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 $\pm 10\%$. 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

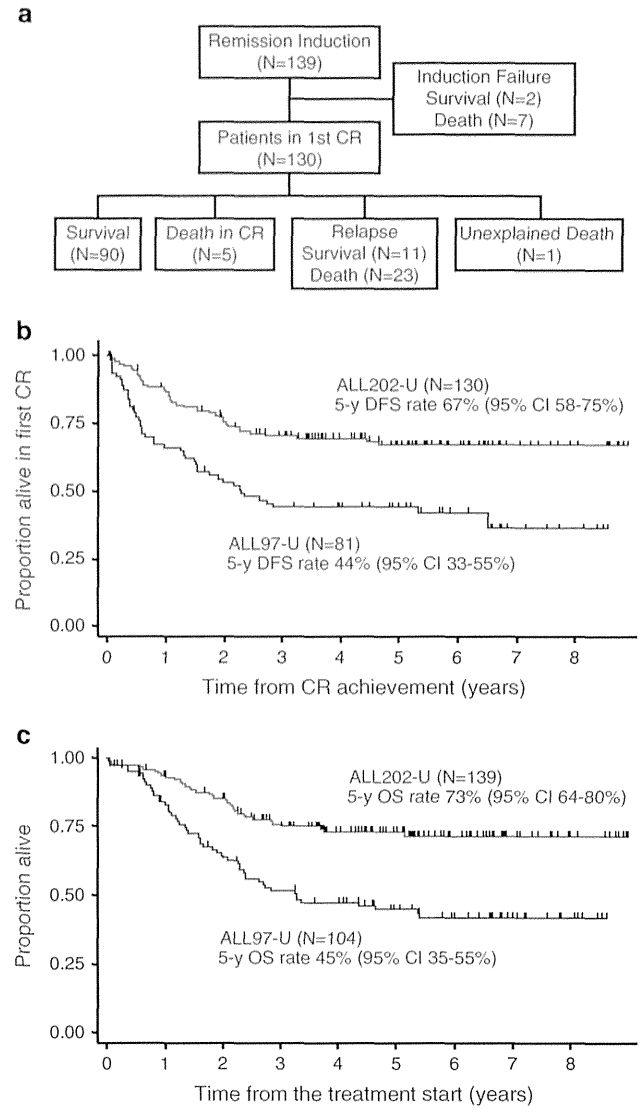

Figure 1. Comparison of DFS and OS rates. (**a**) Patient flow chart. (**b**) Comparison of DFS rates between ALL202-U (red line) and ALL97-U (blue line). The median follow-up times were 5.1 and 5.2 years, respectively. (**c**) Comparison of OS rates between ALL202-U (red line) and ALL97-U (blue line).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

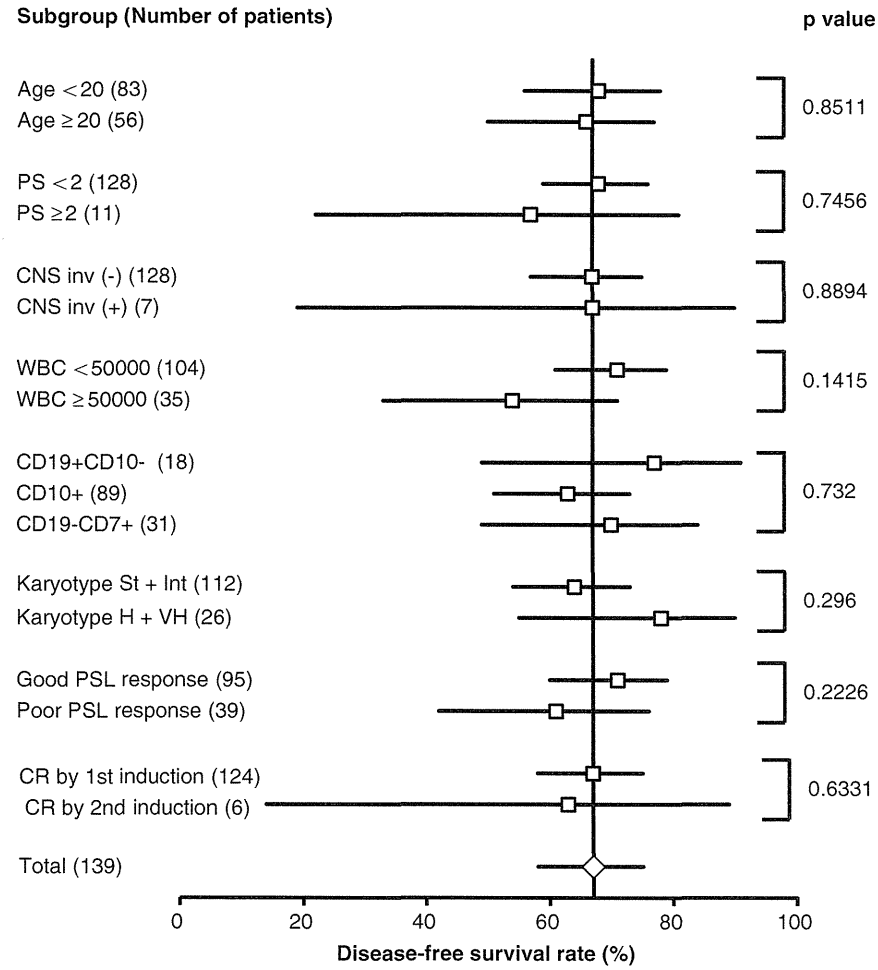

Figure 2. Forest plot of subgroup analysis for DFS rates. 5-year DFS rate of each subgroup was calculated and compared by the log-rank test. Patients undergoing transplantation were not censored. The 5-year DFS rate with 95% CIs are plotted and *P*-values of the log-rank test are shown. Numbers following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

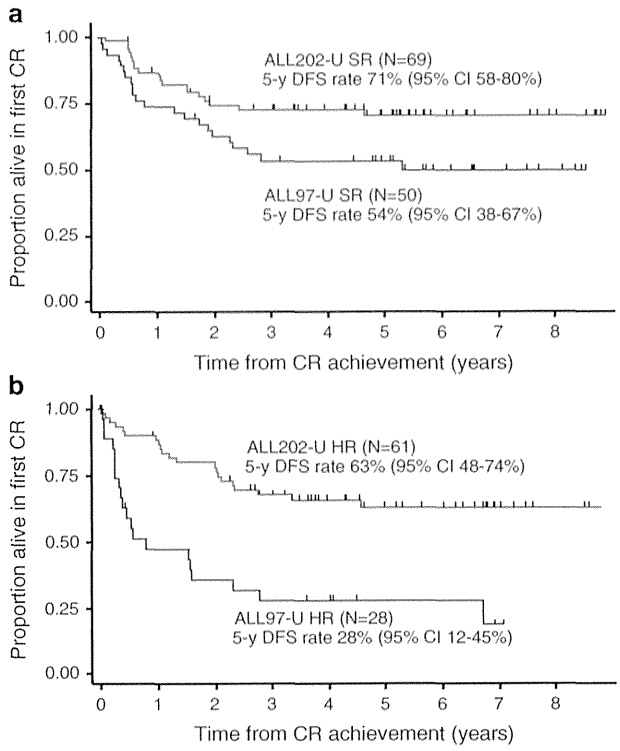

Figure 3. Comparison of the DFS rate in each risk group. (**a**) Comparison between ALL202-U standard-risk (SR) patients (red line) and ALL97-U SR patients (blue line). (**b**) Comparison between ALL202-U high-risk (HR) patients (red line) and ALL97-U HR patients (blue line).

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 $\leqslant 30\times10^9$/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

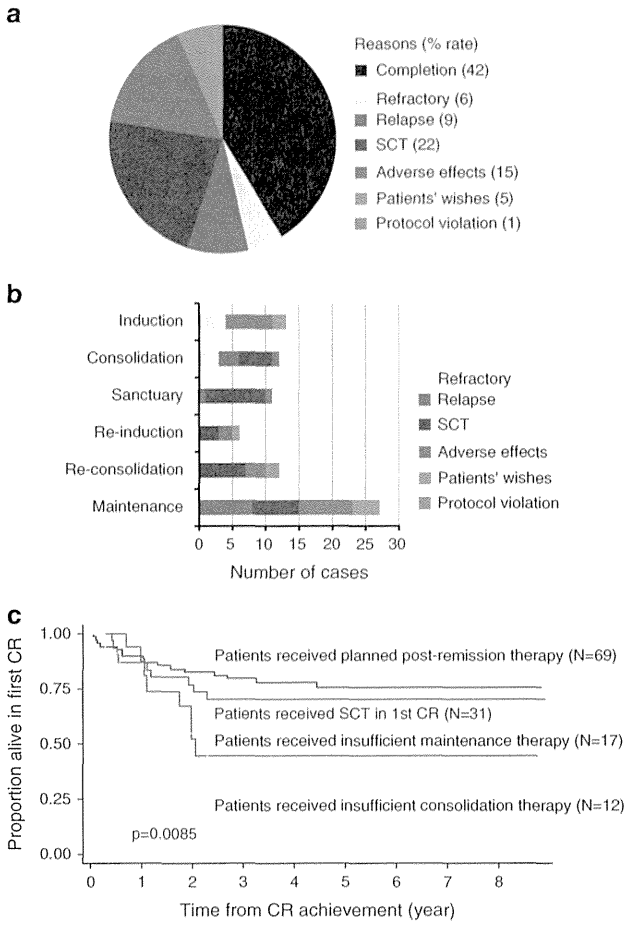

Figure 4. Analysis of protocol therapy termination. (**a**) The reasons for and frequencies of protocol therapy termination. (**b**) The periods of and reasons for protocol therapy termination. (**c**) The effect of therapy insufficiency on the DFS rate. DFS rates were compared among groups of patients 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 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.

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.

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

Comprehensive analysis of genetic alterations and their prognostic impacts in adult acute myeloid leukemia patients

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To clarify the cooperative roles of recurrently identified mutations and to establish a more precise risk classification system in acute myeloid leukemia (AML), we comprehensively analyzed mutations in 51 genes, as well as cytogenetics and 11 chimeric transcripts, in 197 adult patients with *de novo* AML who were registered in the Japan Adult Leukemia Study Group AML201 study. We identified a total of 505 mutations in 44 genes, while only five genes, *FLT3, NPM1, CEBPA, DNMT3A* and *KIT*, were mutated in more than 10% of the patients. Although several cooperative and exclusive mutation patterns were observed, the accumulated mutation number was higher in cytogenetically normal AML and lower in AML with *RUNX1-RUNX1T1* and *CBFB-MYH11*, indicating a strong potential of these translocations for the initiation of AML. Furthermore, we evaluated the prognostic impacts of each sole mutation and the combinations of mutations and/or cytogenetics, and demonstrated that AML patients could be clearly stratified into five risk groups for overall survival by including the mutation status of *DNMT3A*, *MLL*-PTD and *TP53* genes in the risk classification system of the European LeukemiaNet. These results indicate that the prognosis of AML could be stratified by the major mutation status in combination with cytogenetics.

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Keywords: acute myeloid leukemia; gene mutations; prognosis; risk factor

INTRODUCTION

Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease.[1,2] Although about 80% of younger adults with AML achieve complete remission (CR) with induction chemotherapy, more than half of the CR patients relapse, even if they receive intensive consolidation therapies. Allogeneic hematopoietic stem cell transplantation (allo-SCT) is applied to the patients who have risk factors for relapse, and it has been demonstrated by meta-analysis that allo-SCT at the first CR improves the long-term prognosis of the cytogenetically intermediate- and adverse-risk groups.[3] Cytogenetic-risk classification for AML is well established and commonly used as criteria for the application of allo-SCT at the first CR, whereas there is clinical heterogeneity in the intermediate-risk group, particularly cytogenetically normal (CN)-AML.[4] Recent advances and the accumulation of information on the prognostic relevance of recurrent genetic alterations have made more detailed risk stratification possible in AML patients.[5–19] The European LeukemiaNet (ELN) has recommended a novel risk classification system on the basis of the cytogenetic and genetic status.[2] In this system, CN-AML is stratified into two risk groups according to the mutation status of *FLT3*, *NPM1* and *CEBPA*: patients with *NPM1* mutation but not *FLT3*-ITD and those with *CEBPA* mutation are included in the favorable-risk (FR) group, and patients with *FLT3*-ITD and those with neither *NPM1* mutation nor *FLT3*-ITD are categorized into the intermediate-I-risk (IR-I) group. Long-term prognosis according to the ELN classification system was retrospectively evaluated in well-established cohorts, and it has

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been demonstrated that the ELN system is useful for further risk stratification of younger adult patients with CN-AML.[20,21] However, it has been reported that another genetic status, such as mutations in epigenetic modifier-encoding genes, could more precisely distinguish the prognosis in each ELN-risk group.[5,22] In addition, the prognostic impacts of recently identified mutations in spliceosome and cohesin complex genes on AML remain unclear.

The Japan Adult Leukemia Study Group (JALSG) conducted six phase III trials for adult *de novo* AML from 1987 (AML87, AML89, AML92, AML95, AML97 and AML201).[23] In the JALSG AML201 study, we prospectively compared a standard dose of idarubicin (IDR) with a higher dose of daunorubicin (HiDNR) in combination with cytarabine (Ara-C) as induction therapy, and three courses of high-dose Ara-C (HiDAC) with four courses of conventional standard-dose multiagents as consolidation therapy in CR patients.[24,25] We demonstrated that HiDNR was equivalent to IDR, as induction therapy and HiDAC was of benefit only to patients with core-binding factor (CBF)-AML as consolidation therapy. Although the CR rate remained at 75–80% during the six JALSG studies, 7-year overall survival (OS) was improved to 48% in the AML201 study from 29% in the AML87 study. Allo-SCT was conducted in only 7.1% of registered patients in the AML87 study, whereas 45.8% of registered patients received allo-SCT not only at the first CR but also after relapse or primary induction failure in the AML201 study, indicating that active application of allo-SCT even after relapse or primary induction failure might contribute to the improvement of OS. These results collectively suggested that a novel risk stratification system for decision making of allo-SCT at the first CR is required.

In this study, we comprehensively analyzed mutations in 51 genes that have been recurrently identified in myeloid neoplasm as well as cytogenetics, and evaluated the association of genetic status with prognostic and clinical features in patients who were registered in the AML201 study.

PATIENTS AND METHODS

Patients and samples

The study population included 197 newly diagnosed *de novo* AML patients, except for those with acute promyelocytic leukemia, who were registered in the JALSG AML201 study (UMIN Clinical Trials Registry C000000157, http://www.umin.ac.jp/ctrj/). The diagnosis of AML was on the basis of the French–American–British (FAB) classification.[26] Median follow-up time was 32.5 months. The age distribution is presented in Table 1. In the AML201 study, patients were randomly assigned to receive either IDR or HiDNR for induction therapy, and those who achieved CR were again randomized to receive either four courses of conventional consolidation therapy or three courses of HiDAC therapy.[24,25] Of the 197 patients, 98 and 99 patients were assigned to IDR and HiDNR arms for induction therapy, respectively. CR was achieved in 161 of 197 (81.7%) patients, and 80 and 77 patients were assigned to HiDAC and conventional consolidation therapies, respectively (Table 1).

High molecular weight DNA and total RNA were extracted from bone marrow samples using standard methods.[27–29]

Cytogenetic G-banding analysis was performed by standard methods. We also examined 11 chimeric gene transcripts (Major: *BCR-ABL1*, Minor: *BCR-ABL1*, *PML-RARA*, *RUNX1-RUNX1T1*, *CBFB-MYH11*, *DEK-NUP214*, *NUP98-HOXA9*, *MLL-MLLT1*, *MLL-MLLT2*, *MLL-MLLT3*and *MLL-MLLT4*) by reverse transcriptase-mediated quantitative PCR as previously reported.[30]

Morphological diagnosis, the FAB classification and karyotypes were reviewed and confirmed by the central review committees of the JALSG using the BM samples obtained at diagnosis.

We obtained informed consent from all patients to use their samples for banking and molecular analysis, and approval was obtained from the ethics committees of the participating institutes.

Screening for mutations in 51 genes

A custom-made oligonucleotide probe library was designed to capture the exons of 51 genes that have been recurrently identified in myeloid neoplasm (Supplementary Table 1). Captured and enriched exons were subjected to sequencing on an Illumina HiSeq (Illumina, San Diego, CA, USA).[31–33] Sequence variation annotation was performed using known polymorphism databases, followed by mutation characterization. Each predicted variant sequence was confirmed by Sanger sequencing. Internal tandem duplication of the *FLT3* gene (*FLT3*-ITD) and partial tandem duplication of the *MLL* gene (*MLL*-PTD) were examined as previously reported.[28,34]

Table 1. Characteristics of the 197 patients

Characteristics	*Number*	*(%)*
Age (year)		
15–19	6	3.0
20–29	32	16.2
30–39	35	17.8
40–49	33	16.8
50–59	69	35.0
60–64	22	11.2
FAB subtype		
M0	7	3.6
M1	36	18.3
M2	89	45.2
M4	34	17.3
M4Eo	9	4.6
M5	21	10.7
M6	1	0.51
Cytogenetic-risk group		
Favorable	55	27.9
RUNX1-RUNX1T1	41	20.8
CBFB-MYH11	14	7.1
Intermediate	100	50.8
Normal cytogenetics	72	36.5
Unfavorable	23	11.7
Complex karyotype	16	8.1
t(11q23) excluding t(9;11) and t(11;19)	3	1.5
t(9;22)	2	1.0
−7	1	0.5
Not determined	19	9.6
Induction therapy		
IDR + Ara-C	98	49.7
DNR + Ara-C	99	50.3
Achieving CR	161	81.7
Consolidation therapy		
High-dose Ara-C	80	51.0
Multiagent CT	77	49.0

Abbreviation: IDR, idarubicin. The study population included 197 newly diagnosed *de novo* AML patients except for acute promyelocytic leukemia, and equally assigned to induction and consolidation arms. Nine patients showed the M4Eo FAB type, and all of them harbored the *CBFB-MYH11* transcript.

Statistical analysis

Differences in continuous variables were analyzed by the unpaired *t*-test or the Mann–Whitney *U*-test for distribution between two groups. Analysis of frequencies was performed using Fisher's exact test for 2×2 tables or Pearson's χ^2 test for larger tables. A multivariate analysis to identify risk factors for achieving CR was performed by the logistic regression model. Survival probabilities were estimated by the Kaplan–Meier method, and differences in the survival distributions were evaluated using the log-rank test. OS was defined as the time from the date of entry into the AML201 study to death due to any cause or last follow-up. Disease-free survival (DFS) was defined as the time from the day of achieving CR to relapse, death due to any cause or last follow-up. Patients undergoing SCT were not censored at the time of transplantation. The prognostic significance of the clinical variables was assessed using the Cox proportional hazards model. These statistical analyses were performed with Prism 5 (GraphPad Software, La Jolla, CA, USA) and JMP Pro10 (SAS Institute Japan,

Tokyo, Japan). For all analyses, the *P*-values were two-tailed, and a *P*-value of less than 0.05 was considered statistically significant.

RESULTS

Cytogenetic analysis

Cytogenetic analysis revealed a normal karyotype in 72 (36.5%) patients and an abnormal karyotype in 106 (53.8%) patients, including 41 AML with t(8;21) (q22;q22); *RUNX1-RUNX1T1* and 14 AML with inv(16) (p13q22); *CBFB-MYH11*. However, karyotypes could not be determined in 19 (9.6%) patients because we could not obtain sufficient mitotic cells. On the basis of the G-banding karyotype and chimeric transcript analyses, patients were assigned to favorable- ($n=55$, 27.9%), intermediate- ($n=100$, 50.8%) and adverse-risk ($n=23$, 11.7%) groups according to the refined MRC criteria (Table 1).[4]

Frequencies of mutations

We identified mutations in 44 of 51 genes analyzed in the 197 AML patients. However, there were only five genes (*FLT3, NPM1, CEBPA, DNMT3A* and *KIT*) that were mutated in more than 10% of the patients (Figure 1a and Supplementary Table 1). Each position and type of mutation is presented in Supplementary Figure 1. As germ-line controls were available in a limited number of patients, we could not completely confirm that all identified mutations were somatic mutations. Therefore, there is a possibility that a part of identified mutations might be rare SNPs. *FLT3* mutation was the most frequently identified (50 patients, 25.4%), followed by *NPM1* (38 patients, 19.2%), *DNMT3A* (32 patients, 16.2%), *CEBPA* (31 patients, 15.7%) and *KIT* mutations (28 patients, 14.2%). Of the 50 patients with *FLT3* mutations, 36 (18.3%) and 17 (8.6%) patients harbored *FLT3*-ITD and *FLT3*-KDM, respectively, and three patients harbored both mutations. Of the 31 patients with *CEBPA* mutations, 19 (9.6%) and 12 (6.1%) patients harbored double *CEBPA* (*CEBPA*-D) mutations and a single *CEBPA* (*CEBPA*-S) mutation, respectively. Of the 28 patients with *KIT* mutations, 4, 2 and 23 patients harbored mutations in exon 8, exons 10–11 and exon 17 of the *KIT* gene, respectively, and one patient harbored mutations in both exons 10–11 and exon 17.

Although mutations in the 51 analyzed genes were not identified in 14 (7.1%) patients, 183 (92.9%) patients harbored one or more mutations; one mutation in 36, two mutations in 56, three mutations in 40, four mutations in 27, five mutations in 17, six mutations in five and seven mutations in two patients. The mean mutation number per patient was 2.56 ± 0.11 in all patients, whereas it was significantly higher in patients with a normal karyotype (3.18 ± 0.16) than in those with an aberrant karyotype (2.10 ± 0.15) ($P<0.0001$). Furthermore, mean mutation numbers per patient in AML with *RUNX1-RUNX1T1* (1.68 ± 0.17) and *CBFB-MYH11* (1.57 ± 0.20) were significantly lower than that in all samples ($P=0.0008$ and 0.0123, respectively) (Figure 1b).

The mean mutation number per patient aged 60–64 years (3.18 ± 0.41) tended to be higher, although there was no significant difference between the mean mutation number and age (Supplementary Figure 2).

Genetic alterations found in AML have been conceptually grouped into class I mutation, which causes constitutive activation of intracellular signals that contribute to the growth and survival, and class II mutation that blocks differentiation and/or enhance self-renewal by altered transcription factors.[35–37] Recently, it has been suggested that mutations that modify the epigenetic status generate a new class because of their overlap mutations both with class I and class II mutations.[13,38] In this study, Class II mutations (*NPM1, CEBPA, RUNX1* and *GATA2* mutations, and *RUNX1-RUNX1T1* and *CBFB-MYH11*) were the most frequently identified (138/197; 70.1%), followed by Class I mutations (*FLT3, KIT, N/KRAS, PTPN11, JAK1/3* and *TP53* mutations) (116/197; 58.9%) and mutations that modify the epigenetic status (*ASXL1, ATRX, EZH2, TET2, PBRM1, DNMT3A, IDH1/2, KDM6A, MLL* and *DOT1L* mutations) (91/197; 46.2%). Furthermore, mutations of NOTCH family genes (*NOTCH1 and NOTCH2*), cohesin complex genes (*STAG2, SMC1A, SMC3* and *RAD21*), BCOR family genes (*BCOR* and *BCORL1*), NCOR family genes (*NCOR1, NCOR2* and *DIS3*) and spliceosome genes (*SF3B1, U2AF1, SRSF2* and *ZRSR2*) were identified in 19 (9.6%), 22 (11.2%), 17 (8.6%), 22 (11.2%) and 9 patients (4.6%), respectively (Figure 1c).

Association between gene mutations and cytogenetics

The prevalence of each gene mutation differed among the cytogenetic-risk groups. *KIT* mutations were preferentially identified in the favorable cytogenetic-risk group. *FLT3*-ITD, *NPM1, CEBPA* and *DNMT3A* mutations were preferentially identified in the intermediate-risk group, particularly in patients with a normal karyotype. *BCORL1* and *TP53* mutations were preferentially identified in the poor-risk group; in particular, *TP53* mutations were frequent in patients with a complex karyotype. In addition, *PHF6* mutations were also frequently identified in patients with a complex karyotype (Figure 2 and Supplementary Table 2).

Overlap mutations

Several patterns of overlap mutations were identified in this comprehensive mutation analysis (Supplementary Figures 3 and 4). Significantly overlapped mutations were observed between *FLT3* mutations and *NPM1, DNMT3A* and *MLL*-PTD mutations; *NPM1* mutations and *DNMT3A, IDH1* and *IDH2* mutations; *CEBPA* mutations and *TET2* mutations; *ASXL1* mutations and spliceosome gene mutations; *DIS3* mutations and *MLL* mutations; *DNMT3A* mutations and *PTPN11* mutations; *GATA2* mutations and *CEBPA*-D mutations; *K/NRAS* mutations and *WT1* mutations and *BCOR/BCORL1* mutations; *RUNX1* mutations and *U2AF1, MLL-PTD, BCOR/BCORL1* and *PHF6* mutations; *SF3B1* mutations and *NRAS* mutations; and *TET2* mutations and *STAG2* mutations. In contrast, mutually exclusive mutations were observed between *FLT3* mutations and *KIT, K/NRAS* and *CEBPA*-D mutations; *NPM1* mutations and *CEBPA*-D and *RUNX1* mutations; and *CEBPA* mutations and *IDH1/2* mutations.

According to the conceptual classification of the mutated genes, overlap mutations between Class I, Class II and epigenetic modifying gene mutations were frequently observed. However, these major mutations widely coexisted with other family gene mutations, such as the cohesin complex, BCOR family and spliceosome gene mutations (Figure 1d). Although biological functions of mutated genes have not been fully clarified, we also present frequencies and associations of mutated genes according to the provisional gene function in the Supplementary Figure 5.

Association of gene mutations with clinical characteristics

Several associations between mutations and clinical characteristics were observed. *DNMT3A* mutations and *MLL*-PTD were more frequently identified in patients over 50 years old than in those less than 50 years old ($P=0.0064$ and $P=0.0121$, respectively), whereas the other mutations were not significantly associated with age (Supplementary Table 3).

Several mutations were associated with the white blood cell count at diagnosis. *FLT3-ITD, NPM1, DNMT3A* and *NOTCH1* mutations were significantly associated with the high white blood cell count. In contrast, *ASXL1* and *IDH1* mutations were associated with a lower white blood cell count (Supplementary Table 4).

Association of gene mutations with the CR rate

We analyzed the association of mutations with the CR rate. By Fisher's exact test, *RUNX1-RUNX1T1* or *CBFB-MYH11, KIT, NPM1* and *CEBPA*-D mutations were identified as favorable factors for

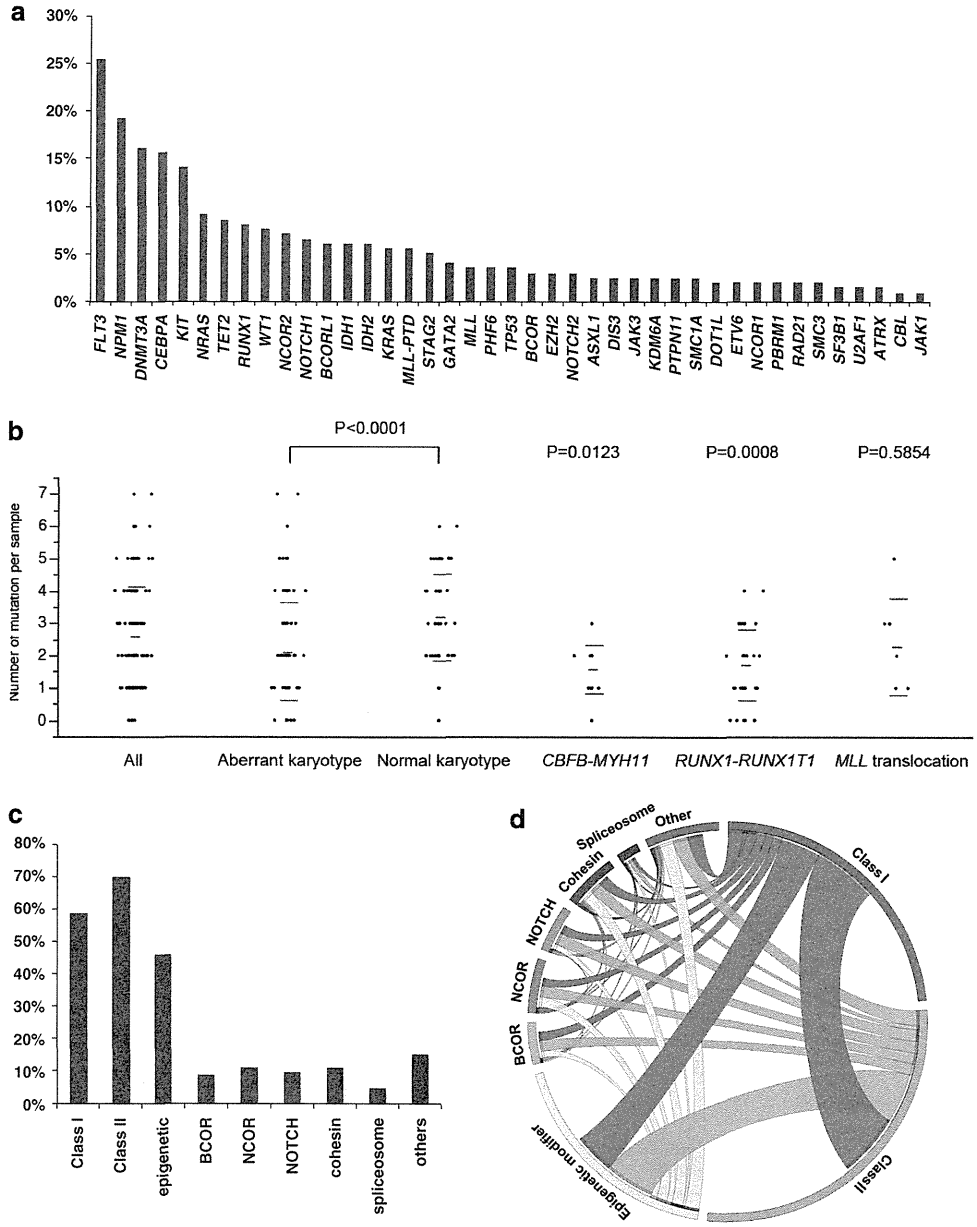


Figure 1. Frequencies and associations of mutated genes. (**a**) Frequencies of analyzed gene mutations. Frequency of each mutated gene is shown. Mutations were identified in 44 genes of 51 genes analyzed in 197 AML patients. Only five genes (*FLT3, NPM1, CEBPA, DNMT3A* and *KIT*) were mutated in more than 10% of the patients. (**b**) Mutated gene numbers according to the cytogenetics. Mean mutation number ± s.d. is indicated by horizontal lines. Mean mutation number per one patient in patients with normal karyotype (3.18 ± 0.16) was significantly higher than in that with aberrant karyotype (2.10 ± 0.15) ($P<0.0001$). Those in AML with *RUNX1-RUNX1T1* (1.68 ± 0.17) and *CBFB-MYH11* (1.57 ± 0.20) were significantly lower than that in all samples ($P=0.0008$ and $P=0.0123$, respectively). That in AML with *MLL*-translocation tended to be lower but not statistically significant (2.28 ± 0.57, $P=0.5854$). (**c**) Frequencies of mutations according to the conceptual classification. Mutations in Class I, Class II and epigenetic modifying genes were frequently identified. (**d**) Association of mutated genes according to the conceptual classification. Circos plot of mutated genes according to the function is shown.[45] Overlap mutations between Class I, Class II and epigenetic modifying genes mutations were frequently observed. These major mutations were widely coexistent with another family genes, such as cohesin complex, BCOR family and spliceosome genes.

achieving CR, and *TP53* mutation was an unfavorable factor; however, multivariate logistic regression analysis including all analyzed mutations showed that only *NPM1* (Hazard ratio (HR): 96.206, 95% Confidence interval (CI): 2.247–411.9, $P=0.0172$) and *TP53* (HR: 22.222, 95% CI: 1.597–333.3, $P=0.0172$) mutations were identified as favorable and unfavorable factors for achieving CR, respectively (Table 2 and Supplementary Table 5).

Importantly, *KIT* mutations were closely associated with *RUNX1-RUNX1T1* or *CBFB-MYH11*, whereas the other mutations that confer the achievement of CR were mutually exclusive (Supplementary Figures 3 and 6). In the patients with *RUNX1-RUNX1T1* or *CBFB-MYH11*, *NPM1* and *CEBPA*-D mutations, the CR rate (106/112; 94.6%) was significantly higher than for those with the other genotypes (55/85; 64.7%) ($P<0.0001$).

Prognostic impacts of mutations

We next analyzed the prognostic impact of each mutation. By univariate analysis, *FLT3*-ITD (HR: 1.805, 95% CI: 1.130–2.885, $P=0.0135$), *DNMT3A* (HR: 1.696, 95% CI: 1.055–2.725, $P=0.0291$),

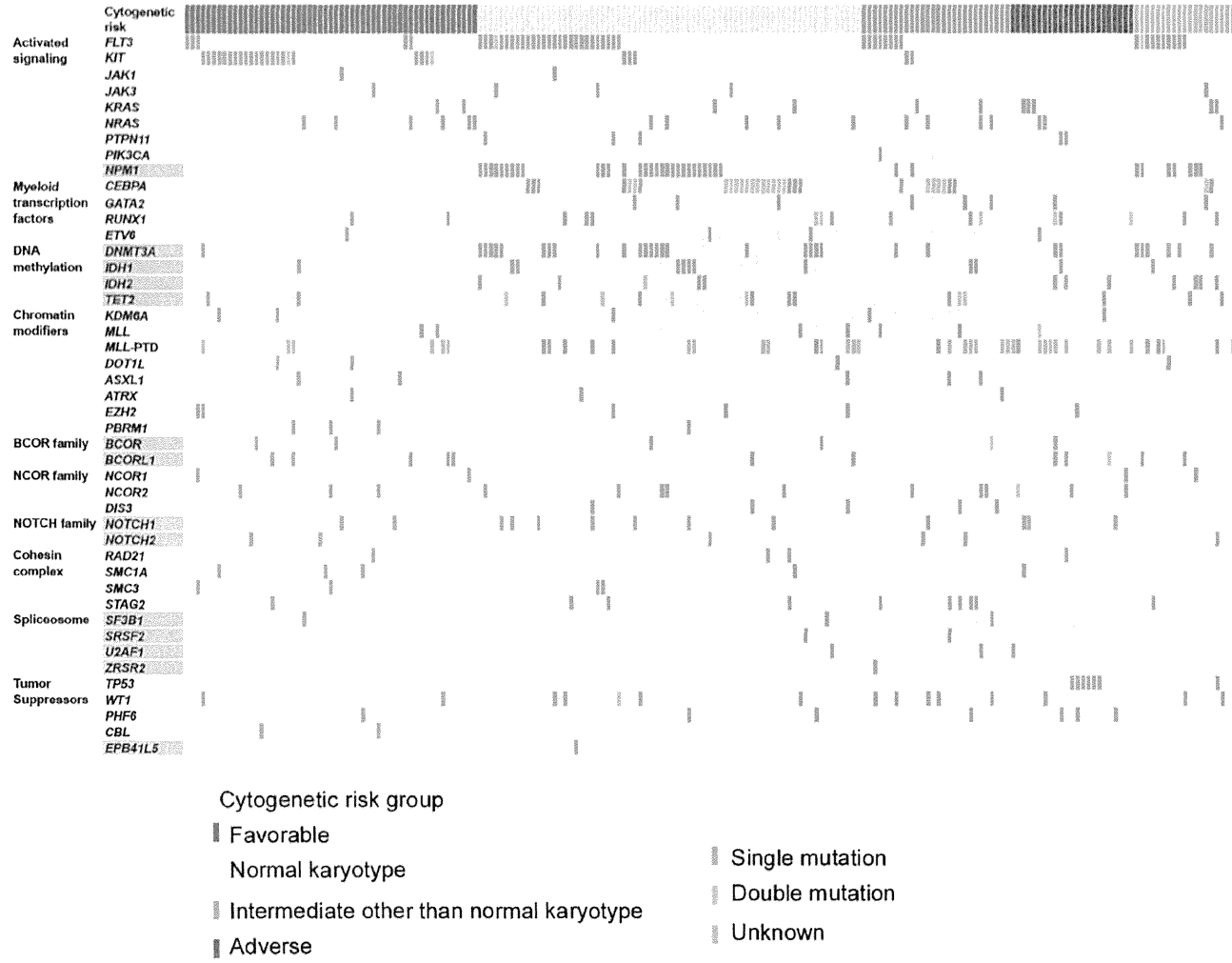


Figure 2. Mutation status according to the cytogetics-risk groups. Identified mutations in analyzed AML patients are shown according to the cytogenetic-risk groups. Pink boxes indicate single mutations and orange boxes indicate double mutations.

Table 2. Gene mutations affecting the CR achievement

Mutations	*CR rate (%)*		P-*value*
	Positive	*Negative*	
Fisher's exact test			
NPM1	97	78	0.0041
CEBPA-D-Mt.	100	80	0.0273
KIT	96	79	0.0326
RUNX1-RUNX1T1 or *CBFB-MYH11*	91	78	0.0409
TP53	14	84	0.0002

Mutations	*HR (95% CI)*	P-*value*
Multivariate analysis		
Wild-*NPM1*	96.206 (2.247–411.9)	<0.0001
TP53 mutation	22.222 (1.597–333.3)	0.0172

Abbreviations: CI, confidence interval; CR, complete remission; HR, hazard ratio. By the Fisher's exact test, *RUNX1-RUNX1T1* or *CBFB-MYH11*, *KIT*, *NPM1* and *CEBPA*-D mutations were identified as the favorable factor for achieving CR, and *TP53* mutation was for the unfavorable factor. The multivariate logistic regression analysis including all analyzed mutations showed that only wild-*NPM1* and *TP53* mutation were identified as unfavorable factors for achieving CR.

TP53 (HR: 15.167, 95% CI: 6.555–35.094, $P<0.001$), *MLL*-PTD (HR: 3.782, 95% CI: 1.948–7.346, $P<0.001$) and *RUNX1* (HR: 2.301, 95% CI: 1.278–4.146, $P=0.0055$) mutations and the karyotypes other than *RUNX1-RUNX1T1* or *CBFB-MYH11* (HR: 2.786, 95% CI: 1.608–4.831, $P=0.0003$) were identified as unfavorable prognostic factors for OS (Table 3 and Supplementary Figure 7). Multivariate Cox regression analysis with stepwise selection showed that *TP53* (HR: 14.803, 95% CI: 6.259–35.009, $P<0.001$), *MLL*-PTD (HR: 2.853, 95% CI: 1.401–5.810, $P=0.0039$) and *RUNX1* (HR: 1.965, 95% CI: 1.054–3.663, $P=0.0336$) mutations and the karyotypes other than *RUNX1-RUNX1T1* or *CBFB-MYH11* (HR: 2.353, 95% CI: 1.342–4.132, $P=0.0028$) were independent poor prognostic factors for OS (Table 3).

In this cohort, mutations of NOTCH family, the cohesin complex, BCOR family and spliceosome genes were frequently identified. NOTCH family and BCOR family genes were not associated with the CR rate, OS and DFS. Although mutations of cohesin complex genes were not associated with the CR rate and DFS, the patients harboring those mutations revealed better OS than those without mutations ($P=0.0274$) (Figure 3). The CR rate and DFS of patients with spliceosome gene mutations tended to be lower than for those without mutations, although both differences were not statistically significant: the CR was achieved in five of the nine (55.6%) and 156 of the 188 (83.0%) patients ($P=0.0601$), and 3-year DFS were 0% and 38.9% ($P=0.1117$) in those with and

without mutations, respectively (Supplementary Table 3 and Supplementary Figure 8).

When the patients were stratified into the risk groups recommended by ELN, that is, FR, IR-I, IR-II and AR groups included 92, 35, 42 and 28 patients, respectively. The ELN system well stratified the long-term prognosis of adult AML patients, whereas the OSs of IR-I and AR groups were the same in the present cohort (Supplementary Figure 9). Therefore, we analyzed whether another mutations could further stratify the prognosis in each ELN-risk group. *MLL*-PTD, *GATA2* and *TP53* mutations were identified as further poor prognostic factors in IR-I, IR-II and AR groups, respectively. Furthermore, we identified that the *DNMT3A* mutation was a poor prognostic factor in the FR group except for the AML with t(8;21) (q22;q22); *RUNX1-RUNX1T1* or inv(16) (p13q22); *CBFB-MYH11* (CBF-AML) (Figure 4).

Table 3. Unfavorable prognostic factors for overall survival (OS)

Mutations	*HR (95% CI)*	*P-value*
Univariate analysis		
TP53	15.167 (6.555–35.094)	<0.0001
MLL-PTD	3.782 (1948–7.346)	<0.0001
Non CBF	2.786 (1.608–4.831)	0.0003
RUNX1	2.301 (1.278–4.146)	0.0055
FLT3-ITD	1.805 (2.247–4119)	0.0135
DNMT3A	1.696 (1.055–2.725)	0.0291
Multivariate analysis		
TP53	14.803 (6.259–35.009)	<0.0001
MLL-PTD	2.853 (1.4017–5.810)	0.0039
Non CBF	2.353 (1.342–4.132)	0.0028
RUNX1	1.965 (1.054–3.663)	0.0336

Abbreviations: CI, confidence interval; HR, hazard ratio. By the univariate analysis, *FLT3*-ITD, *DNMT3A*, *TP53*, *MLL*-PTD and *RUNX1* mutations and the karyotypes other than *RUNX1-RUNX1T1* or *CBFB-MYH11* were identified as adverse prognostic factors for OS. Multivariate Cox regression analysis with stepwise selection showed that *TP53*, *MLL*-PTD and *RUNX1* mutations and the karyotypes other than *RUNX1-RUNX1T1* or *CBFB-MYH11* were independent poor prognostic factors for OS.

In addition, we also evaluated two recently reported risk stratification systems on the basis of genetic status in our cohort.[5,39] Patel *et al.*[5] reported a risk stratification system on the basis of cytogenetics and genetic status According to their system, our patients were clearly stratified into three risk groups, although the patients in the intermediate cytogenetic risk with a favorable mutational risk profile and those in the favorable cytogenetic-risk profile showed the same probability of OS (Supplementary Figure 10a). Grossmann *et al.*[39] reported a prognostic model solely on the basis of molecular mutations. Although our cohort did not include AML with *PML-RARA*, our patients were clearly stratified into four risk groups. However, the patients in the very favorable group and those in the favorable group showed the same probability of OS (Supplementary Figure 10b).

On the other hand, *KIT* mutations were frequently identified in CBF-AML, while they were not a poor prognostic factor for either OS or DFS (Supplementary Figure 11a). According to the types of *KIT* mutations, CBF-AML patients harboring mutations in exon 17 of the *KIT* gene showed worse prognosis than those harboring the other types of *KIT* mutation, although this was not statistically significant (Supplementary Figure 11b). Notably, in the CBF-AML patients harboring *KIT* mutations, OS and DFS of those treated with three courses of HiDAC consolidation therapy tended to be better than those treated with four courses of conventional standard-dose multiagent therapy (Supplementary Figure 11c).

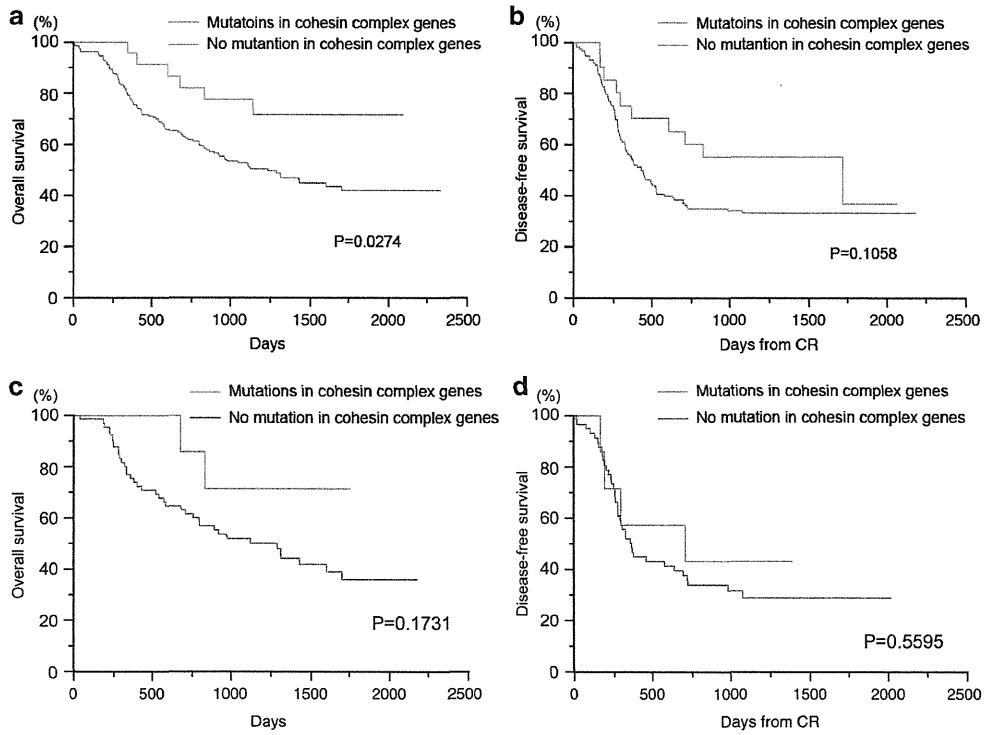


Figure 3. Prognostic impact of mutations in cohesin complex genes. Kaplan–Meier curves for OS and DFS according to the mutations in cohesin complex genes are shown. (**a**) OS in the total patients, (**b**) DFS in the total patient, (**c**) OS in the patients with normal karyotype, (**d**) DFS in the patients with normal karyotype. Although mutations of cohesin complex genes were not associated with the CR rate and DFS, the patients harboring those mutations revealed better OS than those without mutations ($P = 0.0274$). In the patients with normal karyotype, OS of the mutated patients tended to be better that that of unmutated patients, though statistical significance was not observed ($P = 0.1731$).

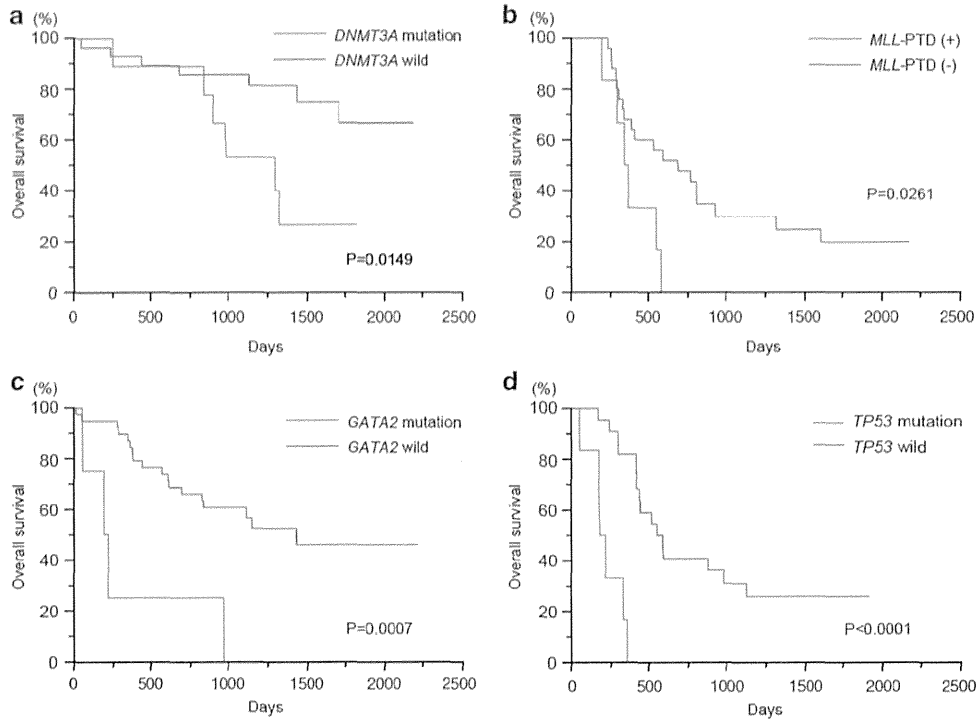


Figure 4. Mutations that could further stratify the ELN-risk groups into two risk groups. (**a**) *DNMT3A* mutation was a poor prognostic factor in the FR group except for the AML with t(8;21) (q22;q22); *RUNX1-RUNX1T1* or inv(16) (p13q22); *CBFB-MYH11* (CBF-AML). (**b**) *MLL*-PTD was a poor prognostic factor for the OS in the ELN IR-I group. (**c**) *GATA2* mutation was a poor prognostic factor for the OS in the ELN IR-II group. (**d**) *TP53* mutation was a poor prognostic factor for the OS in the ELN AR group.

In CBF-AML, the accumulated mutation number was lower than in the other types of AML, although many kinds of mutation were identified (Supplementary Figure 12). However, we could not identify a gene that affects the prognosis of CBF-AML.

Taking these results together, we tried to modify the genetic criteria for the ELN stratification system. When the CN-AML patients with *DNMT3A* mutations of the FR group and the patients with *MLL*-PTD of the IR-I group were included in the IR-I and the AR group, respectively, we could more clearly stratified the patients into four risk groups for OS than the original ELN system (Figures 5a and b). Furthermore, as the prognosis of the patients with *TP53* mutations were very unfavorable as previously reported,[39] we could more clearly stratify the patients into five risk groups for OS by classifying the *TP53*-mutated patients as the very adverse-risk group (Figures 5c and d).

DISCUSSION

In this study, we comprehensively analyzed mutations of 51 genes by the targeting sequence, and identified a total of 505 mutations in 44 genes in 197 adult *de novo* AML patients except for APL. The whole-genome and -exome analysis demonstrated recurrent mutations in a total of 260 genes in 200 AML patients, suggesting that another mutations might be accumulated in the presently analyzed AML cells.[40] However, frequencies of most mutated genes were reportedly less than 10%. In consistence, only five genes (*FLT3, NPM1, CEBPA, DNMT3A* and *KIT*) were mutated in more than 10% patients in our study, and each mutation frequency was almost the same as previous reports.[5,17,19,39] The frequency of *KIT* mutation was relatively higher in our study than previous reports,[5,40] while this is caused by the higher frequency of CBF-AML (28.0%) in the Japanese patients, in which *KIT* mutations are frequently identified. Our study, therefore, essentially includes major genetic regions, which may affect the pathogenesis and prognosis of AML. However, mutation analyses were not thoroughly performed in all subtypes of AML, such as acute erythroid leukemia and acute megakaryoblastic leukemia because of their lower frequencies. Further analyses are required to fully clarify the genetic alterations in AML.

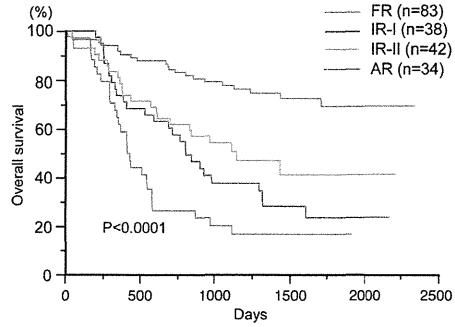
The whole-genome and -exome analysis demonstrated that an average of mutated genes in coding regions per sample was 5.24.[40] Of note was that there were significant differences in mutated gene numbers among the types of cytogenetics and mutations: the mean mutation numbers were higher in AML with *RUNX1-RUX1T1*, and are lower in that with *PML-RARA* and *MLL* translocations than that of all samples. As analyzed gene numbers were limited, mean mutated gene number per sample (2.56 ± 0.11) was low in our study; however, there were different features from the previous report. In our study, higher mutation number was observed in CN-AML (3.18 ± 0.16), and lower was in CBF-AML. Furthermore, the mutation number in AML with *MLL*-translocation tended to be lower (2.28 ± 0.57, $P = 0.5854$). These results collectively suggested that recurrent cytogenetic abnormalities, such as *RUNX1-RUNX1T1, CBFB-MYH11* and *MLL*-translocation, have a strong potential for the initiation of AML, and that most of accumulated mutations in AML with these cytogenetics might be passenger mutations.

It has been reported that common mutations in AML, such as *DNMT3A, NPM1, CEBPA, IDH1/2* and *RUNX1*, were mutually exclusive of the transcription-factor fusions, indicating the high potential for leukemia initiation.[10,11,13,40,41] Consistently, *DNMT3A, NPM1* and *CEBPA* mutations were not identified in CBF-AML, but frequent in CN-AML. In addition, we identified that *MLL*-PTD mutation was also exclusive of CBF-AML. In CBF-AML, *KIT* mutations were preferentially identified, whereas several types of mutations were also accumulated, suggesting that many mutations could act as a driver mutation for the clonal expansion of the initiating clone with *RUNX1-RUNX1T1* and

a

Genetic group	Subsets
Favorable	• CBF-AMLs • CN-AMLs with mutated *NPM1* without *FLT3*-ITD and mutated *DNMT3A* (normal karyotype) • CN-AMLs with mutated *CEBPA* without mutated *DNMT3A* (normal karyotype)
Intermediate-I	• CN-AMLs with mutated *DNMT3A* • CN-AMLs except for those included in the FR and AR
Intermediate-II	• AMLs included in the IR-II of the ELN stratification
Adverse	• CN-AMLs with *MLL*-PTD • AMLs included in the AR of the ELN stratification

b



c

Genetic group	Subsets
Favorable	• CBF-AMLs • CN-AMLs with mutated *NPM1* without *FLT3*-ITD and mutated *DNMT3A* (normal karyotype) • CN-AMLs with mutated *CEBPA* without mutated *DNMT3A* (normal karyotype)
Intermediate-I	• CN-AMLs with mutated *DNMT3A* • CN-AMLs except for those included in the FR and AR
Intermediate-II	• AMLs included in the IR-II of the ELN stratification except for those with mutated *TP53*
Adverse	• CN-AMLs with *MLL*-PTD • AMLs included in the AR of the ELN stratification except for those with mutated *TP53*
Very Adverse	• AMLs with mutated *TP53*

d

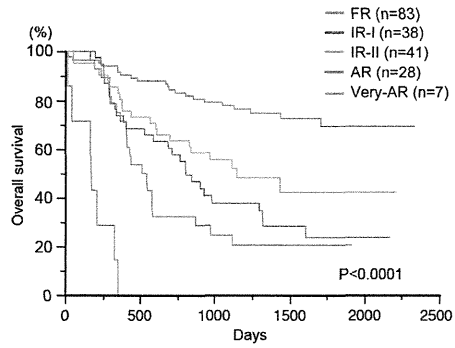


Figure 5. Risk stratification by modifying the ELN stratification system. When the CN-AML patients with *DNMT3A* mutations of the FR group and the patients with *MLL*-PTD of the IR-I group were included in the IR-I and the AR group, respectively (**a**), we could more clearly stratify the patients into four risk groups for OS than the original ELN system (**b**). When the patients with *TP53* mutations were classified as the very adverse-risk group (**c**), we could more clearly stratify the patients into five risk groups for OS (**d**).

CBFB-MYH11. Further study is required to clarify which combination is necessary for the clonal expansion, and whether different combinations cause clinical and phenotypical varieties.

After the completion of genetic alterations in AML, the most important issue is to clarify the prognostic impact of each mutation and/or co-occurring mutations.[7,42] The recently recommended ELN classification system is the first system that includes both cytogenetics and mutation status. Several groups reported that the ELN system clearly stratified the long-term prognosis of AML patients. However, the prognosis of FR groups except for the CBF-AML is still controversial. Paschka *et al.*[43] reported that the *IDH1/2* mutation was a poor prognostic factor in CN-AML with mutated *NPM1* without *FLT3*-ITD. In contrast, Patel *et al.*[5] reported that the *IDH1/2* mutation was a favorable prognostic factor in AML with mutated *NPM1* without *FLT3*-ITD. Furthermore, it has been reported that the *TET2* mutation was an adverse prognostic factor in AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD.[22] In the present cohort, we could not observe the statistically significant effects of *IDH1/2* and *TET2* mutations on the prognosis of CN-AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD, while we identified that *DNMT3A* mutation is an adverse prognostic factor in CN-AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD. In addition, we could not find the better prognostic impact of the *CEBPA* double mutations on the FR group recommended by the ELN in contrast to previous reports.[39,44]

Although different mutations might further stratify the prognosis of AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD, it was noteworthy that all mutations belonged to the class modifying methylation status.[13,38] These results collectively suggested that the epigenetic deregulation might contribute the pathogenesis of AML with mutated *NPM1* or *CEBPA* without *FLT3*-ITD. Prospective and large-scale study is necessary to clarify what genetic alterations influence the prognosis of AML with these genotypes.

In this study, we demonstrated that the prognosis of adult AML patients could be more clearly stratified by including the *DNMT3A* and *MLL*-PTD mutation status than the original ELN system, and that *TP53* mutations have a very adverse effect on the prognosis of AML patients. However, as most recurrently identified mutations were observed less than 5% of AML, it is highly expected to refine the genetic-based risk stratification system by much larger-scale studies. In addition, it is also important to evaluate the prognostic effects according to the functions of mutated genes rather than each sole mutation.

In the JALSG AML201 study, patients were randomized to the standard dose of IDR + Ara-C or HiDNR + Ara-C induction therapy, and the CR patients were again randomized to three courses of HiDAC or four courses of conventional standard-dose multiagent consolidation therapy. Therefore, we analyzed whether therapeutic regimens affect the CR rate and long-term survivals according to the mutation status and risk groups on the basis of the genetic status, while we could not observe any significant differences between therapeutic regimens and genetic status. Furthermore, we could not demonstrate that allo-SCT could improve the prognosis of the patients falling in the intermediate- and adverse-risk groups because of the small number of patients who underwent allo-SCT in the first CR in this cohort. It is, therefore, required to evaluate whether therapeutic regimens and allo-SCT affect the prognosis according to the genetic status.

In conclusion, we comprehensively analyzed 51 genes mutations in 197 *de novo* adult AML patients who were registered to a single prospective clinical study, and demonstrated that cooperative and exclusive mutation patterns and their prognostic impacts. Furthermore, we demonstrated that the prognosis of adult AML patients could be more clearly stratified by including the *DNMT3A, MLL*-PTD and *TP53* mutation status than the original ELN system. However, prognostic impacts of some mutation status are different from the previous reports. We must refine the risk

stratification system by considering all known-risk factors in a large-scale and well-established cohort, although molecular genetic status has a strong impact on the prognosis of AML patients. We are now conducting a prospective large-scale study to confirm the present results.

CONFLICT OF INTEREST

H Kiyoi: Research funding from Bristol-Myers Squibb, Novartis Pharma, Chugai Pharmaceutical Co., Ltd. and Kyowa Hakko Kirin Co., Ltd. YM: Honoraria from Bristol-Myers Squibb, Novartis Pharma, Chugai Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd. and Celgene Japan; Research funding from Bristol-Myers Squibb, Novartis Pharma, Chugai Pharmaceutical Co., Ltd. and Kyowa Hakko Kirin Co., Ltd. NU: Consultant for Kyowa Hakko Kirin Co., Ltd.; Honoraria from Bristol-Myers Squibb, Novartis Pharma and Chugai Pharmaceutical Co., Ltd.; Research funding from Bristol-Myers Squibb, Novartis Pharma, Chugai Pharmaceutical Co., Ltd. and Kyowa Hakko Kirin Co., Ltd. TN: Research funding from Bristol-Myers Squibb, Novartis Pharma, Chugai Pharmaceutical Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Dainippon Sumitomo Pharma and Zenyaku Kogyo. The other authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

H Kiyoi, S Ogawa and TN designed the study, interpreted the data and wrote the manuscript; RK, YN, T Kato, EY, KS and FC performed molecular analysis and interpreted the data; YN, YS, KC, HT, SM and S Ogawa performed bioinformatics; NA, S Ohtake, SM, YM, TS, YO, N Usui, H Kanamori, T Kiguchi, KI, N Uike, FK, KK, CN, MO, AT, FI, HS, YK and HM collected samples and clinical data, contributed to the interpretation of the data and critically reviewed the draft; and all authors approved the final version submitted for the publication.

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Regular Article

CLINICAL TRIALS AND OBSERVATIONS

Pretransplant administration of imatinib for allo-HSCT in patients with *BCR-ABL*–positive acute lymphoblastic leukemia

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Key Points

- Pretransplant imatinib improved both relapse and nonrelapse mortality in patients with *BCR-ABL*–positive acute lymphoblastic leukemia.

We aimed to evaluate the impact of pretransplant imatinib administration on the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in adults with Philadelphia chromosome-positive (Ph^+) acute lymphoblastic leukemia (ALL). We retrospectively analyzed 738 patients with Ph^+ ALL that underwent allo-HSCT between 1990 and 2010 using data from the Transplant Registry Unified Management Program of the Japan Society of Hematopoietic Cell Transplantation. We compared the allo-HSCT outcomes between 542 patients who received imatinib before allo-HSCT during the initial complete remission period (imatinib cohort) and 196 patients who did not receive imatinib (non-imatinib cohort). The 5-year overall survival after allo-HSCT was significantly higher in the imatinib cohort than in the non-imatinib cohort (59% vs 38%; 95% confidence interval [CI], 31-45%; $P < .001$). Multivariate analysis indicated that pretransplant imatinib administration had beneficial effects on overall survival (hazard ratio [HR], 0.57; 95% CI, 0.42-0.77; $P < .001$), relapse (HR, 0.66; 95% CI, 0.43-0.99; $P = .048$), and nonrelapse mortality (HR, 0.55; 95% CI, 0.37-0.83; $P = .005$). In conclusion, our study showed that imatinib administration before allo-HSCT had advantageous effects on the clinical outcomes of allo-HSCT in patients with Ph^+ ALL. (*Blood.* 2014;123(15):2325-2332)

Introduction

The treatment of Philadelphia chromosome–positive (Ph^+) acute lymphoblastic leukemia (ALL) has changed dramatically since the introduction of imatinib. Most imatinib-treated patients achieve complete remission (CR), and hematopoietic stem cell transplantation (HSCT) can be performed in a substantial proportion of patients who have achieved major or complete molecular remission.[1-4] Several studies have shown improvements in overall survival (OS) since the incorporation of imatinib-based therapy.[5-9] However, the possible benefits of imatinib administration before HSCT have not been extensively examined. In Japan, imatinib was initially used to treat Ph^+ ALL in the Japan Adult Leukemia Study Group (JALSG) ALL202 study, which began in February 2002, and has been widely used since 2005.[4] A comparison of the clinical outcomes of the 60 patients enrolled in the JALSG ALL202 study with those of patients from the pre-imatinib era strongly suggested that Ph^+ ALL patients who received imatinib before allogeneic HSCT (allo-HSCT) during the initial CR period had significantly improved OS compared with those who did not receive imatinib.[10] In the present study, we used data from the Transplant Registry Unified Management Program of the Japan Society of Hematopoietic Cell Transplantation (JSHCT) to perform a large retrospective analysis of the clinical impact of imatinib administration before allo-HSCT.[11,12]

Methods

Data source and patient selection criteria

For this retrospective observational study, patient data were provided by the JSHCT, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network.[11] In the Transplant Registry Unified Management Program, patient survival, disease status, and long-term complications, including chronic

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Table 1. Characteristic of 738 patients with Ph$^+$ ALL who received allo-SCT

Characteristic	Non-imatinib cohort (n = 196)	Imatinib cohort (n = 542)	*P*
Age at SCT, years (%)			
≤29	48 (24)	99 (18)	
30-54	140 (71)	365 (67)	
≥55	8 (4)	78 (14)	
Median	39	42	<.001
Gender (male/female)	109/87	293/249	.708
Donor status (%)			
Related	121 (62)	178 (33)	
Unrelated	75 (38)	364 (67)	<.001
HLA disparity (%)			
Matched	139 (71)	330 (61)	
Mismatched	56 (29)	211 (39)	
Unknown	1 (0)	1 (0)	.010
Stem cell source (%)			
Bone marrow	151 (77)	345 (64)	
Peripheral blood	36 (18)	86 (16)	
Cord blood	9 (5)	111 (20)	<.001
PS at SCT (%)			
0	70 (36)	311 (57)	
1-4	45 (23)	218 (40)	
Unknown	81 (41)	13 (2)	.632
Days from diagnosis to SCT (%)			
<180	94 (48)	286 (53)	
≤180	98 (50)	255 (47)	
Unknown	4 (2)	1 (0)	.352
BCR-ABL subtype (%)			
Major	24 (12)	70 (13)	
Minor	69 (35)	352 (65)	
Major and minor	1 (0)	18 (3)	
Unknown	102 (52)	102 (19)	.039
Donor recipient gender match (%)			
Male-male	43 (22)	180 (33)	
Male-female	38 (19)	129 (24)	
Female-male	35 (18)	97 (18)	
Female-female	33 (17)	111 (20)	
Unknown	47 (24)	25 (5)	.463
Conditioning regimen (%)			
Reduced intensity	1 (1)	44 (8)	
Myeloabrative	121 (62)	479 (88)	
Unknown	74 (38)	19 (4)	<.001
WBC at diagnosis (%)			
<30 000/μL	109 (56)	288 (53)	
≤30 000/μL	74 (38)	247 (46)	
Unknown	13 (7)	7 (1)	.178
GVHD prophylaxis (%)			
CyA/methotrexate	133 (68)	228 (42)	
Tacrorimus/methotrexate	46 (23)	266 (49)	
Other/unknown	17 (9)	48 (9)	<.001
Cytogenetics (%)			
t(9;22) only	180 (92)	461 (85)	
Other abnormality	16 (8)	81 (15)	.016
ABO blood type disparity (%)			
Match	65 (33)	266 (49)	
Minor	24 (12)	119 (22)	
Major	37 (19)	152 (28)	
Unknown	70 (36)	5 (1)	.747
Transplant year (%)			
1990-2005	183 (93)	139 (26)	
2006-2010	13 (7)	403 (74)	<.001
MRD status at SCT			
Positive	44 (22)	144 (27)	
Negative	23 (12)	256 (47)	<.001

CyA, cyclospoline.

graft-versus-host disease (GVHD) and secondary malignancies, are reviewed annually using follow-up forms.[12] Ph$^+$ ALL was diagnosed by the presence of the *Ph* chromosome using cytogenetics and/or fluorescence in situ hybridization analysis and the determination of *BCR-ABL* fusion transcript positivity via real-time quantitative polymerase chain reaction (PCR) analysis. Grafts from unrelated donors were exclusively bone marrow derived because peripheral blood stem cell donation from unrelated donors was not approved in Japan during the study period. The timing and procedure of allo-HSCT, including the conditioning regimens, GVHD prophylaxis, and *BCR-ABL* transcript level assessments, were determined at each institution. *BCR-ABL* transcript levels were not compensated with a correction factor. In most laboratories, *BCR-ABL* mRNA copy numbers were normalized relative to glyceraldehyde-3-phosphate dehydrogenase mRNA copy numbers and expressed as copies per microgram of RNA. The quantification threshold was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}; nondetection of *BCR-ABL* or samples below this threshold was designated as "not detected" or "<50 copies/μg" (presented herein as PCR negative). Minimum residual disease (MRD) was evaluated using real-time quantitative PCR within a 30-day period before transplantation. Therapeutic decisions regarding tyrosine kinase inhibitor (TKI) administration after allo-HSCT were made at each institution. This study was approved by the data management committees of the JSHCT, the Japan Marrow Donor Program, and the Japan Cord Blood Bank Network and by the Institutional Review Board of the Fujita Health University. This study was conducted in accordance with the Declaration of Helsinki.

Patient selection

To attain an adequate level of comparability in terms of the allo-HSCT regimens, the following inclusion criteria were used: (1) presence of de novo Ph$^+$ ALL; (2) age of 16 to 59 years; (3) allo-HSCT during the first CR; and (4) initial HSCT between 1990 and 2010. Additional data on pretransplant imatinib administration and MRD at the time of allo-HSCT were also collected for this study. Of the 865 patients who fulfilled these criteria, information on pretransplant imatinib administration was available for 739 patients. One patient was excluded because of missing information on the date of relapse. Finally, 738 patients with Ph$^+$ ALL who underwent allo-HSCT during the initial CR were analyzed.

Statistical considerations

The primary end point of our study was OS after allo-HSCT. Secondary end points included the incidence of nonrelapse mortality (NRM) and relapse. The observation periods for OS were calculated from the date of transplantation until the date of the event or the last known date of follow-up. The OS probabilities were estimated according to the Kaplan-Meier product limit method. The cumulative relapse and NRM incidences were estimated while considering the competing risk, as described elsewhere.[13] For each estimate of the cumulative event incidence, death without an event was defined as a competing risk. Risk factors were evaluated using a combination of univariate and multivariate analyses. The following variables were evaluated: imatinib use before HSCT (yes vs no), age group in years (40-54 and 55-59 vs <40), donor and stem cell source (bone marrow from unrelated donor, peripheral blood from related donor or cord blood vs bone marrow from related donor), human leukocyte antigen (HLA) disparity (matched [HLA identical siblings or 6/6 allele-matched unrelated] vs mismatched), performance status (PS) at allo-HSCT (0 vs 1-4), time from diagnosis to allo-HSCT (<180 vs ≥180 days), BCR-ABL subtype (major vs minor vs major and minor), donor-recipient gender match (male-male vs male-female vs female-male vs female-female), conditioning regimen (decreased intensity vs myeloablative), white blood cell (WBC) count at diagnosis (<30 000/μL vs ≥30 000/μL), GVHD prophylaxis (CyA/methotrexate vs tacrolimus/methotrexate), cytogenetics [t(9;22) only vs more/other abnormalities], and ABO blood type compatibility (match, minor mismatch, or major mismatch). Continuous CR was defined as the absence of any hematological recurrence. We defined the following dosages as decreased-intensity regimens: busulfan, <9 mg/kg; melphalan, ≤140 mg/m^2; and total body irradiation, <500 cGy (single or fractionated) or 500 to 800 cGy (fractionated).[14] Donor and recipient pairs were considered matched when the