was shown in Supplementary Table 1. The X^2 test was used to statistically analyze characteristic differences between patient groups. The Kaplan–Meier product limit method was performed to estimate DFS and OS. Patients were divided into two groups in some analyses. Patients whose WBC counts were $< 30\times109$/l and karyotype risks that were standard or intermediate in the modified MRC UKALLXII/ECOG E2993ALL cytogenetic classification[16] were defined as the standard-risk group, and others were defined as the high-risk group. The DFS rates of each group were analyzed separately. To compare DFS and OS rates, the log-rank test was used for univariate analysis, and a Cox proportional hazard model for uni- and multivariate analyses. To evaluate maintenance therapy insufficiency, we treated the termination of maintenance therapy as a time-varying covariate. Stata SE 11.2 (Stata Co., College Station, TX, USA) was used for all statistical analyses.

RESULTS

Patient entry and characteristics

Between August 2002 and October 2009, 150 patients from 59 hospitals participating in the JALSG were enrolled in this study. Eleven patients were excluded because two had been misdiagnosed (one with acute myeloid leukemia and one with *BCR–ABL*-positive ALL), four had dropped out before starting the treatment, four had been registered after prephase therapy and one was registered before protocol approval by the Institutional Review Board. Therefore, we here reported the outcomes of 139 eligible patients. The diagnosis of *BCR–ABL* negativity was based on the Multiplex RQ-PCR assay ($n=124$), *BCR–ABL* RQ-PCR assay ($n=1$), fluorescent *in situ* hybridization analysis ($n=7$) and chromosome karyotype assay ($n=7$). The pretreatment characteristics of ALL202-U and ALL97-U were summarized in Table 2. The median age was 19 years and there were 78 men (56%) and 61 women. Cytogenetic evaluations were performed in all 139 patients, and revealed that all were Ph-negative. Results were classified according to the modified MRC UKALLXII/ECOG E2993ALL cytogenetic subgroups:[16] the very high-risk group ($n=15$) included t(4;11), complex karyotype, defined as >5 abnormalities

without known translocations or low hypodiploidy/near triploidy; the high-risk group ($n=8$) included other MLL translocations, monosomy 7 with <5 abnormalities or t(1;19); the intermediate-risk group ($n=110$) included a normal karyotype or other miscellaneous abnormal karyotypes; the standard-risk group ($n=2$) included high hyperdiploidy. The multiplex RQ-PCR assay

Table 1. JALSG-ALL202-U schedule

Phases/drugs	*Route*	*Doses*	*Days*
Induction therapy (weeks 1–5)			
Methotrexate	IT	12 mg/body	1
Prednisolone	PO/IV	60 mg/m^2	1–7
Dexamethasone	IV	10 mg/m^2	8–14
Vincristine	IV	1.5 mg/m^2 [a]	8, 15, 22, 29
THP-adriamycin	IV	25 mg/m^2	8, 9
Cyclophosphamide	IV	1200 mg/m^2	10
L-asparaginase	IV/IM	6000 U/m^2	15, 17, 19, 21, 23, 25, 27, 29
Prednisolone	PO	40 mg/m^2	15–28
IT-triple[b]	IT		8, 22[c]
Consolidation thrapy (weeks 6–9)			
Cyclophosphamide	IV	750 mg/m^2	1, 8
THP-adriamycin	IV	25 mg/m^2	1, 2
Cytarabine	IV	75 mg/body	1–6, 8–13[d]
Mercaptopurine	PO	50 mg/m^2	1–14
IT-triple[b]	IT		1, 8
Sanctuary therapy (weeks 10–11)			
Methotrexate[e]	IV (24 h)	3 g/m^2	1, 8
IT-triple[b]	IT		2, 9
Reinduction therapy (weeks 12–15)			
Vincristine	IV	1.5 mg/m^2 [a]	1, 8, 15
THP-adriamycin	IV	25 mg/m^2	1, 8
Cyclophosphamide	IV	500 mg/m^2	1, 8
L-asparaginase	IM	6000 U/m^2	1, 3, 5, 8, 10, 12
Prednisolone	PO	40 mg/m^2	1–14
IT-triple[b]	IT		1
Reconsolidation therapy (weeks 16–19)			
Same as consolidation therapy			
Maintenance therapy 1-A (weeks 20–25) for CNS-invasion-negative cases			
Methotrexate	IV	150 mg/m^2	1, 15, 29
Mercaptopurine	PO	50 mg/m^2 [f]	1–28
IT-triple[b]	IT		29
Maintenance therapy 1-B (weeks 20–25) for CNS-invasion-positive cases			
Cranial irradiation		1.5 Gry × 8	1–12[g]
Methotrexate	IV	150 mg/m^2	29
Mercaptopurine	PO	50 mg/m^2 [f]	1–28
IT-triple[b]	IT		1, 8
Maintenance therapy 2 (weeks 26–29, 46–49, 66–69, 86–89)			
Vincristine	IV	1.5 mg/m^2 [a]	1, 8, 15
Cyclophosphamide	IV	600 mg/m^2	8
L-asparaginase	IM	10 000 U/m^2	1, 8, 15
Prednisolone	PO	40 mg/m^2	1–14
Maintenance therapy 3 (weeks 30–35, 40–45, 50–55, 60–65, 70–75, 80–85, 90–95)			
Methotrexate	IV	150 mg/m^2	1, 15, 29
Mercaptopurine	PO	50 mg/m^2 [f]	1–28
IT-triple[b]	IT		29[h,i]
Maintenance therapy 4 (weeks 36–39, 56–59, 76–79, 96–98)			
Vincristine	IV	1.5 mg/m^2 [a]	1, 8, 15
THP-adriamycin	IV	25 mg/m^2	8
L-asparaginase	IM	10 000 U/m^2	1, 8, 15
Prednisolone	PO	40 mg/m^2	1–14

Abbreviations: CNS, central nervous system; JALSG, Japan Adult Leukemia Study Group; IM, intramuscularly; IT, intrathecally; IV, intravenously; PO, per os; WBC, while blood cell. [a]Maximum dose was 2 mg per body. [b]IT-triple consisted of methotrexate 12 mg, cytarabine 30 mg and hydrocortisone 25 mg. [c]On days 8, 11, 15, and 22, when CNS invasion was positive. [d]Administration was stopped, when neutrophil count went down to 0/l. [e]With folinic acid rescue (15 mg/m^2, IV, six times every 6 h), beginning 42 h after the start of methotrexate infusion. [f]Dose should be adjusted to keep WBC count from 2000 to 3000/ul. [g]Eight times during this period. [h]For CNS-invasion-negative cases. [i]Not on weeks 74 and 94.

Table 2. Patient characteristics

Characteristics	*ALL202-U (n = 139)*	*ALL97*[a] *(n = 104)*	*P-value*
	No. (%)	*No. (%)*	
Sex			
Male	78 (56)	58 (56)	
Female	61 (44)	46 (44)	0.957
Age			
Median	19	19	
Age < 20	83 (60)	54 (52)	
Age ⩾ 20	56 (40)	50 (48)	0.226
PS			
0–1	128 (92)	93 (89)	
2–4	11 (8)	11 (11)	0.474
WBC count (/μl)			
Median	10 500	11 480	
WBC < 50 000	104 (75)	79 (76)	
WBC ⩾ 50 000	35 (25)	25 (24)	0.838
Serum LDH level			
Normal	20 (14)	14 (13)	
Elevated	119 (86)	90 (87)	0.415
Phenotype			
CD19+, CD10-	18 (13)	20 (19)	
CD10+	89 (64)	69 (66)	
CD19−, CD7+	31 (22)	14 (14)	0.591[b]
Unknown	1 (1)	1 (1)	
Karyotype[c]			
Standard risk	2 (1)	5 (5)	
Intermediate risk	110 (79)	74 (71)	
High risk	11 (8)	7 (7)	
Very high risk	15 (11)	7 (7)	0.322[b]
Unknown	1 (1)	11 (10)	
Chimera mRNA			
E2A-PBX1	6 (5)		
SIL-TAL1	4 (3)		
TEL-AML1	2 (2)		
MLL-AF4	1 (1)		
MLL-ENL	1 (1)		
CNS involvement			
Negative	128 (95)	103 (99)	
Positive	7 (5)	1 (1)	0.072

Abbreviations: CNS, central nervous system; LDH, lactic acid dehydrogenase; PS, performance status; WBC, white blood cell. [a]Ph-negative patients under 25 years were extracted. [b]Analyzed excluding unknown cases. [c]Modified MRC UKALLXII/ECOG E2993ALL cytogenetic subgroups.

was performed for 124 patients. Twelve sets of primers were used to detect *WT1*, *MDR1*, and nine distinct fusion gene transcripts, namely, major and minor *BCR–ABL*, *TEL-AML1*, *E2A-PBX1*, *MLL-AF4*, *MLL-AF6*, *MLL-AF9*, *MLL-ENL*, *SIL-TAL1* and *GAPDH* as an internal control. One hundred eight samples were analyzed by the full set of primers, eight samples by primers not including *MDR1* and eight samples by primers not including *MDR1*, *MLL-ENL* and *SIL-TAL1*. Six patients were positive for *E2A-PBX1*, two for *TEL-AML1*, one for *MLL-AF4* and one for *MLL-ENL*. ALL patients were negative for *MLL-AF6* and *MLL-AF9*.

Response to induction therapy

The results of therapy are summarized in Supplementary Table 2. A total of 130 (94% (95% CI 88–97%)) of 139 evaluated patients

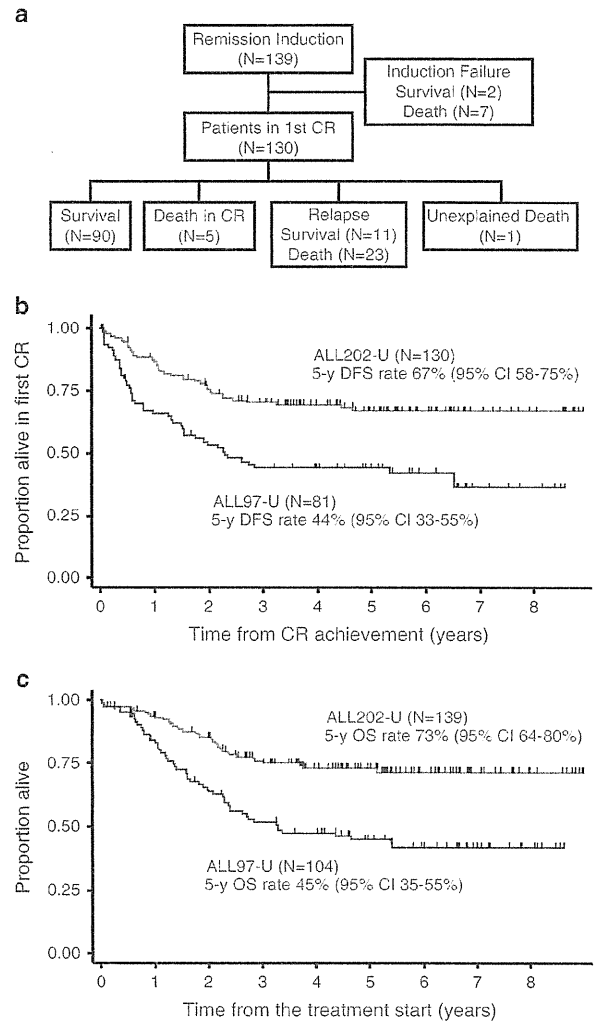

Figure 1. Comparison of DFS and OS rates. (**a**) Patient flow chart. (**b**) Comparison of DFS rates between ALL202-U (red line) and ALL97-U (blue line). The median follow-up times were 5.1 and 5.2 years, respectively. (**c**) Comparison of OS rates between ALL202-U (red line) and ALL97-U (blue line). The median follow-up times were 5.1 and 5.8 years, respectively.

achieved CR: 124 after the first treatment and 6 after the second course. Four patients died of sepsis during the first induction therapy before their remission status could be ascertained, and these were the only deaths that occurred during induction therapy. Three patients failed to achieve CR after two courses of therapy. Two patients dropped out of the study without starting the second therapy, because the first therapy failed to achieve CR. These results were markedly better than ALL97-U. The CR rate was 84% (95% CI 75–90%) and 12 patients died during induction therapy in ALL97-U.

Survival

Nine out of 139 eligible patients did not achieve CR and 7 of them died. Of the 130 CR patients, 5 patients died in remission, 1 died for an unknown reason and 34 patients relapsed; 19 of them received SCT and 23 relapsed patients died. A total of 36 patients died (Figure 1a). The estimated 5-year DFS rate was 67% (95% CI 58–75%, Figure 1b) and the estimated probability of the OS rate at

5 years was 73% (95% CI 64–80%; Figure 1c). Both the DFS rate and OS rate were markedly better than those of ALL97-U patients (44 and 45%, respectively; Figures 1b and c).

The results of univariate analysis on the effects of clinical and biological features on the DFS rate are summarized in Figure 2 as a forest plot. Age, performance status, CNS involvement, WBC counts, immunophenotype, cytogenetics, PSL response and CR achievement by the second induction therapy did not correlate with DFS.

We stratified patients with widely accepted risk factors, WBC counts and karyotypes as described in the Patients and Methods section, and analyzed survival in each group. Sixty-nine and 61 patients in ALL202-U were classified into the standard-risk group and high-risk group, respectively, and 50 and 28 patients in ALL97-U were classified in a similar manner. The DFS rate of ALL202-U patients was markedly better than that of ALL97-U patients both in the standard-risk group (71% vs 54%) and high-risk group (63% vs 28%; Figures 3a and b). As a result, no significant difference was observed in the DFS rate between the standard-risk and high-risk groups in ALL202-U (71% vs 63%, $P=0.4291$; compare red lines in Figures 3a and b), however, it was significant in ALL97-U (54% vs 28%, $P=0.0053$; compare blue lines in Figures 3a and b).

Some patient groups with possible poor prognostic factors, such as severe leukocytosis, pro-B and T-cell phenotypes, and poor PSL responses, contained more patients who received SCT in the first remission (Supplementary Table 3), which suggested that good survival outcome of ALL202-U was the result of the rescue of high-risk patients by SCT, however, no significant difference was observed in the DFS rate between patients that received SCT and those who did not, even in the high-risk group (Supplementary Figures 1A and B). These results suggested that the effect of the possible rescue of high-risk patients by SCT, if any, was not marked.

Toxicity

A full assessment of toxicity was performed in 1688 courses of chemotherapy (139 induction therapies, 126 consolidation therapies, 113 sanctuary therapies, 102 reinduction therapies, 98 reconsolidation therapies and 1110 maintenance therapies). Ninety-nine percent of patients developed grade 4 neutropenia during induction therapy, however, it was difficult to distinguish this from hematopoietic disorders by leukemia. The grade 3–4 adverse events observed during induction therapy were as follows: febrile neutropenia, sepsis and other infections occurred in 46.5%, 15% and 4.4% of patients, respectively. Elevated alanine aminotransferase levels, pancreatitis and ileus were observed in 27.8%, 6.6% and 3.6%, respectively. Eighteen (13.2%) and 10 (7.2%) patients developed disseminated intravascular coagulopathy and gastrointestinal bleeding, respectively. Hyperglycemia, neuropathy and tumor lysis syndrome occurred in 4.4%, 3.6% and 3.6%, respectively. Diarrhea, heart disease, creatinine elevations

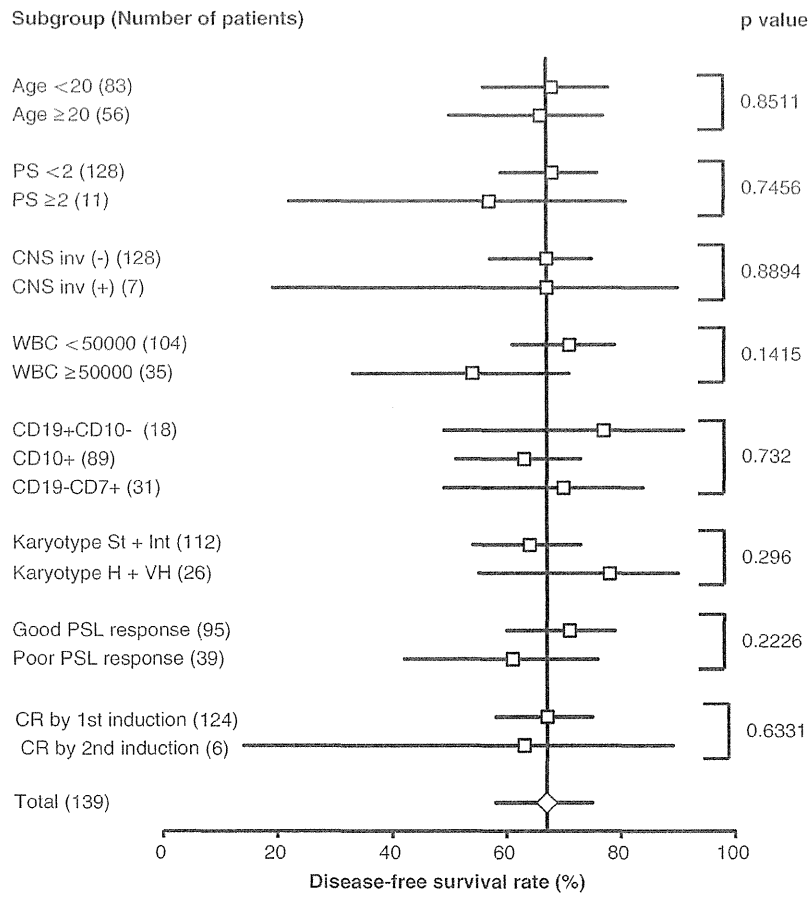

Figure 2. Forest plot of subgroup analysis for DFS rates. 5-year DFS rate of each subgroup was calculated and compared by the log-rank test. Patients undergoing transplantation were not censored. The 5-year DFS rate with 95% CIs are plotted and *P*-values of the log-rank test are shown. Numbers following subgroup names indicate the number of cases in the groups.

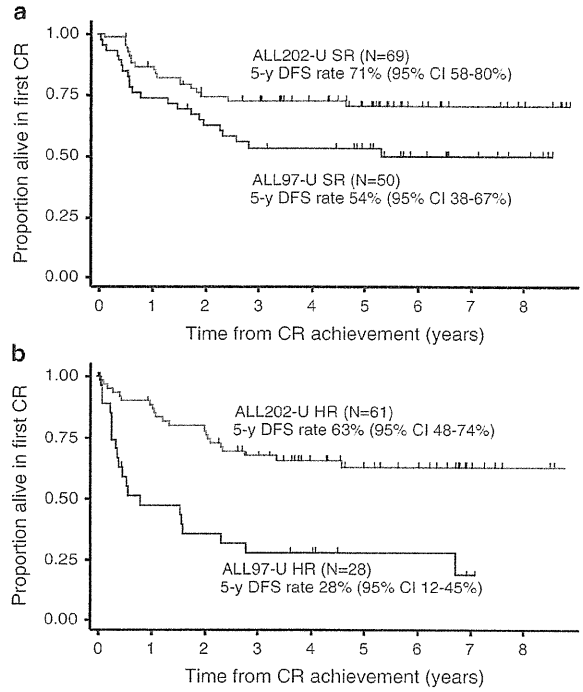

Figure 3. Comparison of the DFS rate in each risk group. (**a**) Comparison between ALL202-U standard-risk (SR) patients (red line) and ALL97-U SR patients (blue line). (**b**) Comparison between ALL202-U high-risk (HR) patients (red line) and ALL97-U HR patients (blue line).

and brain bleeding were observed in $< 1\%$ of patients. Severe adverse events such as neutropenia, thrombocytopenia, febrile neutropenia, sepsis, hepatic toxicity, pancreatitis and neuropathy occurred frequently during post-remission therapy. These have been summarized in Table 3. Toxicity was evaluated in the ALL97 study with the toxicity grading criteria of the Japan Clinical Oncology Group (JCOG). ALL202-U results were compared with those of ALL97-U in the points where the criteria coincided between NCI-CTC version 2 and the JCOG (Table 3). Sepsis, hepatic toxicity and neuropathy were more frequent in ALL202-U, although no patient died from the adverse events associated with chemotherapy during post-remission therapy in this study. In the pediatric study, JACLS ALL-02, patients in the high-risk group were treated with the ALL-02 HR protocol, which was the same as JALSG ALL202-U; 136 patients aged 10–18 years (90% patients were < 15 years old) were treated with ALL-02 HR. Severe adverse events, except for pancreatitis, occurred more frequently in pediatric patients (Table 3).

Protocol adherence

Therapies were delayed in many patients, and this was attributed to the adverse events associated with chemotherapy, however, some patients could proceed to the next therapy earlier than planned. The median delays from the planned schedule were 7 (range 0 to 171), 7 (range −9 to 35), 9 (range 0 to 36), 6 (range −8 to 70) and 19 (range −5 to 62) days in induction, consolidation, sanctuary, reinduction and reconsolidation therapy, respectively. As for the 57 patients who completed maintenance therapy, the median duration of maintenance therapy was 633 (range 553–881) days, which was 80 (range 0–328) days more than the planned schedule. No patients could complete the whole therapy without delays.

L-asparaginase dose reductions were required for 48 (35%), 18 (18%) and 38 (47%) patients because of its adverse events in induction, reinduction and maintenance therapy, respectively. Seventeen (30%) patients could complete the whole therapy without dose reductions in any drugs.

Fifty-seven (41%) patients could complete the whole therapy and 81 (59%) dropped out of the protocol therapy. The reasons, frequencies and periods of protocol therapy terminations have been summarized in Figures 4a and b. Seven (6%) patients were primary refractory, including early death, and 12 (9%) relapsed. Thirty-one (22%) patients dropped out of the study in the first remission to receive SCT. Twenty-two (16%) patients terminated protocol therapy because of severe adverse events. Eight (6%) patients dropped out of the study for their own reasons. One (1%) patient received the same maintenance therapy as ALL97 because of a doctor's mistake. This case was treated as a dropout because of a protocol violation. A significantly large number of patients dropped out after reconsolidation therapy for reasons other than relapse and SCT, and subsequently received no or insufficient maintenance therapy. In order to analyze the effects of insufficient maintenance therapy on survival, patients who achieved CR were divided into four groups: patients who did not drop out for reasons other than relapse (patients received planned post-remission therapy), those who dropped out because of SCT (patients received SCT in first CR) and those who dropped out for reasons other than relapse and SCT before and after the completion of drug administration in reconsolidation therapy (patients received insufficient consolidation therapy and patients received insufficient maintenance therapy, respectively). The estimated 5-year DFS rates of these groups were 76% (95% CI 63–84%), 70% (95% CI 50–83%), 29% (95% CI 5–59%) and 45% (95% CI 19–68%), respectively (Figure 4c). The DFS rate of patients who received insufficient maintenance therapy was compared with others using a proportional hazard model with time-varying covariates. The hazard ratio of insufficient maintenance therapy was 5.59 (95% CI 2.52–12.41, $P < 0.001$) in univariate analysis and 5.60 (95% CI 2.36–13.26, $P < 0.001$) in multivariate analysis (Table 4).

DISCUSSION

The results of this prospective study indicate that the pediatric protocol, ALL202-U, enabled markedly better survival rates to be achieved by AYA with ALL than the conventional adult protocol, ALL97. The OS rate reported here was similar to those reported in previous retrospective studies: 78% (age 15–20),[3] 71% (age 15–17),[4] 79% (age 15–18)[6] and 67% (age 16–20). [5] It was also similar to previous prospective studies by two pediatric groups (81% in those aged 15–18 years and 78% in those aged 16–21 years)[7,8] and an adult group (6-year OS rate for standard-risk ALL patients: 69% in those aged 15–30 years).[11] Patients who received the pediatric protocol treatment were mainly adolescents and $> 80\%$ were 18 years and under in all retrospective studies and prospective studies by pediatric groups.[7,8] Regardless of the prospective or retrospective design, comparisons with pediatric group studies could not conclude the efficacy of the pediatric protocol in young adults, however, the only study of an adult group included only standard-risk ALL patients (WBC count $\leqslant 30 \times 10^9$/l, and absence of t(9;22), t(1;19), t(4;11) or any other 11q23 rearrangements).[11] Ours is the only study on whole Ph-negative AYA ALL that used an unmodified pediatric protocol. The results obtained in our study demonstrated for the first time that a pediatric protocol was feasible and could also markedly improve survival in Ph-negative high-risk young adult ALL patients.

Concerning the key difference between pediatric protocols and adult protocols, pediatric protocols use more non-myelosuppressive drugs, such as glucocorticoids and L-asparaginase and fewer myelosuppressive drugs, such as anthracycline,[5]

Table 3. Comparison of adverse effect

ALL202-U vs ALL97

Therapy	*G4 neutropenia (%)*	*G3-G4 thrombocytopenia (%)*	*Sepsis (%)*	*G3-G4 hepatic toxicity (%)*	*G3-G4 neuropathy (%)*
ALL202-U (age 15–24)					
Induction			15	27.8	3.6
Consolidation	99.2	97.8	7.4	13.5	2.4
Sancturary	12	19.7	2.6	13.2	1.8
Reinduction	65	42.6	3.9	16.7	0
Reconsolidation	99	100	9.1	5.1	4
ALL97 (age 15–24)					
Induction			3.8	11.2	0
C1	73.7	10.5	0	4.2	0
C2	61.7	9.9	0	0	0
C3	64.6	3.8	0	0	0
C4	97.1	97.1	0	0	0
C5	41.9	1.6	0	0	0
C6	58.9	25	0	0	0
C7	86.8	9.4	0	0	0
C8	98	100	2.2	0	0

AYA vs pediatrics

Therapy	*Febrile neutropenia (%)*	*G3-G4 pancreatitis (%)*	*G4 hepatic toxicity (%)*	*G3-G4 neuropathy (%)*
ALL202-U (age 15–24)				
Induction	46.5	6.6	1.0	3.6
Consolidation	44.4	0.0	0.0	2.4
Sancturary	9.7	0.0	0.0	1.8
Reinduction	25.5	5.8	1.0	0.0
Reconsolidation	55.6	0.0	0.0	4.0
Maintenance	0.8	0.3	0.3	0.2
ALL-02-HR (age 10–18)				
Induction	63.9	5.0	3.4	9.3
Consolidation	58.0	1.1	3.5	6.5
Sancturary	37.8	0.0	0.0	13.3
Reinduction	51.8	1.1	1.1	3.3
Reconsolidation	73.7	0.0	0.0	0.0
Maintenance	8.5	0.3	0.6	0.2

Abbreviation: AYA, adolescent and young adult.

which was applied to the comparison between ALL202-U and ALL97. Many differences existed between these two protocols, such as the cumulative doses and dose intensities of each drug, treatment durations and prophylaxis of CNS involvement; therefore, we cannot identify the key difference responsible for the different treatment outcomes by comparing the whole protocols. However, this comparison becomes simple by focusing on induction therapy. The treatment schedules were similar between the protocols, except that ALL202-U had three treatments with intrathecal injections and prephase therapy of PSL for 7 days. Therefore, marked differences were observed in the cumulative doses. The main difference noted was that ALL202-U used more L-asparaginase (48 000 vs 18 000 U/m^2) and glucocorticoids (980 mg/m^2 PSL and 70 mg/m^2 dexamethasone vs 840 mg/m^2 PSL) and less anthracycline (50 mg/m^2 THP-adriamycin vs 135 mg/m^2 daunorubicin). Owing to the upper limit of the dose (2 mg per body), the planned cumulative doses of vincristine were almost identical (8.0 vs 7.8 mg per body). Therefore, these differences have been implicated in the marked difference observed in the CR rate of the first induction therapy (89.2% vs 76.0%), which suggests that the increased doses of L-asparaginase and glucocorticoids partly contributed to the improved survival in the ALL202-U study.

The indication for allo-SCT in the first remission remains a controversial issue in ALL. SCT is currently recommended in Japan in the first remission for high-risk ALL, defined by WBC $>30\times10^9$/l, a high-risk karyotype such as t(9;22), t(4;11), t(1;19), and +8, age $\geqslant 30$, or late CR achievement. Therefore, it was unavoidable that unignorable number of patients received allo-SCT, which made the interpretation of the results of this study difficult, however, SCT did not affect the DFS rate in multivariate analysis (hazard ratio 1.01; Table 3) and did not improve the DFS rate of ALL202-U patients, even in the high-risk group ($P=0.9394$; Supplementary Figures 1A and B). Therefore, the superiority of ALL202-U to historical control was not impaired. Based on the good outcomes observed in this study, SCT will no longer be recommended in the first remission for this type of high-risk ALL of AYA if patients are treated by this protocol. The indication for SCT in the first remission for ALL of AYA should be similar to that for pediatric patients. Children with Ph-negative ALL in Japan are recommended to receive SCT in the first remission if they are positive for

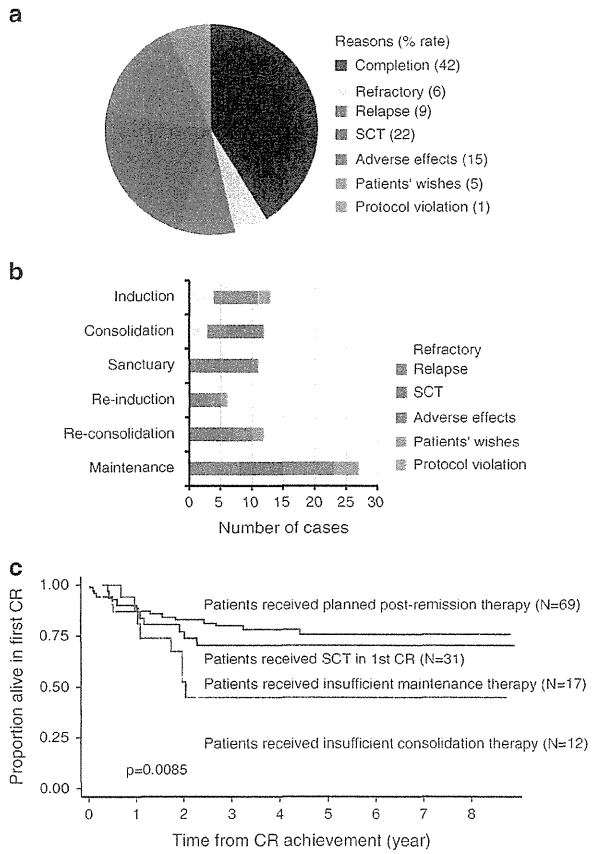

Figure 4. Analysis of protocol therapy termination. (**a**) The reasons for and frequencies of protocol therapy termination. (**b**) The periods of and reasons for protocol therapy termination. (**c**) The effect of therapy insufficiency on the DFS rate. DFS rates were compared among groups of patients who received planned post-remission therapy (blue line), those who received SCT in first CR (green line), those who received insufficient consolidation therapy (yellow line) and those who received insufficient maintenance therapy (red line).

the chromosome 11q23 abnormality, show a poor PSL response, or achieve CR later than 6 weeks from the treatment start.

A poor PSL response was previously shown to be a stronger prognostic factor than age and the WBC count in pediatric B-ALL.[17,18] The JACLS ALL-02 study and other pediatric studies used the PSL response for risk stratification. In our study, a poor PSL response did not significantly worsen the prognosis of patients (Figure 2). Patients with a poor PSL response received SCT during the first remission more frequently than those with a good PSL response (33% vs 22%; Supplementary Table 3), and 22% of patients had T-ALL in this study (Table 2). These results should be considered, however, a poor PSL response was not a significant risk factor in multivariate analysis (Table 4). The prognostic impact of the PSL response should be investigated in a larger number of patients. Considering the rarity of ALL in AYA, this should be examined in patients with a wider age range. Among other patient characteristics and therapy responses, the risks of late CR achievement and presence of t(4;11), other 11q23 rearrangements and t(1;19) could not be determined in this study because of the small number of patients (6, 1, 1 and 6, respectively) and high frequency of receiving SCT (50%, 100%, 0% and 67%, respectively).

The toxicity of the ALL202-U protocol appeared to be high because severe adverse events occurred more frequently in this

Table 4. Multivariate analysis of the effect of biological and clinical features on DFS

Parameters	*Hazard ratio (95% CI)*	*P-value*
Insufficient maintenance therapy	5.60 (2.36–13.26)	< 0.001
Age ⩾ 20	1.25 (0.62–2.52)	0.531
PS ⩾ 2	1.28 (0.42–3.91)	0.662
CNS involvement (+)	0.93 (0.19–4.50)	0.927
WBC ⩾ 50000	1.63 (0.77–3.43)	0.195
Karyotype high + very high	0.72 (0.27–1.92)	0.516
B-cell phenotype	1.36 (0.58–3.21)	0.484
Poor PSL response	1.52 (0.71–3.27)	0.284
CR by 2nd induction	1.64 (0.34–7.98)	0.538
SCT in 1st remission	1.01 (0.43–2.37)	0.980

Abbreviations: CI, confidence interval; CNS, central nervous system; CR, complete remission; DFS, disease-free survival; PS, performance status; PSL, prednisolone; SCT, stem cell transplantation; WBC, white blood cell.

study, however, the death rate during induction therapy was lower in this study than ALL97 (3% vs 12%; Supplementary Table 2), and this may have been because patients achieved CR more frequently and quickly in this study. In addition, no chemotherapy-related deaths were observed during post-remission therapy in this study. These results indicate the tolerability of this protocol by AYA. Children treated with the same protocol in the JACLS ALL-02 study exhibited severe adverse events more frequently than AYA in this study. Our results indicated that AYA tolerated chemotherapy better than children, except for L-asparaginase-induced pancreatitis. These results suggested that ALL202-U was feasible as a treatment for AYA with BCR–ABL-negative ALL.

Adherence to the protocol was not good in this study, and this was mainly due to the high toxicity of this treatment. Protocol therapy was frequently terminated because of adverse events and the patients' wishes. Such therapy terminations were the most frequent during maintenance therapy (Figure 4b), although the frequency of severe adverse events was markedly less during maintenance therapy than all other post-remission therapies (Table 3). This result suggested maintenance therapy may have been terminated because of less severe adverse events. Another reason is the difficulty in maintaining motivation for therapy in AYA against their psychosocial conditions. Our results clearly showed the significant importance of completing maintenance therapy. This information will help to maintain motivation for therapy, and may lead to further improvements in the outcomes of patients.

Taken together, ALL202-U caused high, but acceptable toxicity and led to a markedly better outcome than the previous study and is thought to be a feasible and highly effective treatment for AYA with BCR–ABL-negative ALL, including high-risk cases.

CONFLICT OF INTEREST

Consultancy: NU (Pfizer) and TN (Pfizer). Honoraria: OS (Kyowahakko-Kirin); HH (Kyowahakko-Kirin, Nippon Shinyaku); YA (Kyowahakko-Kirin, Shionogi); NU (Kyowahakko-Kirin, Nippon Shinyaku, Shionogi, Pfizer); YM (Kyowahakko-Kirin, Nippon Shinyaku, Pfizer); YK (Nippon Shinyaku); TN (Kyowahakko-Kirin, Nippon Shinyaku, Shionogi). Research funding: JM (Kyowahakko-Kirin, Shionogi, Pfizer); NU (Kyowahakko-Kirin, Nippon Shinyaku, Shionogi, Pfizer, Meiji Seika Pharma); HK (Bristol-Myers Squibb, Chugai Pharmaceutical, Kyowahakko-Kirin, Dainippon Sumitomo Pharma, Zenyaku Kogyo and FUJIFILM Corporation); YM (Kyowahakko-Kirin, Pfizer); YK (Pfizer); TN (Kyowahakko-Kirin).

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ORIGINAL ARTICLE

Soluble interleukin-2 receptor level on day 7 as a predictor of graft-versus-host disease after HLA-haploidentical stem cell transplantation using reduced-intensity conditioning

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Abstract In the present study, we analyzed the kinetics of serum soluble interleukin-2 receptor (sIL-2R) using data from 77 patients undergoing HLA-haploidentical transplantation using reduced-intensity conditioning (RIC), who were at an advanced stage or at high risk for relapse, to clarify the usefulness of sIL-2R as a biomarker of acute graft-versus-host disease (GVHD). Anti-T-lymphocyte globulin and methylprednisolone were used as GVHD prophylaxis. While the median sIL-2R in 38 patients not developing GVHD was suppressed at levels <740 U/ml, sIL-2R in 25 patients developing severe GVHD peaked on day 11 (1,663 U/ml), and thereafter decreased to <1,000 U/ml after day 30. The occurrence of GVHD was not limited to times of high sIL-2R level, but occurred at any time point on the sIL-2R curve. Most patients developing GVHD, however, experienced a higher sIL-2R level early in their transplant course. The combination of RIC and glucocorticoids sufficiently suppressed sIL-2R levels after HLA-haploidentical transplantation. In a multivariate analysis to identify factors associated with GVHD, day 7 sIL-2R >810 U/ml was the only factor significantly associated with the occurrence of severe GVHD ($p = 0.0101$).

Keywords Allogeneic stem cell transplantation · Graft-versus-host disease · Soluble interleukin-2 receptor · Alloreactive response · HLA-haploidentical transplantation

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Introduction

Bone marrow transplantation (BMT) from siblings genotypically matched for human leukocyte antigen (HLA) improves long-term survival in patients with hematologic malignancies [1]. However, more than 70 % of patients who could benefit from allogeneic BMT do not have a matched sibling donor. On the other hand, there is a >90 % chance of promptly identifying an HLA-haploidentical donor within the family; therefore, the number of patients receiving HLA-haploidentical stem cell transplantation (SCT) is gradually increasing [2–6]. The major drawback of HLA-haploidentical SCT is graft-versus-host disease (GVHD). To overcome GVHD after HLA-haploidentical SCT, several breakthroughs in transplant methodology, including drastic ex vivo T cell purging coupled with the use of megadose of stem cells [2], and in vivo T cell purging through the use of anti-T-lymphocyte globulin (ATG) [4, 5, 7], or the use of cyclophosphamide at post-transplant, have been done [6]. We and others have been studying HLA-haploidentical SCT using in vivo T cell purging method using ATG [4, 5, 7]. In this transplant setting, although the severity of GVHD is within a permissible range, GVHD still continues to be the problem, but an appropriate monitoring method of GVHD has not been established yet.

Basically, GVHD is induced by the immunological response of donor T cells. In general, once activated, T cells express the interleukin-2 receptor (IL-2R), consisting of at least three subunits (α, β and γc) on their membrane

[8, 9]. The soluble form of IL-2R is produced by proteolytic cleavage of IL-2Rα, and the release of soluble interleukin-2 receptor (sIL-2R) into the circulation has been found to be proportional to its membrane bound expression [10, 11]. Thus, serum sIL-2R levels reflect the magnitude of the T cell immunological response and are associated with the incidence and severity of GVHD in allogeneic BMT settings. In fact, sIL-2R is reported to be the most reliable biomarker among several useful biomarkers [12].

The role of sIL-2R as a GVHD biomarker has been studied mainly in the transplant settings of HLA-matched myeloablative SCT for patients mostly in complete remission (CR) [12–17]. Reduced-intensity conditioning (RIC), which has been used also in HLA-haploidentical transplant settings, may contribute to the reduction of the incidence and severity of GVHD [18–20]. We and others reported that HLA-haploidentical reduced-intensity conditioning stem cell transplantation (RIST) was useful for patients who did not have a suitable HLA-matched donor [5, 7]; however, there are no reports analyzing whether sIL-2R is still a useful biomarker of GVHD in this transplant setting.

Despite the usefulness of sIL-2R as a GVHD biomarker, transplant-related complications, including severe infection, graft rejection, and hepatic veno-occlusive disease, are known to increase sIL-2R levels [13, 15, 21]. Furthermore, leukemia- or lymphoma-associated elevation of serum sIL-2R levels has been reported [22–25]. The coexistence of these conditions could reduce the value of sIL-2R as a biomarker of GVHD.

Therefore, in the present study, after excluding data of patients with conditions that increase sIL-2R levels other than GVHD, we retrospectively studied the usefulness of sIL-2R as a GVHD biomarker using data from 77 patients, with poor prognosis or in an advanced stage of disease, who underwent HLA-haploidentical RIST.

Patients and methods

Patients

To retrospectively evaluate the role of the sIL-2R level as a biomarker of acute GVHD, we analyzed data from patients who underwent HLA-haploidentical RIST at the Hospital of Hyogo College of Medicine between January 2009 and June 2012. All patients had hematologic malignancies and were at an advanced stage or had a poor prognosis at the time of transplantation.

The inclusion criteria were as follows: donor-type engraftment, survival for at least 30 days after transplantation, the absence of hepatic veno-occlusive disease, and severe infections (CRP >10), including sepsis [13, 15, 21]. Furthermore, to avoid the effect of tumor-associated sIL-2R [22–25], data from patients who showed a tumor-associated increase in sIL-2R >2,000 U/ml before conditioning, which did not decrease to <1,000 U/ml on day 0, were excluded. Consequently, data from 20 % of the total transplant patients were excluded based on the exclusion criteria described above, and we analyzed data from 77 patients who underwent transplantation using a graft from an HLA-haploidentical donor (2–3 antigen-mismatches in GVH direction). The patients' characteristics are shown in Table 1.

Table 1 Patients' characteristics

	GVHD Grade 0	Grade I	Grade II–III
Number of patients	38	14	25
Sex			
Male/female	24/14	5/9	10/15
Age (years)			
Median (range)	42.5 (17–63)	46.5 (20–61)	55 (14–65)
Disease			
AML/MDS	17	10	12
ALL	11	0	3
Lymphoma	6	2	6
Others	4	2	4
Disease status			
Good (CR/RA/CP)	2	0	2
Intermediate (PR/RAEB/AP)	4	1	1
Poor	32	13	22
HLA disparity in GVH direction			
2 antigen	18	9	13
3 antigen	20	5	12
Number of times of transplant			
First	17	7	20
Second or later	21	7	5
Conditioning combination chemotherapy			
Busulfan-containing	8	3	9
Melphalan-containing	26	8	15
Others	4	3	1
TBI			
Containing	25	8	15
Non-containing	13	6	10

Institutional review board approval was obtained for the treatment protocol, and written informed consent was obtained from the patients and their families.

Preparative regimen for transplantation

Sixty-nine patients received a regimen consisting of fludarabine (30 mg/m^2/day on days −9 to −4), cytarabine (2 g/m^2 on days −9 to −6), ATG (thymoglobulin: total