Table 13 Case registration institutions

Institutions (in alphabetical order according to the Japanese syllabary)	
Iwate Medical University School of Medicine	NTT Kanto Medical Center
The Cancer Institute Hospital of JFCR	Kitasato University Hospital
Gunma Prefectural Cancer Center	Gunma University Hospital
Showa General Hospital	Saitama Red Cross Hospital
Jichi Medical University Hospital	Showa University Hospital
Showa University Fujigaoka Hospital	Tokai University Hospital
Tokyo Medical University Hospital	Tokyo Medical and Dental University Hospital Faculty of Medicine
Tokyo Women's Medical University Hospital	Tokyo Women's Medical University Medical Center East
Tokyo Metropolitan Fuchu Hospital	Toranomon Hospital
Nagaoka Red Cross Hospital	Niigata Cancer Center Hospital
Niigata University Medical and Dental Hospital	Japanese Red Cross Medical Center
University of Yamanashi Hospital	Yokohama City University Medical Center
Yokohama Rosai Hospital	

antibiotic administration prior to onset of pyrexia [23]. In fact, as many as 98 patients in our series had an indwelling catheter.

With regard to safety, adverse reactions (20 events) were reported in 13 patients (6.4%). Most of these were expected reactions and mild, such as liver-function-related events and rash and drug eruptions and did not pose any particular safety concern. Thus, BIPM appears to be promising as an initial-stage therapeutic agent in patients with FN complicating hematopoietic disease, as it was demonstrated to afford prompt clinical benefit in these patients.

Acknowledgments We are indebted to all doctors involved in this group study from the institutions listed in Table 13.

References

1. Hughes WT, Armstrong D, Bodey GP, Feld R, Mandell GL, Meyers JD, From the Infectious Diseases Society of America, et al. Guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. J Infect Dis. 1990;161:381–96.
2. Hughes WT, Armstrong D, Bodey GP, Brown AE, Edwards JE, Feld R, et al. 1997 guidelines for the use of antimicrobial agents in neutropenic patients with unexplained fever. Infectious Diseases Society of America. Clin Infect Dis. 1997;25:551–73.
3. Hughes WT, Armstrong D, Bodey GP, Bow EJ, Brown AE, Calandra T, et al. 2002 guidelines for the use of antimicrobial agents in neutropenic patients with cancer. Clin Infect Dis. 2002; 34:730–51.
4. Masaoka T. Conclusions and recommendations: evidence-based recommendations for antimicrobial use in febrile neutropenia in Japan. Int J Hematol. 1998;68:S39.
5. Hikida M, Kawashima K, Yoshida M, Mitsuhashi S. Inactivation of new carbapenem antibiotics by dehydropeptidase-1 from porcine and human renal cortex. J Antimicrob Chemother. 1992; 30:129–34.
6. Sakai H, Sanada M, Shimamoto K, Azuma R, Harada H, Mori H, et al. Clinical evaluation of biapenem for febrile neutropenia in patients with hematological disorders. Jpn J Antibiot. 2007; 60:125–31 (Japanese).
7. Kasahara S, Hara T, Tsurumi H, Goto N, Kanemura N, Yoshikawa T, et al. Clinical effects of biapenem on febrile neutropenia in patients with hematological malignancy. Jpn J Antibiot. 2008;61:115–21 (Japanese).
8. Sasada M. Anti-infection measures. Nippon Rinsho. 2007; 65:S445–9 (Japanese).
9. Yamaguchi K, Ishii Y, Iwata M, Watanabe N, Uehara N, Yasujima M, et al. Nationwide surveillance of parenteral antibiotics containing meropenem activities against clinically isolated strains in 2006. Jpn J Antibiot. 2007;60:344–77 (Japanese).
10. Matsumoto F, Inoue M, Sakurai I, Ishida Y, Osonoi T, Itoh H, et al. A comparative study of biapenem and imipenem/cilastatin in lower respiratory infections. Jpn J Chemother. 2000;48:45–67 (Japanese).
11. Shibata H, Yamane T, Sakamoto E, Nakamae H, Ohta K, Hino M. Clinical analysis of antibiotic treatment for febrile neutropenia. Jpn J Antibiot. 2005;58:382–7 (Japanese).
12. Urabe A, Mutoh Y, Mizoguchi H, Toyama K, Oshimi K, Suzuki K, et al. Efficacy and safety of panipenem/betamipron (PAPM/BP) in the treatment of infections accompanying hematologic diseases. Antibiot Chemother. 1998;14:119–26 (Japanese).
13. Sawae Y, Niho Y, Okamura T, Gondo H, Suehiro Y, Kumakawa M, et al. Comparison between monotherapy with imipenem/cilastatin sodium (IPM/CS) and combinations of IPM/CS and other drugs for treating bacterial infections in patients with hematopoietic disorders. Jpn J Antibiot. 1996;49:1049–61 (Japanese).
14. Bodey GP, Rodriguez V, Chang HY, Narboni G. Fever and infection in leukemic patients: a study of 494 consecutive patients. Cancer. 1978;41:1610–22.
15. Craig WA. Interrelationship between pharmacokinetic and pharmacodynamic in determining dosage regimens for broad-spectrum cephalosporins. Diagn Microbiol Infect Dis. 1995;22:89–96.
16. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin Infect Dis. 1998;26:1–12.
17. Craig WA. The role of pharmacodynamics in effective treatment of community-acquired pathogens. Adv Stud Med. 2002; 2:126–34.

18. Drusano GL. Prevention of resistance: a goal for dose selection for antimicrobial agents. Clin Infect Dis. 2003;36:S42–50.
19. Takata T, Aizawa K, Shimizu A, Sakakibara S, Watabe H, Totsuka K. Optimization of dose and dose regimen of biapenem based on pharmacokinetic and pharmacodynamic analysis. J Infect Chemother. 2004;10:76–85.
20. Tamura K, Matsuoka H, Tsukada J, Masuda M, Ikeda S, Matsuishi E, et al. Cefepime or carbapenem treatment for febrile neutropenia as a single agent is as effective as a combination of 4th-generation cephalosporin + aminoglycosides: comparative study. Am J Hematol. 2002;71:248–55.
21. Tamura K, Imajo K, Akiyama N, Suzuki K, Urabe A, Ohyashiki K, et al. Randomized trial of cefepime monotherapy or cefepime in combination with amikacin as empirical therapy for febrile neutropenia. Clin Infect Dis. 2004;39:S15–24.
22. Klastersky J. Treatment of neutropenic infection: trends towards monotherapy? Support Care Cancer. 1997;5:365–70.
23. Ramphal R. Changes in the etiology of bacteremia in febrile neutropenic patients and the susceptibilities of the currently isolated pathogens. Clin Infect Dis. 2004;39:S25–31.

A Markov decision analysis of allogeneic hematopoietic cell transplantation versus chemotherapy in patients with acute myeloid leukemia in first remission

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Various prospective trials have been performed to assess the roles of allogeneic hematopoietic cell transplantation (allo-HCT) and chemotherapy in patients with acute myeloid leukemia (AML) in first complete remission (CR1). However, the results have not always been consistent, and there has been a limited evaluation of quality of life (QOL) in these postremission strategies. We performed a Markov decision analysis that enabled us to compare survival outcomes with a QOL evaluation using a database of 2029 adult AML patients who achieved CR1. The Markov decision model compared 2 strategies: allo-HCT or chemotherapy in CR1. Patients who had intermediate- or unfavorable-risk AML had a longer life expectancy when they received allo-HCT in CR1 than patients treated with chemotherapy alone. Likewise, patients who had a suitable related donor who received allo-HCT in CR1 had a longer life expectancy. The life expectancy was shortened to a greater degree by adjustment for QOL in the allo-HCT group. Nevertheless, QOL-adjusted life expectancies in most of the subgroups remained longer in the allo-HCT group than in the chemotherapy group. Our results showed that older patients with a related donor and younger patients with unfavorable cytogenetics benefited the most from allo-HCT in CR1. (*Blood*. 2011;117(7):2113-2120)

Introduction

Although 60%-80% of patients with acute myeloid leukemia (AML) achieve first hematologic complete remission (CR1) with chemotherapy, a substantial number of patients have an individualized risk of relapse.[1] Allogeneic hematopoietic cell transplantation (allo-HCT) has been established as a powerful treatment method to reduce the risk of relapse in patients with AML. However, this approach still leaves concerns associated with a certain probability of nonrelapse mortality. Although several prospective trials that used genetic allocation have been performed to clarify the roles of postremission strategies, the results have not always been consistent.[2-9] The role of allo-HCT in patients with AML in certain subgroups, including patients with intermediate-risk AML and elderly patients who have remained in CR1, remains unclear. A large meta-analysis that considered many of these prospective studies reported that allo-HCT in CR1 provided survival advantages not only in an unfavorable-risk group but also in an intermediate-risk group.[10] Even with these numerous studies performed in a prospective setting, it is still controversial to simply define allo-HCT as a better decision because of concerns about various late effects such as graft-versus-host disease (GVHD) that might lower the quality of life (QOL) after cure of the disease.

A decision analysis is a statistical technique that is used to help decision making under uncertain conditions with the assumption of a QOL evaluation.[11] When it is combined with a Markov process, it gives a flexible analytical method that makes it possible to track clinical events that occur after a certain decision with different probabilities and desirability over time.[12] This technique can offer valuable information about what clinical decision should be taken by quantitatively integrating the risks and benefits of a certain decision, and, hence, has been widely applied in making decisions in various fields. For example, in the field of hematology, on the basis of the results of a Markov decision analysis, Lee et al[13] reported the indications of allo-HCT for chronic myeloid leukemia in the era before imatinib, and Cutler et al[14] elucidated the recommended timing of allo-HCT for younger patients with myelodysplastic syndrome. Regarding AML, Sung et al[15] reported the results of a decision analysis with a conventional decision tree concerning consolidation strategies for patients in CR1. However, a Markov decision analysis has not yet been reported for postremission strategies in AML in CR1. To address this point, we performed a Markov decision analysis with the use of clinical information collected from 2029 patients.

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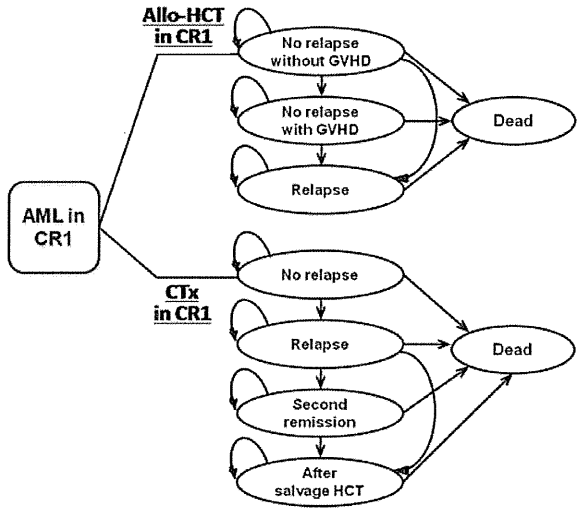

Figure 1. Markov decision model. Markov model that compares allo-HCT in CR1 and chemotherapy in CR1 is shown. Possible health states for each of the 2 groups are indicated in circles. Arrows indicate possible transitions between states. CR1 indicates first complete remission; allo-HCT, allogeneic hematopoietic cell transplantation; CTx, chemotherapy; and GVHD, graft-versus-host disease.

Methods

Data source

The study protocol was approved by the Institutional Review Board at National Cancer Center Hospital. We constructed a new database that included the clinical data of adult patients (age 16-70 years) whose conditions were diagnosed as AML by the World Health Organization classification between 1999 and 2006 and who had achieved CR1 after 1 or 2 courses of induction chemotherapy. Clinical information on >2600 patients was collected from 70 institutions across the country. Patients with biphenotypic leukemia who were treated with chemotherapy for acute lymphocytic leukemia; patients who had extramedullar AML without marrow invasion, an extramedullary lesion that did not totally disappear after remission induction chemotherapy, or acute promyelocytic leukemia; and patients who received autologous HCT in CR1 were excluded from the analysis. Consequently, a total of 2029 patients were considered for this analysis.

Decision strategy

The primary decision examined in this study was whether to perform allo-HCT in patients with AML who remained in CR1. Statistical analyses were performed as of January 2010 with the use of the software package TreeAge Pro 2009 (TreeAge Software Inc) and the SPSS software package (SPSS Inc).

Markov model. We constructed a Markov decision model to compare 2 strategies: performing allo-HCT in CR1 (HCT group) and continuing chemotherapy without allo-HCT in CR1 (CTx group; Figure 1). The possible health states that were considered to occur after each decision/strategy included, for the HCT group, (1) no relapse without GVHD, (2) no relapse with GVHD, (3) relapse, and (4) dead, and for the CTx group, (1) no relapse, (2) relapse, (3) second remission, (4) after salvage allo-HCT, and (5) dead. The "GVHD" state included chronic extensive GVHD. The "dead" state included death from any cause. A schematic of the tree file is shown in supplemental Figure 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article.

State transition probabilities. Transition probabilities between the states were calculated from the information in the database collected for this analysis as described in "Data source." The probabilities of state transition were allowed to vary over time. As a result, patients were distributed in various health states with different proportions along with cycle advances, that is, as time advanced from CR1, as shown in Figure 2. To take into account patients who were unable to receive allo-HCT in CR1 even though they had made a decision to receive allo-HCT, patients who died or relapsed within 3 months from CR1 were excluded from the database when we calculated the probabilities. The cycle length between state transitions has previously been set at the time considered to represent the clinical features and decision-making process for the target disease. In a Markov decision analysis that targeted myelodysplastic syndrome,[14] the cycle length was set at 6 months. In this analysis that targets patients with AML, we chose a shorter cycle length (3 months), and the analysis was performed for 40 cycles (10 years). The results are presented as life expectancy (LE), which is the average duration of life when patients are followed up for 10 years.

QOL utilities. We also assessed QOL-adjusted life expectancy (QALE) for the HCT and CTx groups. The time spent in each health state was adjusted for the estimated QOL that patients experienced while they remained in that state, which was represented by a utility value. In this study, utility values were derived from a questionnaire (supplemental Figure 2) that used a visual analog scale and was presented to 35 physicians who were familiar with the treatment of AML. Among them, 25 were physicians who were mainly involved in transplantation, and 10 were physicians mostly involved in chemotherapy with knowledge of transplantation. The utility values were expressed as numerical values between 0 (a

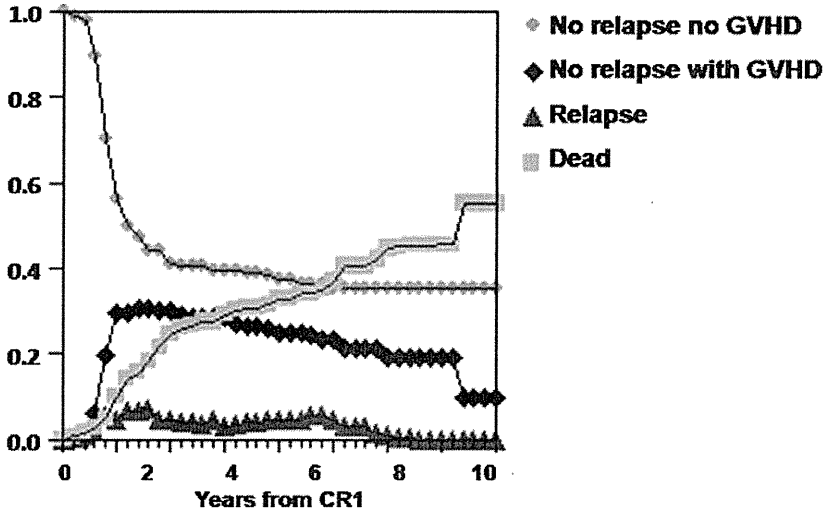

Figure 2. Distribution of patients in each health state. Distribution of patients with intermediate-risk AML in each health state is shown. Transition probabilities between the states were calculated for each subgroup with the use of the database. The probabilities of state transition were allowed to vary along with the cycle (1 cycle = 3 months) advances, depending on the states that the cohorts move from and to. As a result, the patients were distributed in each health state in changing proportions at different times from CR1. GVHD, indicates graft-versus-host disease; and CR1, first complete remission.

Table 1. Quality-of-life utilities

	Median	Range
Allo-HCT in CR1		
No relapse without GVHD	0.90	0.60-1.00
No relapse with GVHD	0.60	0.40-0.80
Relapse	0.30	0.20-0.70
Chemotherapy in CR1		
No relapse	0.90	0.80-1.00
Relapse	0.50	0.20-0.80
Second remission	0.80	0.40-0.95
After salvage allo-HCT	0.66	0.10-1.00

Allo-HCT indicates allogeneic hematopoietic cell transplantation; CR1, first complete remission; and GVHD, graft-versus-host disease.

health state equivalent to dead) and 1 (perfect health) (Table 1) and were used to adjust for QOL by being multiplied by the expected length of life for each state in each cycle. For long-term survivors who developed chronic extensive GVHD, the utility value was changed on the basis of the previously reported probability of the discontinuation of immunosuppressive treatment.[16,17]

Comparison of HCT with CTx in CR1 and sensitivity analyses. Both LE and QALE were analyzed for the HCT group and the CTx group. LE and QALE, which represent the average expected duration of life in 10-year follow-up from CR1, were obtained from the area under the survival curves depicted by TreeAge Pro software. An annual discount rate of 3% was used for all analyses. Subgroup analyses were performed on the basis of patient age, the Southwest Oncology Group (SWOG) cytogenetic classification,[2] and donor availability. We performed sensitivity analyses to test the robustness of our conclusions. Variable measures that were tested in the sensitivity analysis included the range of patients who were excluded from the database on the assumption that they were unable to receive the decided treatment, the plausible range of QOL utilities, 95% confidence intervals of the state transition probabilities, and the age range of subgroups.

Results

Patients

A total of 2029 patients were eligible for this analysis (Table 2). The median age was 50 years, and the median follow-up of the surviving patients was 49.8 months (range, 0.2-116.3 months). The proportions of patients with favorable, intermediate, unfavorable, and unknown cytogenetic risk according to the SWOG criteria were 19%, 52%, 18%, and 11%, respectively. Therapies performed at CR1 were allo-HCT in 494 patients (24%) and chemotherapy in 1535 patients (76%). The HCT group included all the 494 patients who received allo-HCT in CR1. The median interval from CR1 to allo-HCT was 4.7 months (range, 0-37 months). Among patients who were treated with chemotherapy in CR1, 118 patients who died or relapsed within 3 months were excluded when calculating state transition probabilities on the assumption that they might have decided to receive allo-HCT while they remained in CR1. As a consequence, 1417 patients, including 478 who received allo-HCT after their first relapse, were included in the CTx group (Figure 3). The patients in the HCT group were younger and were more often associated with unfavorable features compared with those in the CTx group. Table 3 and Figure 3 show donor availability and actual application of allo-HCT in CR1. Among 1076 patients for whom human leukocyte antigen (HLA) was typed in CR1, 431 had HLA-matched or 1-antigen (Ag)–mismatched related donors (40%). Donor group included the 431 patients who had a suitable related donor. Among them, 243 actually received allo-HCT in CR1 (related donor, 240; unrelated donor, 3). The no-donor group included the 645 patients who did not find a related donor and 953 for whom HLA was not typed in CR1. Among them, 251 received allo-HCT in CR1 from an alternative donor (unrelated bone marrow, 177; unrelated cord blood, 62; haploidentical related donor, 12). In both the donor and no-donor groups, subgroup analyses were separately performed by comparing patients who received allo-HCT in CR1 (HCT group) and patients who did not (CTx group). Overall survival curves obtained by a Kaplan-Meier estimation of all of the patients registered in our original database stratified according to the SWOG classification and the treatment chosen in CR1 are shown in supplemental Figure 3. Survival curves depicted by TreeAge Pro are shown in supplemental Figure 4.

Markov decision analysis

The discounted LE and QALE for the HCT and CTx groups were analyzed for patients of all ages, younger patients (16-49 years) and older patients (50-70 years; Table 4). In each age group, LE and QALE were analyzed in different cytogenetic subgroups and donor-availability subgroups.

Analysis of all patients. An analysis that included patients of all ages showed that LE in the HCT group was 3 months longer than that in the CTx group (69.7 vs 66.7 months; Table 4). After we adjusted for QOL, QALE in the HCT group was only 0.5 months longer than that in the CTx group (55.9 vs 55.4 months). The LE was generally shortened to a greater degree in the HCT group after adjustment for QOL. This trend was consistent throughout all of the subgroups.

We performed subset analyses according to cytogenetic risk stratified according to the SWOG criteria. Patients with favorable-risk AML in the CTx group had a longer LE than patients in the HCT group. In contrast, patients with intermediate, unfavorable, and unknown-risk AML in the HCT group had a longer LE than patients in the CTx group (intermediate, 73.6 vs 66.4 months; unfavorable, 61.6 vs 53.4 months). Although QALE was shortened to a greater degree in the HCT group, we found that QALE

Table 2. Patient characteristics

Characteristics	Allo-HCT in CR1	CTx in CR1	All patients	*P**
No. of patients	494	1535	2029	
Median age, y	42	53	50 (16-70)	< .001
Cytogenetic risks (SWOG)				< .001
Favorable, n (%)	29 (6)	360 (23)	389 (19)	
Intermediate, n (%)	272 (55)	777 (51)	1049 (52)	
Unfavorable, n (%)	115 (23)	246 (16)	361 (18)	
Unknown, n (%)	78 (16)	152 (10)	230 (11)	
FAB				< .001
M1, 2, 4, 5, n (%)	339 (81)	1345 (93)	1684 (90)	
M0, 6, 7, n (%)	81 (19)	104 (7)	185 (10)	
WBC count				.123
≤ 20 000 μL, n (%)	303 (65)	887 (61)	1190 (62)	
> 20 000 μL, n (%)	163 (35)	570 (39)	733 (38)	
Remission induction courses				< .001
1 course, n (%)	340 (69)	1276 (83)	1616 (80)	
2 courses, n (%)	154 (31)	259 (17)	413 (20)	
Dysplasia				< .001
No, n (%)	337 (68)	1264 (83)	1601 (79)	
Yes, n (%)	156 (32)	268 (17)	424 (21)	

Allo-HCT indicates allogeneic hematopoietic cell transplantation; CTx, chemotherapy; SWOG, Southwest Oncology Group; FAB, French-American-British; and WBC, white blood cell.

*Comparing "Allo-HCT in CR1" with "CTx in CR1."

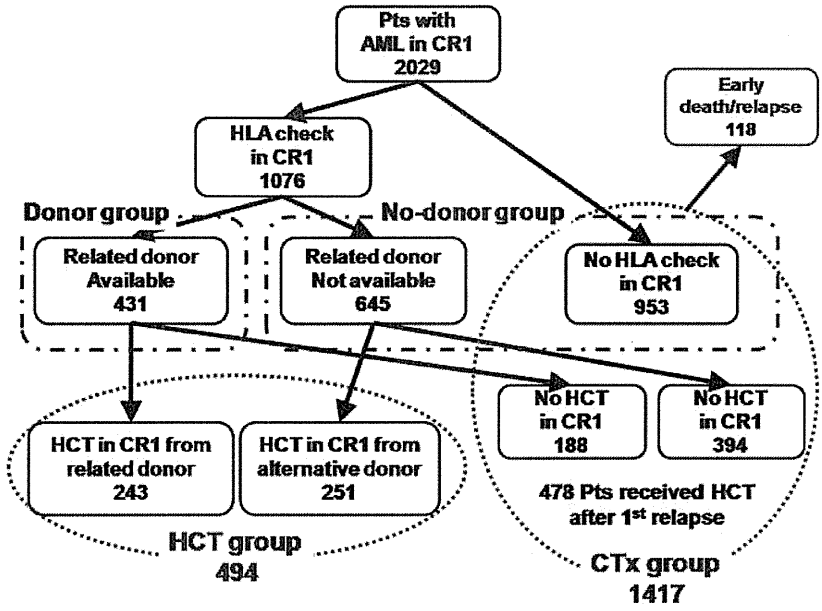

Figure 3. Patient flow. The flow of HLA check, donor availability, and actual application of allo-HCT in CR1 are shown. Among the total of 2029 patients with AML in CR1, 494 received allo-HCT in CR1 and were included in the HCT group. Among the remaining 1535 patients, 118 patients who died or relapsed within 3 months were excluded to take into account patients who were unable to receive allo-HCT in CR1 even though they had made a decision to receive HCT in CR1. Consequently, 1417 patients were included in the CTx group. Among them, 478 received allo-HCT after first relapse. The donor group included the 431 patients who had a suitable related donor. The no-donor group included the 645 patients who did not find a related donor and 953 for whom HLA was not typed in CR1. CR1 indicates first complete remission; and HCT, hematopoietic cell transplantation.

remained longer in the HCT group for all cytogenetic risks except for the favorable-risk group (favorable, 56.0 vs 64.3 months; intermediate, 59.4 vs 55.6 months; unfavorable, 47.6 vs 44.4 months). In the analysis of AML other than favorable risk, patients in the HCT group had a longer LE and a longer QALE than patients in the CTx group (LE, 69.5 vs 62.5 months; QALE, 55.8 vs 52.0 months).

We also performed subset analyses on the basis of the availability of a related donor. Patients who were known to have an HLA-matched or 1-Ag– mismatched related donor (donor group) in the HCT group had a longer LE and a longer QALE than patients in the CTx group (LE, 72.2 vs 63.0 months; QALE, 57.6 vs 49.9 months). However, in patients who did not have a suitable related donor (no-donor group), there were no differences in LE or QALE between the HCT and CTx groups (LE, 67.7 vs 67.0 months; QALE, 54.6 vs 54.4 months). Analyses of the donor and no-donor groups were also conducted with the database whereby the favorable-risk patients were excluded. There was almost no change in LE and QALE in the HCT group (less than a month) compared with the results obtained with the whole database. However, LE and QALE in the CTx group were shortened by several months by excluding the patients with favorable-risk AML from analysis. Consequently, in the donor group, the differences of LE and QALE between the HCT and CTx group increased (LE, 72.0 vs 60.5 months; QALE, 57.2 vs 47.6 months). Meanwhile in the no-donor group, LE and QALE in the HCT group became longer than those in the CTx group (LE, 67.3 vs 64.2 months; QALE, 54.5 vs 52.2 months). Survival curves that compare the HCT and CTx groups in these subgroups depicted by TreeAge Pro software are shown in Figure 4.

Analysis of younger patients. For younger patients, LE and QALE were analyzed with the data from patients aged 16-49 years

Table 3. Donor availability and transplantation in CR1

Characteristics	No HLA check in CR1	HLA check in CR1 (n = 1076)			
		Related donor available/HCT+	Related donor available/HCT–	Related donor not available/HCT+	Related donor not available/HCT–
Total no. of patients	953	243	188	251	394
Cytogenetic risks (SWOG)					
Favorable, n (%)	233 (24)	12 (5)	47 (25)	17 (7)	80 (20)
Intermediate, n (%)	496 (52)	140 (58)	84 (45)	132 (53)	197 (50)
Unfavorable, n (%)	139 (15)	52 (21)	38 (20)	63 (25)	69 (18)
Unknown, n (%)	85 (9)	39 (16)	19 (10)	39 (16)	48 (12)
No. of younger patients, n (%)	257	167	127	175	267
Cytogenetic risks					
Favorable, n (%)	106 (41)	8 (5)	35 (28)	16 (9)	60 (22)
Intermediate, n (%)	101 (39)	97 (58)	55 (43)	82 (47)	125 (47)
Unfavorable, n (%)	30 (12)	39 (23)	27 (21)	49 (28)	50 (19)
Unknown, n (%)	20 (8)	23 (14)	10 (8)	28 (16)	32 (12)
No. of older patients, n (%)	696	76	61	76	127
Cytogenetic risks					
Favorable, n (%)	127 (18)	4 (5)	12 (20)	1 (1)	20 (16)
Intermediate, n (%)	395 (57)	43 (57)	29 (48)	50 (66)	72 (57)
Unfavorable, n (%)	109 (16)	13 (17)	11 (18)	14 (18)	19 (15)
Unknown, n (%)	65 (9)	16 (21)	9 (15)	11 (14)	16 (13)

CR1 indicates first complete remission; HLA, human leukocyte antigen; HCT, allogeneic hematopoietic cell transplantation; and SWOG, Southwest Oncology Group.

Table 4. Discounted life expectancy

	All patients				Younger patients (median age, 35 y)				Older patients (median age, 60 y)			
	LE		QALE		LE		QALE		LE		QALE	
Decision at CR1	Allo-HCT	CTx	Allo-HCT	CTx	Allo-HCT	CTx	Allo-HCT	CTx	Allo-HCT	CTx	Allo-HCT	CTx
Total	69.7	66.7	55.9	55.4	71.4	73.2	57.7	60.2	65.8	60.0	52.1	50.6
Cytogenetic risks (SWOG)												
Favorable	69.6	77.0	56.0	64.3	67.0	82.3	53.8	67.6				
Intermediate	73.6	66.4	59.4	55.6	76.2	75.1	62.0	62.4	68.5	60.7	54.5	51.4
Unfavorable	61.6	53.4	47.6	44.4	62.8	55.3	48.7	44.8	61.6	53.3	46.0	45.0
Unknown	65.6	59.3	54.1	46.8	67.4	68.3	56.3	53.6	63.1	48.8	50.6	38.9
Other than favorable	69.5	62.5	55.8	52.0								
Donor availability												
Related donor	72.2	63.0	57.6	49.9	73.0	67.6	58.3	54.2	73.4	53.2	57.7	40.4
No related donor	67.7	67.0	54.6	54.4	71.0	70.7	57.7	57.2	57.4	57.7	45.4	46.8
Donor availability (other than favorable-risk)												
Related donor	72.0	60.5	57.2	47.6								
No related donor	67.3	64.2	54.5	52.2								

Life expectancies are shown in months.
LE indicates life expectancy; QALE, quality of life–adjusted life expectancy; allo-HCT, allogeneic hematopoietic cell transplantation; and CTx, chemotherapy.

(median 35 years). In the HCT group, LE in younger patients was 6 months longer than that in older patients (71.4 vs 65.8 months). In the CTx group, LE in younger patients was longer than that in older patients by more than a year (73.2 vs 60.0 months).

Younger patients with favorable-risk AML had both a longer LE and a longer QALE in the CTx group than in the HCT group. Allo-HCT in CR1 among younger patients was associated with a longer LE in both the unfavorable-risk group (62.8 vs 55.3 months) and donor group (73.0 vs 67.6 months). After we adjusted for QOL, these patients in the HCT group had a longer QALE than those in the CTx group (unfavorable, 48.7 vs 44.8 months; donor group, 58.3 vs 54.2 months). Younger patients with intermediate-risk AML in the HCT group had a slightly longer LE than those in the CTx group (76.2 vs 75.1 months). However, QALE did not improve when they received allo-HCT in CR1 (62.0 vs 62.4 months).

Analysis of older patients. The outcomes for older patients were analyzed with the data from patients aged 50-70 years (median, 60 years). Older patients who received allo-HCT in CR1 had a longer LE than patients who received chemotherapy in all subgroups, except for the no-donor group (intermediate, 68.5 vs 60.7 months; unfavorable, 61.6 vs 53.3 months; donor group, 73.4 vs 53.2 months). The data available for favorable-risk patients who received allo-HCT in CR1 were insufficient to perform an

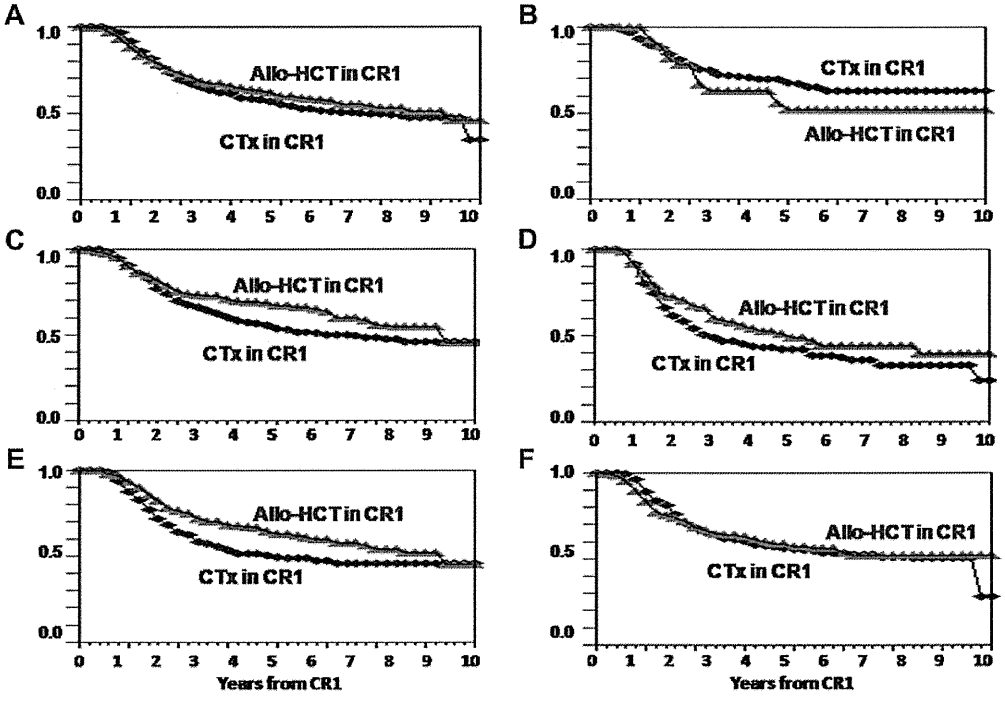

Figure 4. Survival curves of allo-HCT versus CTx by TreeAge. The overall survival curves of the HCT and CTx groups depicted by TreeAge Pro 2009 in (A) total patients, (B) SWOG favorable-risk group, (C) intermediate-risk group, (D) unfavorable-risk group, (E) donor group, and (F) no-donor group. allo-HCT indicates allogeneic hematopoietic cell transplantation; CTx, chemotherapy; and CR1, first complete remission.

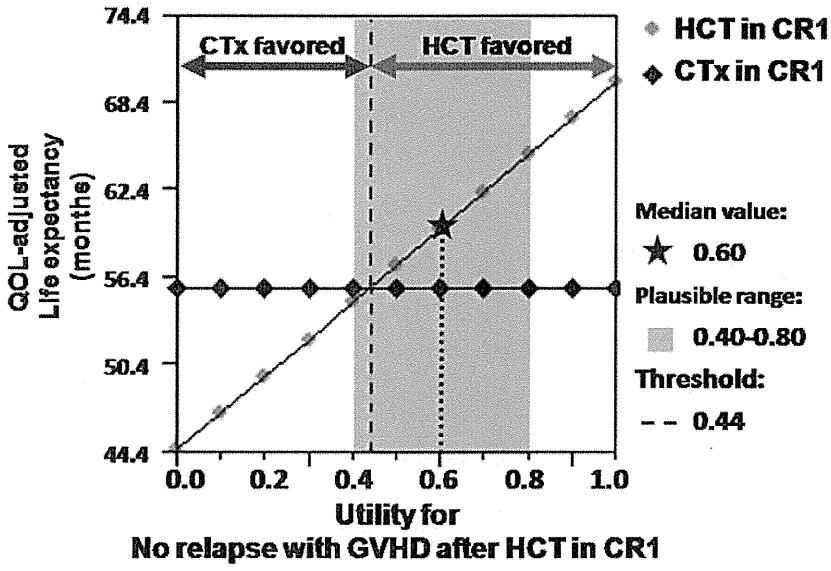

Figure 5. One-way sensitivity analysis. One-way sensitivity analysis for the utility of the state "No relapse with GVHD" after allogeneic transplantation in CR1 among patients with intermediate-risk AML is shown. The green dot represents the QOL-adjusted life expectancy when allo-HCT was performed in CR1. The blue dot represents the QOL-adjusted life expectancy when treated with chemotherapy in CR1. The median value of the utility for this state provided by physicians was 0.60, shown as a red star. At the median value, QOL-adjusted life expectancy in the HCT group is shown to outweigh that in the CTx group. The threshold value at which the favored decision is altered was 0.44, shown as a black dotted line. The plausible range of the utility provided by physicians was 0.40-0.80, shown as a red transparent square. Because the threshold value, 0.44, was included within the plausible range, this sensitivity analysis indicates that this result favoring HCT may be altered, depending on how the QOL of chronic GVHD is evaluated. Such results that favored a decision may change within the plausible range are interpreted as "sensitive." If the plausible range was provided in 0.50-0.80, this result would turn to "not sensitive," indicating that the favored decision does not change. QOL indicates quality of life; CR1, first complete remission; HCT, allogeneic hematopoietic cell transplantation; CTx, chemotherapy; and GVHD, graft-versus-host disease.

analysis. Because of the large decrease in LE in the CTx group among older patients, differences in LE between the HCT and CTx groups became more prominent in older patients than in younger patients. Although the difference in the duration of life between the HCT and CTx groups decreased after we adjusted for QOL, we found that older patients in the HCT group had a longer QALE in the intermediate- and unfavorable-risk groups. The difference in QALE between the HCT and CTx groups was most prominent among older patients who had a suitable related donor (donor group, 57.7 vs 40.4 months).

Sensitivity analysis and external validation. Sensitivity analyses were performed for the assumption of "patients who were unable to receive allo-HCT in CR1 despite the decision to perform allo-HCT," the plausible range of QOL utilities (Figures 5-6; supplemental Figure 5), 95% confidence intervals of the state transition probabilities, and the age range. We found that the optimal decisions could be altered in both directions, allo-HCT favored versus CTx favored, by changing the population that was excluded from the database, changing the utility values within the plausible range of physicians' opinions, changing the state transition probabilities within the range of the confidence interval, and changing the cutoff point for the age at which the age subgroups were divided. We also compared the overall survival curves depicted by TreeAge Pro software with the use of our database with those obtained by a Kaplan-Meier estimation as reported in prospective studies from other countries.[2,6] The curves had similar shapes (supplemental Figure 4).

Discussion

We performed a decision analysis that applied a Markov process to evaluate 2 postremission strategies: allo-HCT and CTx in AML in

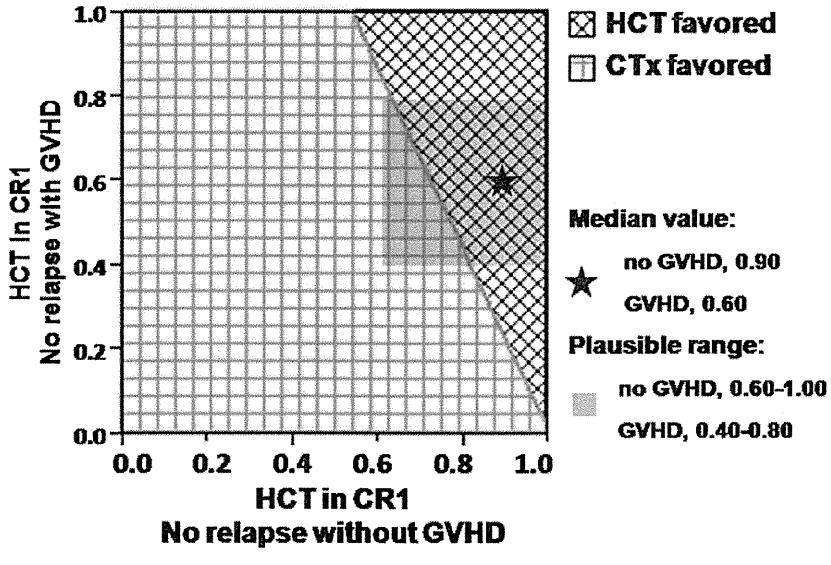

Figure 6. Two-way sensitivity analysis. Two-way sensitivity analysis for the utilities of the states "No relapse without GVHD" and "No relapse with GVHD." The blue area represents the range in which HCT is favored. The green area represents the range in which CTx is favored. Although the median value (0.90 for "without GVHD" and 0.60 for "with GVHD," shown as a red star) indicates that HCT in CR1 is favored, the plausible range (0.60-1.00 for "without GVHD" and 0.40-0.80 for "with GVHD," shown as a red transparent square) overlaps the threshold line. This result is interpreted as "sensitive," which means the outcome is changeable within the plausible range of QOL evaluation provided by physicians. CR1 indicates first complete remission; HCT, allogeneic hematopoietic stem cell transplantation; CTx, chemotherapy; and GVHD, graft-versus-host disease.

CR1. Our results showed that the LE of patients with intermediate- and unfavorable-risk AML were longer when they received allo-HCT in CR1. We also found that patients who were known to have a suitable related donor had a longer LE in the HCT group. After adjustment for QOL, QALE in most of these subgroups remained longer in patients who received allo-HCT in CR1 than in patients who received chemotherapy.

In subset analyses according to the cytogenetic risk, we showed that favorable-risk patients had a longer LE and a longer QALE in the CTx group, which is consistent with previous reports. However, the results in favorable-risk patients may not be reliable because only a few patients with favorable-risk AML received allo-HCT in CR1 and patients in the HCT group may have had specific reasons (eg, 2 courses of remission induction chemotherapy or antecedent hematologic dysplasia).

In intermediate-risk and unfavorable-risk patients, LE was longer in the HCT group. This result was consistent with that of a large meta-analysis.[10] If we integrate the assumption about the QOL obtained after the 2 strategies using utility values provided by physicians, the LE was shortened to a greater degree in the HCT group. This observation may indicate that there are more concerns about the deterioration of the QOL after allo-HCT than after chemotherapy alone. However, we still found that the QALE was longer in the HCT group, except for younger intermediate-risk patients.

In subset analyses that were based on donor availability, we found that patients who had an HLA-matched or 1-Ag–mismatched related donor had a longer LE and a longer QALE when allo-HCT was performed during CR1. A purposeful delay of allo-HCT has not been fully studied in patients with AML when they have a suitable related donor.[6] This result may recommend that we consider allo-HCT in CR1 rather than wait until after relapse when a suitable related donor is available. LE in older patients who received allo-HCT from a suitable related donor was even comparable to that in younger patients (73.0 vs 73.4 months), which led to a more conspicuous superiority of allo-HCT compared with CTx when older patients had a suitable related donor. In addition, the QALE of older patients with a related donor was 17 months longer in the HCT group than in the CTx group. This result suggests that allo-HCT in CR1 from a suitable related donor for older patients may provide an improved outcome even after we take into account transplantation-related toxicities, which are generally a greater concern among older patients.[18] However, among patients who did not have a suitable related donor, we did not find any advantages of allo-HCT from an alternative donor in CR1 compared with the CTx group. In recent years, the outcomes of allo-HCT from a matched related donor and that from a matched unrelated donor have been reported to be comparable.[19] Because this database included the clinical information of patients treated between 1999 and 2006, most of the unrelated bone marrow (BM) donor sources were selected on the basis of HLA serum matches and not on allele matches. In addition, our database included 1-Ag–mismatched unrelated BM and unrelated cord blood as alternative donors. Regarding the indications for allo-HCT from an alternative donor, further studies may be needed to evaluate whether there is a population that benefits from allo-HCT from well-matched unrelated BM.

The ability to consider QOL is one of the advantages of performing a decision analysis. We adjusted for QOL by applying QOL utility values provided by physicians. Utility values for various health states were obtained over a wide range. This observation may indicate that, even for the same patient, different therapeutic strategies may be chosen at the discretion of the physician. Another reason why the range of utility was broad may be the diverse symptoms and QOL within the same health state, such as the severity of "extensive chronic GVHD."[20,21] Consequently, in our study, sensitivity analyses showed that a better decision with a higher QALE was frequently altered to the other decision within the plausible range of utility values provided by physicians. There were no significant difference between the values provided by transplantation physicians and chemotherapy physicians. However, interestingly, median values of QOL utility in our study were lower than those used in prior analyses performed in North America. For example, although the utility for "no relapse with GVHD" was set at 0.6 (range, 0.4-0.8) in our study, this value was set at around 0.9 in other studies.[13-15,22] This trend was more prominent in the HCT group, which might indicate differences in approaches to estimating the same complications that may be due to ethnicity or differences in the contents of questionnaires.

It might be ideal to evaluate QALE based on QOL utility values obtained from patients who actually live with various disease states.[23,24] However, most prior studies on decision analysis in this field have used utility values provided by physicians.[13-15] Sung et al[15] stated that their utility values provided by physicians were consistent with those provided by patients in the European Organization for Research and Treatment of Cancer and Gruppo Italiano Malattie Ematologiche dell' Adulto trial.[24] Patients may even give diverse QOL values for a certain health state according to differences in age, background, and philosophy. We believe that a QOL validation by patients is an important issue and is worth being pursued in another study.

Our data surely reflect the nature of a retrospectively collected database, including a diverse heterogeneity in treatment strategies chosen after the achievement of CR1. However, it may be difficult to obtain a database that was collected purely prospectively, especially in patients who were treated with chemotherapy alone. Therefore, we considered that this database, which consists of the clinical information for 2029 patients, was sufficient for us to perform this analysis. Another concern is that, because we collected clinical data on Japanese patients, the application of these results to other ethnic populations needs to be carefully evaluated. However, we have shown that the survival curves obtained from this analysis are similar to those reported in prospective studies from other countries. In decision analysis, the P value is not used to show the "significantly" better decision. Sensitivity analysis is a way to investigate the robustness of our conclusions when various parameters are changed within a possible range. It might be difficult to draw a definite conclusion in this study because, as a result of the sensitivity analysis, a favorable decision could be switched to the other decision. Nevertheless, we have been able to show that a decision analysis with a Markov model can be effectively used to evaluate the QOL-adjusted survival outcomes of allo-HCT versus chemotherapy in CR1.

In summary, by using a Markov decision analysis that was based on an original database collected for this study, we have shown that patients with intermediate- and unfavorable-risk AML and patients who had a suitable related donor had a longer LE and a longer QALE when they received allo-HCT in CR1. A subgroup analysis showed that older patients with a suitable related donor benefited the most from allo-HCT in CR1. Although it is clear that both methods of treatment still require improvement, we believe

that this observation serves as an important guide for considering the indications for allo-HCT in AML in CR1 by incorporating the evaluation of QOL. Adjustment for QOL with the use of utility values provided by patients who live with the disease should add valuable clues for weighing the value of a postremission strategy for each person. In addition, an investigation that applies a prospectively collected database for a multiethnic population should help to further show the roles of allo-HCT and chemotherapy in AML in CR1.

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Authorship

Contribution: S.K. designed the study, prepared the data file, performed the analysis, interpreted data, and wrote the manuscript; T. Yamaguchi was primarily responsible for the study design, data analysis, and interpretation of the data; S.M., N.U., H.K., K.U., T. Yamashita, M.W., K.Y., S.Y., Y. Nawa, J. Taguchi, J. Takeuchi, J. Tomiyama, and Y. Nakamura obtained the patients' data and interpreted data; I.M. reviewed the cytogenetic reports and interpreted data; Y.K. helped to design the study and to interpret data; Y.T. interpreted data and helped to write the paper; and T.F. was primarily responsible for the entire paper as an accurate and verifiable report.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

1. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med.* 1999;341(14):1051-1062.
2. Slovak ML, Kopecky KJ, Cassileth PA, et al. Karyotypic analysis predicts outcome of preremission and postremission therapy in adult acute myeloid leukemia: a Southwest Oncology Group/Eastern Cooperative Oncology Group Study. *Blood.* 2000;96(13):4075-4083.
3. Suciu S, Mandelli F, de Witte T, et al. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. *Blood.* 2003;102(4):1232-1240.
4. Jourdan E, Boiron JM, Dastugue N, et al. Early allogeneic stem-cell transplantation for young adults with acute myeloblastic leukemia in first complete remission: an intent-to-treat long-term analysis of the BGMT experience. *J Clin Oncol.* 2005;23(30):7676-7684.
5. Burnett AK, Wheatley K, Goldstone AH, Stevens R, Hann I, Hills RK. Long-term results of the MRC AML10 trial. *Clin Adv Hematol Oncol.* 2006;4(6):445-451.
6. Cornelissen JJ, van Putten WL, Verdonck LF, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? *Blood.* 2007;109(9):3658-3666.
7. Basara N, Schulze A, Wedding U, et al. Early related or unrelated haematopoietic cell transplantation results in higher overall survival and leukaemia-free survival compared with conventional chemotherapy in high-risk acute myeloid leukaemia patients in first complete remission. *Leukemia.* 2009;23(4):635-640.
8. Schlenk RF, Benner A, Hartmann F, et al. Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial. *Leukemia.* 2003;17(8):1521-1528.
9. Sakamaki H, Miyawaki S, Ohtake S, et al. Allogeneic stem cell transplantation versus chemotherapy as post-remission therapy for intermediate or poor risk adult acute myeloid leukemia: results of the JALSG AML97 study. *Int J Hematol.* 2010;91(3):471-477.
10. Koreth J, Schlenk R, Kopecky KJ, et al. Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials. *JAMA.* 2009;301(22):2349-2361.
11. Detsky AS, Naglie G, Krahn MD, Naimark D, Redelmeier DA. Primer on medical decision analysis: part 1—getting started. *Med Decis Making.* 1997;17(2):123-125.
12. Naimark D, Krahn MD, Naglie G, Redelmeier DA, Detsky AS. Primer on medical decision analysis: part 5—working with Markov processes. *Med Decis Making.* 1997;17(2):152-159.
13. Lee SJ, Kuntz KM, Horowitz MM, et al. Unrelated donor bone marrow transplantation for chronic myelogenous leukemia: a decision analysis. *Ann Intern Med.* 1997;127(12):1080-1088.
14. Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. *Blood.* 2004;104(2):579-585.
15. Sung L, Buckstein R, Doyle JJ, Crump M, Detsky AS. Treatment options for patients with acute myeloid leukemia with a matched sibling donor: a decision analysis. *Cancer.* 2003;97(3):592-600.
16. Martin PJ, Storer BE, Rowley SD, et al. Evaluation of mycophenolate mofetil for initial treatment of chronic graft-versus-host disease. *Blood.* 2009;113(21):5074-5082.
17. Stewart BL, Storer B, Storek J, et al. Duration of immunosuppressive treatment for chronic graft-versus-host disease. *Blood.* 2004;104(12):3501-3506.
18. Hegenbart U, Niederwieser D, Sandmaier BM, et al. Treatment for acute myelogenous leukemia by low-dose, total-body, irradiation-based conditioning and hematopoietic cell transplantation from related and unrelated donors. *J Clin Oncol.* 2006;24(3):444-453.
19. Appelbaum FR. Allogeneic hematopoietic cell transplantation for acute myeloid leukemia when a matched related donor is not available. *Hematology Am Soc Hematol Educ Program.* 2008;2008:412-417.
20. Lee SJ, Joffe S, Kim HT, et al. Physicians' attitudes about quality-of-life issues in hematopoietic stem cell transplantation. *Blood.* 2004;104(7):2194-2200.
21. Lee SJ, Kim HT, Ho VT, et al. Quality of life associated with acute and chronic graft-versus-host disease. *Bone Marrow Transplant.* 2006;38(4):305-310.
22. Pidala J, Anasetti C, Kharfan-Dabaja MA, Cutler C, Sheldon A, Djulbegovic B. Decision analysis of peripheral blood versus bone marrow hematopoietic stem cells for allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant.* 2009;15(11):1415-1421.
23. Pidala J, Anasetti C, Jim H. Quality of life after allogeneic hematopoietic cell transplantation. *Blood.* 2009;114(1):7-19.
24. Zittoun R, Suciu S, Watson M, et al. Quality of life in patients with acute myelogenous leukemia in prolonged first complete remission after bone marrow transplantation (allogeneic or autologous) or chemotherapy: a cross-sectional study of the EORTC-GIMEMA AML 8A trial. *Bone Marrow Transplant.* 1997;20(4):307-315.

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ORIGINAL ARTICLE

An open-label extension study evaluating the safety and efficacy of romiplostim for up to 3.5 years in thrombocytopenic Japanese patients with immune thrombocytopenic purpura (ITP)

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Abstract Long-term use of the thrombopoietin mimetic romiplostim was examined in Japanese patients with chronic immune thrombocytopenic purpura (ITP) in this open-label extension. The starting dose of romiplostim was the previous trial dose or 3 μg/kg/week, which was titrated up to 10 μg/kg/week to maintain platelet counts between 50 and 200 × 10^9/L. As of April 2010, 44 patients had enrolled; 71 % women, median age 55.5 years, with five patients discontinuing romiplostim due to patient request (2), administrative decision (2), or not achieving study-defined platelet response (1). Median treatment duration was 100 weeks; median average weekly dose was 3.8 μg/kg. Twenty-eight patients (64 %) self-injected romiplostim. The most frequent adverse events were nasopharyngitis and headache. Nine patients (20 %) had a total of 14 serious adverse events (0.31/100 patient-weeks); of these, only oral hemorrhage was considered treatment related. Fifty hemorrhagic adverse events were reported in 20 patients (46 %) (1.12/100 patient-weeks). Ninety-six percent of patients had a platelet response (doubling of baseline platelet count and platelet count ≥50 × 10^9/L). Of the 25 patients receiving concurrent ITP therapy at baseline, all reduced or discontinued the therapy. Eight patients (18 %) received rescue medications. Administration of up to

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3.5 years of romiplostim increased platelet counts and was well tolerated in Japanese patients with chronic ITP.

Keywords Immune thrombocytopenic purpura (ITP) · Romiplostim · Thrombopoietin receptor agonists · Thrombopoietin mimetic

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by isolated thrombocytopenia (i.e., no other hematologic abnormality) with platelet counts below 100×10^9/L, due to both increased platelet destruction and a relatively low level of platelet production [1–5]. Incidence of ITP in Japan is 2.16/100,000/year, with approximately 70 % of cases occurring in patients older than 50 years [6, 7]. Treatment is typically not recommended for patients with platelet counts $>50 \times 10^9$/L [2, 8, 9]. When treatment is necessary, options include corticosteroids and other immunosuppressive agents, splenectomy, and immunoglobulins [8, 9]. However, a significant proportion of ITP patients either will not respond to or will not have a sustained platelet response with these agents, many of which are accompanied by significant side effects [8, 15, 16]. For those ITP patients who have active *Helicobacter pylori* infection, *H. pylori* eradication therapy appears to improve thrombocytopenia in some [6, 10–14].

While, traditionally, options such as those listed above aim to limit platelet destruction, a new class of agents addresses the now understood relative deficiency in platelet production. Romiplostim, a thrombopoietin (TPO) mimetic with no structural overlap with TPO, increases platelet production by a mechanism similar to that of endogenous TPO [5, 17, 18]. Romiplostim, which has been shown to be effective for the treatment of chronic ITP with good tolerability, has been approved in many countries for the treatment of chronic ITP in adult patients with an insufficient response to previous treatments [19]. Specifically, romiplostim (Nplate®) is indicated in the United States for the treatment of thrombocytopenia in patients with chronic ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy [20]. Romiplostim should be used only in patients with ITP whose degree of thrombocytopenia and clinical condition increases the risk for bleeding, but not to normalize platelet counts [20]. In Europe, romiplostim is indicated for the treatment of splenectomized adult chronic ITP patients who are refractory to other treatments and may be considered as second-line treatment for adult non-splenectomized ITP patients for whom surgery is contraindicated [21]. As of January 21, 2011, the Japanese regulatory agency, the Ministry of Health, Labour, and Welfare, approved romiplostim (brand name Romiplate®) for the treatment of thrombocytopenia in adult chronic ITP in patients who have had an inadequate response to or are intolerant of other therapies for ITP [22]. Romiplostim should be used only in those patients with ITP whose degree of thrombocytopenia and clinical condition increase the risk for bleeding [22].

Disease presentation, pharmacokinetics, pharmacodynamics, and safety may be affected by ethnic background [23, 24]. Therefore, the use of romiplostim in Japanese patients with ITP was assessed in clinical studies in Japanese patients with ITP. Similar to early phase studies in other populations, romiplostim was found to be well tolerated and effective at increasing platelet counts in a dose-dependent manner with good tolerability in Japanese patients with ITP [25–27]. Likewise, romiplostim significantly increased and maintained platelet counts and was well tolerated in a phase 3 study of 34 Japanese patients with ITP, with similar dosing to that seen in non-Asian patients [27, 28]. However, there are few reports of the long-term safety and efficacy of romiplostim in clinical trials. We describe here the results of patients from the phase 2 and phase 3 studies who then continued into an open-label extension study for up to 3.5 years of romiplostim treatment.

Materials and methods

Study design

This was an open-label extension study designed to assess the safety and efficacy of long-term romiplostim dosing in thrombocytopenic Japanese patients with ITP (Fig. 1). If patients entered the extension study within 12 weeks of receiving the last romiplostim dose in the previous study and had shown an increase in platelet counts $\geq 20 \times 10^9$/L from baseline at least once during the 13-week treatment period of the original trial (excluding 4 weeks after rescue medication), they were treated with romiplostim at the same weekly dose last received in the previous study. Otherwise, patients were treated with romiplostim at a starting dose of 3 μg/kg. Romiplostim was administered by subcutaneous (SC) injection once per week. Dose adjustment based on platelet counts was permitted throughout the treatment period to allow patients to maintain platelet counts in the target range of $50–200 \times 10^9$/L, up to a maximum permitted dose of 10 μg/kg. Patients who achieved a stable dose of romiplostim for at least 3 consecutive weeks were allowed to self-inject romiplostim away from the clinic. The study began in October 2006 and is ongoing.

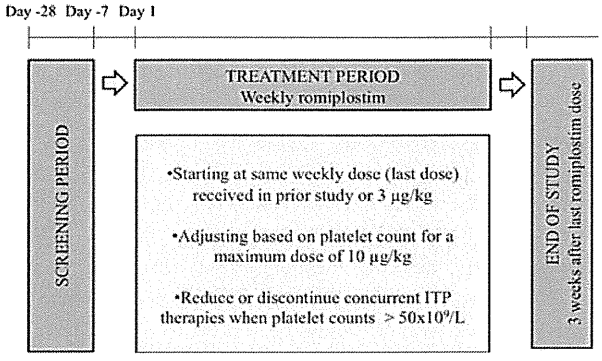

Fig. 1 Study design. This was an open-label extension study of long-term romiplostim dosing in thrombocytopenic Japanese patients with ITP

Eligibility

Patients who had completed any previous romiplostim ITP study in Japan (a phase 2 open-label study and a phase 3 randomized study) were eligible to screen for this study. Additionally, patients were required to provide written informed consent before any study-specific procedures were performed and must have had a platelet count at screening of $<50 \times 10^9$/L. Patients were excluded from the study if they had any significant change in medical history since completion of the previous romiplostim ITP study, including bone marrow stem cell disorders or new active malignancies; tested positive for neutralizing antibodies to romiplostim in the previous romiplostim ITP study; were receiving any treatment for ITP except oral corticosteroids, azathioprine, and/or danazol administered at a constant dose and schedule for at least 4 weeks prior to the screening visit; were pregnant, breastfeeding, or of reproductive potential and not using adequate contraception; had a known severe drug hypersensitivity; or were unlikely to comply with the protocol.

Study endpoints

The primary endpoint of this study was to determine the safety of romiplostim as a long-term treatment in thrombocytopenic Japanese patients with ITP, as measured by the incidence of adverse events, including clinically significant changes in laboratory values. Additional endpoints included incidence of anti-romiplostim antibody formation, incidence of platelet response (doubling of the baseline platelet count at study entry of the previous study and platelet counts $\geq 50 \times 10^9$/L), and proportion of patients able to reduce or discontinue their concurrent ITP therapies (for patients who were receiving oral corticosteroids at a constant dose and schedule at the screening visit). Anti-romiplostim antibodies were assayed at week 1, every 12 weeks during the study and at end of study. Specifically, two validated assays were used: a Biacore 3000 (Biacore International, AB, Uppsala, Sweden) immunoassay and a cell-based bioassay to detect neutralizing or inhibitory effects in vitro [29–31]. If a sample was positive in both assays, a subject was defined as positive for neutralizing antibodies. Throughout the study, investigators could perform a bone marrow biopsy when deemed medically appropriate.

Statistics

The statistical analyses in this open-label extension study were descriptive in nature. Categorical endpoints were summarized by the number and percentage of patients in each category. Continuous endpoints were summarized by number in an eligible subset (n), mean, standard deviation, median, Q1 (25th percentile), Q3 (75th percentile), and minimum and maximum values.

Results

Patient characteristics, disposition, and exposure

As of April 2010, 44 patients who had previously completed either a phase 2 or phase 3 study in Japan [25, 27] enrolled in this open-label extension study. These patients had baseline characteristics of 71 % women, median age 55.5 years (ranging from 25 to 81 years), and median (Q1, Q3) platelet count of 16.5 (6.0, 23.0) $\times 10^9$/L (Table 1). Past treatments included corticosteroids (98 %), IVIg (57 %), *H. pylori* eradication (48 %), splenectomy (39 %), azathioprine (25 %), danazol (23 %), cyclophosphamide (11 %), vincristine/vinblastine (7 %), and rituximab (7 %). Two patients had a past medical history of hepatitis B virus (HBV), three of hepatitis C virus (HCV), and one of HBV and HCV. As of this data cutoff, 5 patients (11 %) discontinued romiplostim due to patient request (2, after 85 and 183 days of treatment, respectively), administrative decision secondary to platelet counts $>200 \times 10^9$/L (2, after 281 and 583 days of treatment, respectively) and platelet counts $\leq 20 \times 10^9$/L after 4 weeks of dosing with 10 μg/kg (1, after 247 days of treatment). The patients who discontinued romiplostim completed an end of study visit 3 weeks after the last administration of romiplostim. All patients received at least one dose of romiplostim, with the mean (SD) treatment duration being 102 (47) weeks (ranging from 12 to 184 weeks) and the mean (SD) average weekly dose being 4.3 (2.7) μg/kg (ranging from 0 to 10 μg/kg). The overall mean weekly dose increase around week 150 corresponds to when the study population consisted of patients from the phase 2 study only (i.e., none

Table 1 Baseline characteristics

	Phase 2 ($N = 11$)	Phase 3 ($N = 33$)	Total ($N = 44$)
Age (years)			
Mean ± SD	55.5 ± 9.8	54.7 ± 13.9	54.9 ± 12.9
Median (min, max)	62.0 (32, 63)	54.0 (25, 81)	55.5 (25, 81)
Sex, *n* (%)			
Female	7 (63.6)	24 (72.7)	31 (70.5)
Male	4 (36.4)	9 (27.3)	13 (29.5)
Baseline platelet count ($\times 10^9$/L)[a]			
Mean ± SD	11.5 ± 10.1	17.7 ± 8.5	16.1 ± 9.2
Median (min, max)	5.5 (3, 31)	19.5 (3, 32)	16.5 (3, 32)
Platelet count prior to the treatment of this study ($\times 10^9$/L)[b]			
Mean ± SD	10.7 ± 8.6	16.3 ± 11.9	14.9 ± 11.3
Median (min, max)	6.0 (3, 25)	11.0 (3, 53)	11.0 (3, 53)

[a] Baseline platelet count in this study was baseline platelet count obtained in the previous study

[b] Platelet count of week 1 or pre-treatment platelet count closest to the first dose of romiplostim in this study if platelet count of week 1 was missing

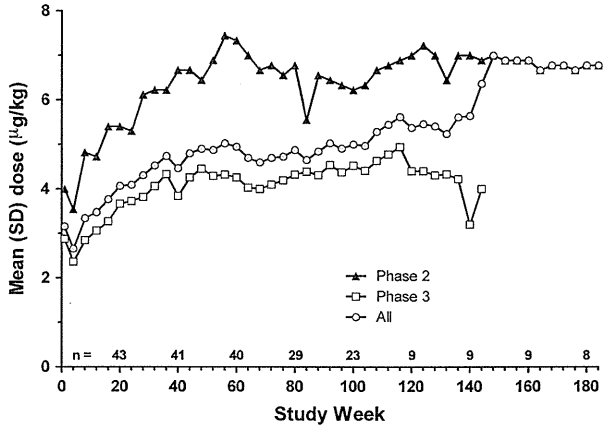

Fig. 2 Mean dose over time. Mean doses for all of the patients, as well as for those who were originally from the phase 2 trial or the phase 3 trial prior to entering the extension, are shown

from the phase 3 study) (Fig. 2). The patients in the phase 2 study had received higher doses throughout this extension. Twenty-eight patients (64 %) received romiplostim by self-injection, beginning after a median (Q1, Q3) of 21 (8.5, 29.0) weeks on study and continuing self-injection for a median (Q1, Q3) duration of 60.0 (28.5, 87.5) weeks. The median (Q1, Q3) percent of weeks these patients were self-injecting was 65 % (42, 81 %). Twelve of these 28 patients (43 %) discontinued self-injection.

Safety

All patients reported at least 1 adverse event after beginning treatment with romiplostim, with 27 patients (61 %) reporting adverse events that were considered by the investigator to be related to the treatment with romiplostim (Table 2). The most frequent adverse events were nasopharyngitis (2.1/100 patient-weeks), headache (0.7/100 patient-weeks), back pain (0.3/100 patient-weeks), contusion (0.3/100 patient-weeks), and malaise (0.3/100 patient-weeks). All nasopharyngitis cases were considered by investigators to not be related to romiplostim, and they were generally mild common upper respiratory tract infections; 6 cases (of 101) were rated as moderate in severity. The most frequently reported treatment-related adverse events were headache (0.52/100 patient-weeks), back pain (0.13/100 patient-weeks), malaise (0.13/100 patient-weeks), and vertigo (0.09/100 patient-weeks).

Nine patients (20 %) reported serious adverse events (duration-adjusted rate of 0.31/100 patient-weeks), with one serious adverse event, mouth hemorrhage, considered by the investigator to be related to the treatment with romiplostim. Other reported serious adverse events included one event each of hemorrhagic anemia, thrombocytopenia, appendicitis, grand mal convulsion, transient ischemic attack, epistaxis, intracranial aneurysm, lumbar spinal stenosis, allergic transfusion reaction, melena, mouth hemorrhage, subcutaneous hematoma, wound, and spinal compression fracture (Table 3). The event of mouth hemorrhage occurred 17 months after initiation of romiplostim in this study. Platelet counts in this patient during romiplostim treatment fluctuated greatly, and thus the dose was frequently adjusted. During one of the times of low platelet counts, the mouth hemorrhage occurred, thus the investigator indicated that there was a reasonable possibility that the hemorrhage was due to romiplostim. As the investigator judged the romiplostim as being effective, treatment with romiplostim was continued. The event of transient ischemic attack occurred 59 days after initiation of romiplostim in this study. The patient had a history of paroxysmal atrial fibrillation, hyperlipidemia, and hyperbilirubinemia. Three days prior to the event, the platelet count was 206 $\times$ 10^9/L. The patient went to the emergency room, where the platelet count was measured at 135 $\times$ 10^9/L. He was not hospitalized, returned home, and the event resolved the next day. Platelet count 4 days after the event was 70 $\times$ 10^9/L. As the investigator judged that the event was caused by transient cerebral hypoperfusion and cerebrovascular spasm, it was considered to not be due to romiplostim, and romiplostim treatment continued. Each of these serious adverse events occurred at a rate of 0.02/100 patient-weeks. There were

Table 2 Overall summary of safety

	N (%)	Rate
Any adverse events (AE)	44 (100)	11.15
Serious AE (SAE)	9 (21)	0.31
Any treatment-related AE	27 (61)	1.70
Any treatment-related SAE	1 (2)	0.02
Death	0 (0)	0
Withdrawal due to AE	0 (0)	0

Rate events per 100 patient-weeks

Table 3 Serious adverse events (SAE)

	N (rate)
All SAE	14 (0.31)
Hemorrhagic anemia	1 (0.02)
Thrombocytopenia	1 (0.02)
Appendicitis	1 (0.02)
Grand mal convulsion	1 (0.02)
Transient ischemic attack	1 (0.02)
Epistaxis	1 (0.02)
Intracranial aneurysm	1 (0.02)
Lumbar spinal stenosis	1 (0.02)
Allergic transfusion reaction	1 (0.02)
Melena	1 (0.02)
Mouth hemorrhage[a]	1 (0.02)
Subcutaneous hematoma	1 (0.02)
Wound	1 (0.02)
Spinal compression fraction	1 (0.02)

Rate events per 100 patient-weeks

[a] Considered by the investigator to be related to romiplostim

no life-threatening adverse events, and no patients died or withdrew from the study.

A total of 50 hemorrhagic adverse events were reported in 20 patients (46 %), with a duration-adjusted rate of 1.12/100 patient-weeks. The most common hemorrhagic adverse events were contusion (0.29/100 patient-weeks), epistaxis (0.16/100 patient-weeks), purpura (0.11/100 patient-weeks), and conjunctival hemorrhage (0.09/100 patient-weeks). Three patients (7 %) had a total of 5 serious hemorrhagic adverse events; one with epistaxis and hemorrhagic anemia, one with melena and subcutaneous hematoma, and one with mouth hemorrhage.

Regarding adverse events of interest, no cases were reported of hematopoietic malignancy, myelodysplastic syndrome, thrombocytosis, or bone marrow reticulin/collagen fibrosis (bone marrow biopsies were performed at investigator discretion). A total of 14 biopsies were performed on 8 patients over a wide range of time, from before the study (1), within the first year (8), to more than 1 year up to over 2 years (5). All biopsies were negative for reticulin and collagen. However, after this data cutoff (on study day 735), one patient experienced a mild non-serious adverse event of increased reticulin that was considered by the investigator to be related to treatment with romiplostim. The only thromboembolic event was a serious adverse event of transient ischemic attack. Additionally, no patients tested positive for neutralizing antibodies to romiplostim or TPO in the antibody assays that were performed every 12 weeks.

Efficacy

Overall, 96 % of patients had a platelet response (doubling of the baseline platelet count at study entry of the previous study and platelet counts $\geq 50 \times 10^9$/L) (response rate over time shown in Fig. 3). Median platelet counts stayed above 50×10^9/L each week from week 2 onward (Fig. 4). Of the 25 patients receiving concurrent ITP therapy at baseline, all were able to reduce or discontinue that therapy: 11 (44 %) had a >25 % reduction in at least 1 concurrent therapy, 5 (20 %) had a >50 % reduction in at least 1 concurrent therapy, and 9 (36 %) discontinued all concurrent therapies. There was an overall decrease over time in the proportion of patients with bleeding events (Fig. 5). Eight patients (18 %) received rescue medications for ITP at some point during the study. These included prednisolone (6 patients), platelet transfusion (5 patients), immunoglobulins (3 patients), dexamethasone and red blood cell transfusion (each 1 patient). Details on individual patients are provided in Table 4.

Discussion

Results of this study indicate that romiplostim administration for up to 3.5 years was well tolerated in Japanese patients with ITP. The reported incidence of adverse events did not increase over time during long-term exposure to romiplostim and were similar to those seen in other romiplostim studies, such as the long-term open-label extension in ITP patients of other ethnic origins ($N = 292$) [32]. In addition, both studies had similar proportions of patients having a platelet response (96 % in this study, 95 % in the other), similar median doses (3.8 vs. 4.0 μg/kg), and a majority of patients initiating self-injection (64 vs. 82 %). In this study, the safety and tolerability of romiplostim self-injection was generally satisfactory; however, please note that self-injection of romiplostim is not approved in Japan. During self-injection, patients continued to have regular platelet count assessments, and, if platelet counts were greater than the target range ($50–200 \times 10^9$/L), romiplostim was discontinued. This discontinuation rule applied to those who received romiplostim from a healthcare provider

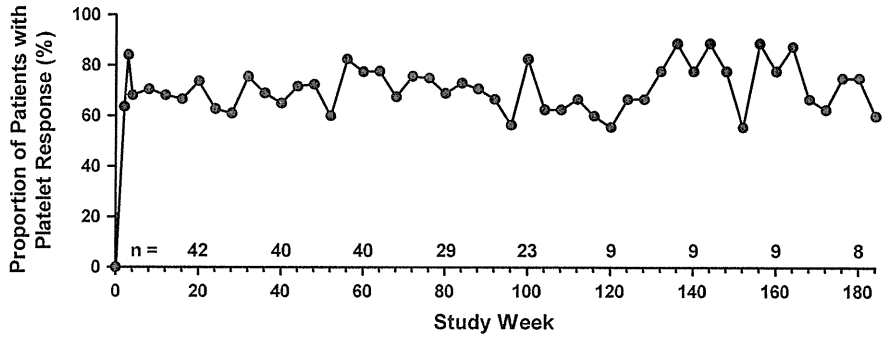

Fig. 3 Platelet response over time

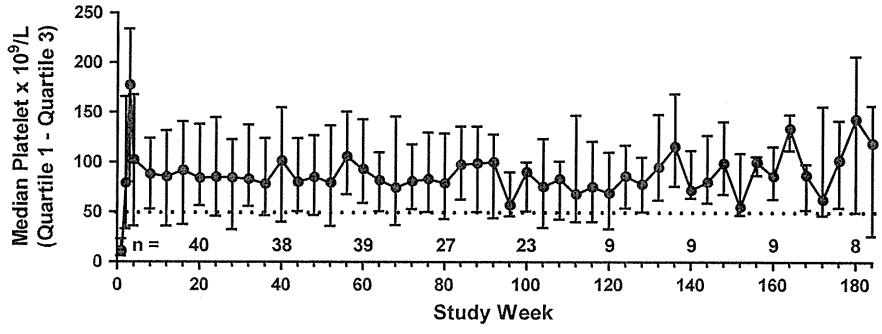

Fig. 4 Platelet count over time

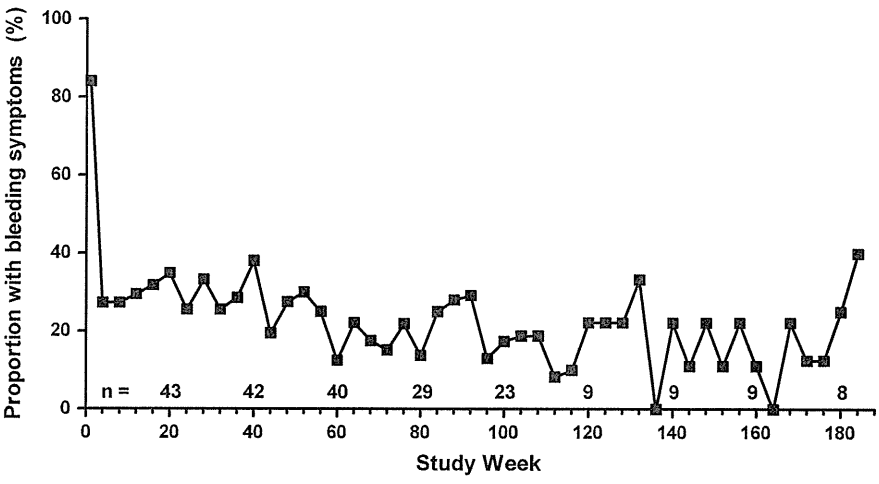

Fig. 5 Bleeding symptoms over time

as well. Concurrent ITP medications at baseline were reduced or discontinued in most patients in both studies (100 vs. 81 %). Overall, the efficacy and safety profile is consistent in these two study populations [28, 33, 34].

During the study, one thromboembolic event of transient ischemic attack was reported. The event occurred in a patient who had a history of paroxysmal atrial fibrillation, hyperlipidemia, and hyperbilirubinemia. Although the transient ischemic attack was not judged to be related to romiplostim by the investigator based on the patient's medical history, this kind of thromboembolic event should be noted and followed carefully during romiplostim treatment. Thus, the benefit:risk ratio of romiplostim should be carefully considered in patients with significant risk factors for thromboembolic events, and platelet counts should be closely monitored. Other reported serious adverse events were mouth hemorrhage, hemorrhagic anemia, thrombocytopenia, appendicitis, grand mal convulsion, epistaxis, intracranial aneurysm, lumbar spinal stenosis, allergic transfusion reaction, melena, mouth hemorrhage, subcutaneous hematoma, wound, and spinal compression fracture, each occurring at a rate of 0.02/100 patient-weeks. Eight patients received rescue medications for ITP, including prednisolone, platelet transfusion,

Table 4 Rescue medication use

Patient	Rescue medication	Number of incidents	Notes[a]
1	Prednisolone	1	
2	Platelet transfusion	2	
3	Prednisolone	3	
4	Prednisolone	2	
	IVIg	1	
5	Dexamethasone	1	
	IVIG	2	GI hemorrhage leading to anemia
	Platelet transfusion	7	One was due to epistaxis, the other 6 due to GI hemorrhage leading to anemia
	Prednisolone	2	
6	Platelet transfusion	1	Mouth hemorrhage
	Prednisolone	5	
	IVIg	3	1 was due to mouth hemorrhage
7	Platelet transfusion	22	2 were due to subcutaneous hematoma
	RBC transfusion	11	All were due to anemia
	Prednisolone	3	
8	Platelet transfusion	4	

GI gastrointestinal

[a] Unless otherwise indicated, use was for thrombocytopenia, not any other specific cause

immunoglobulin, dexamethasone and red blood cell transfusion. There were no deaths and no neutralizing antibodies to romiplostim or TPO. A total of 14 bone marrow biopsies were performed on 8 patients over a wide range of time, with no findings of bone marrow reticulin or collagen as of this data cutoff. Subsequently, there was a mild nonserious adverse event of increased reticulin considered related to romiplostim (study day 735).

A higher romiplostim dose was consistently seen with patients originally from the phase 2 study as compared with those from the phase 3 study. It was thought that this may reflect that the patients from the phase 2 study had a longer history of ITP (median 11.8 vs. 5.8 years for the phase 3 study), and hence likely more advanced disease. To explore this possibility, we performed a post hoc analysis and found that higher romiplostim doses were related to lower platelet count at study entry ($p = 0.0003$)(i.e., inversely related) and inversely related to *H. pylori* eradication prior to study start ($p < 0.0001$), and positively associated with starting dose in this extension study ($p = 0.006$). Of note, the association of ITP duration with romiplostim dose was not statistically significant ($p = 0.1$). Rather, the higher dose in patients originally in the phase 2 study was due to lower platelet count at study entry ($p = 0.01$) and higher starting dose ($p = 0.02$) compared with the phase 3 study, as per study design.

One limitation of this study is the relatively small size (44 patients). Therefore, it is difficult to make conclusions regarding different patient subgroups (such as splenectomized vs. non-splenectomized, etc.). As of this data cutoff, there have been 86 patient-years of romiplostim exposure in this extension study; as this study is ongoing, analyses at future dates will be based on longer exposure time. Another possible limitation is self-selection, as often patients who respond to a medication are more likely to enter an extension study. As a high proportion of patients from earlier studies (44/46, or 96 %) enrolled into this study, it is unlikely that selection bias influenced the results of this extension trial.

In conclusion, similarly to non-Japanese patients, long-term administration of romiplostim was well tolerated in Japanese patients with ITP, with the vast majority of patients achieving a platelet response and no new safety concerns. With the approval of romiplostim in Japan, Japanese patients with ITP will now have access to another option for second-line therapy.

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References

1. Rodeghiero F, Stasi R, Gernsheimer T, Michel M, Provan D, Arnold DM, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. Blood. 2009;113:2386–93.
2. Cines DB, Bussel JB, Liebman HA, Luning Prak ET. The ITP syndrome: pathogenic and clinical diversity. Blood. 2009;113:6511–21.
3. Nugent D, McMillan R, Nichol JL, Slichter SJ. Pathogenesis of chronic immune thrombocytopenia: increased platelet destruction and/or decreased platelet production. Br J Haematol. 2009;146:585–96.
4. McMillan R. The pathogenesis of chronic immune thrombocytopenic purpura. Semin Hematol. 2007;44:S3–11.
5. Molineux G, Newland A. Development of romiplostim for the treatment of patients with chronic immune thrombocytopenia: from bench to bedside. Br J Haematol. 2010;150:9–20.
6. Fujimura K, Kuwana M, Kurata Y, Imamura M, Harada H, Sakamaki H, et al. Is eradication therapy useful as the first line of treatment in Helicobacter pylori-positive idiopathic thrombocytopenic purpura? Analysis of 207 eradicated chronic ITP cases in Japan. Int J Hematol. 2005;81:162–8.

7. Kurata Y, Fujimura K, Kuwana M, Tomiyama Y, Murata M. Epidemiology of primary immune thrombocytopenia in children and adults in Japan: a population-based study and literature review. Int J Hematol. 2011;93:329–35.
8. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood. 2009;115:168–86.
9. Neunert C, Lim W, Crowther M, Cohen A, Solberg L Jr. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood. 2011;117: 4190–207.
10. Arnold DM, Bernotas A, Nazi I, Stasi R, Kuwana M, Liu Y, et al. Platelet count response to *H. pylori* treatment in patients with immune thrombocytopenic purpura with and without *H. pylori* infection: a systematic review. Haematologica. 2009;94:850–6.
11. Arnold DM, Stasi R. Does *Helicobacter pylori* eradication therapy result in a platelet count improvement in adults with immune thrombocytopenic purpura regardless of *H pylori* infection? ASH evidence-based review. Hematology Am Soc Hematol Educ Program. 2008;2008:31–2.
12. Kuwana M, Ikeda Y. *Helicobacter pylori* and immune thrombocytopenic purpura: unsolved questions and controversies. Int J Hematol. 2006;84:309–15.
13. Suzuki T, Matsushima M, Masui A, Watanabe K, Takagi A, Ogawa Y, et al. Effect of *Helicobacter pylori* eradication in patients with chronic idiopathic thrombocytopenic purpura-a randomized controlled trial. Am J Gastroenterol. 2005;100: 1265–70.
14. Tsumoto C, Tominaga K, Okazaki H, Tanigawa T, Yamagami H, Watanabe K, et al. Long-term efficacy of *Helicobacter pylori* eradication in patients with idiopathic thrombocytopenic purpura: 7-year follow-up prospective study. Ann Hematol. 2009;88:789–93.
15. McMillan R. Therapy for adults with refractory chronic immune thrombocytopenic purpura. Ann Intern Med. 1997;126:307–14.
16. Stasi R, Provan D. Management of immune thrombocytopenic purpura in adults. Mayo Clin Proc. 2004;79:504–22.
17. Nomura S, Dan K, Hotta T, Fujimura K, Ikeda Y. Effects of pegylated recombinant human megakaryocyte growth and development factor in patients with idiopathic thrombocytopenic purpura. Blood. 2002;100:728–30.
18. Rank A, Weigert O, Ostermann H. Management of chronic immune thrombocytopenic purpura: targeting insufficient megakaryopoiesis as a novel therapeutic principle. Biologics. 2010;4:139–45.
19. Nplate (romiplostim), European Public Assessment Report summary. 2009.
20. Nplate™ (romiplostim) Prescribing Information. Amgen, Inc., Thousand Oaks, CA. 2011.
21. Nplate™ (romiplostim) Prescribing Information. European Medicines Agency. Amgen, Inc., Thousand Oaks, CA. 2011.
22. Romiplate prescribing information. 2011; http://www.mhlw.go.jp/stf/shingi/2r985200000136yg-att/2r9852000001372i.pdf.
23. Burroughs VJ, Maxey RW, Levy RA. Racial and ethnic differences in response to medicines: towards individualized pharmaceutical treatment. J Natl Med Assoc. 2002;94:1–26.
24. Matthews HW. Racial, ethnic and gender differences in response to medicines. Drug Metabol Drug Interact. 1995;12:77–91.
25. Kumagai Y, Fujita T, Ozaki M, Sahashi K, Ohkura M, Ohtsu T, et al. Pharmacodynamics and pharmacokinetics of AMG 531, a thrombopoiesis-stimulating peptibody, in healthy Japanese subjects: a randomized, placebo-controlled study. J Clin Pharmacol. 2007;47:1489–97.
26. Shirasugi Y, Ando K, Hashino S, Nagasawa T, Kurata Y, Kishimoto Y, et al. A phase II, open-label, sequential-cohort, dose-escalation study of romiplostim in Japanese patients with chronic immune thrombocytopenic purpura. Int J Hematol. 2009;90:157–65.
27. Shirasugi Y, Ando K, Miyazaki K, Tomiyama Y, Okamoto S, Kurokawa M, et al. Romiplostim for the treatment of chronic immune thrombocytopenia in adult Japanese patients: a double-blind, randomized Phase III clinical trial. Int J Hematol. 2011;94: 71–80.
28. Kuter DJ, Bussel JB, Lyons RM, Pullarkat V, Gernsheimer TB, Senecal FM, et al. Efficacy of romiplostim in patients with chronic immune thrombocytopenic purpura: a double-blind randomised controlled trial. Lancet. 2008;371:395–403.
29. Gupta S, Indelicato SR, Jethwa V, Kawabata T, Kelley M, Mire-Sluis AR, et al. Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics. J Immunol Methods. 2007;321:1–18.
30. Mason S, La S, Mytych D, Swanson SJ, Ferbas J. Validation of the BIACORE 3000 platform for detection of antibodies against erythropoietic agents in human serum samples. Curr Med Res Opin. 2003;19:651–9.
31. Mire-Sluis AR, Barrett YC, Devanarayan V, Koren E, Liu H, Maia M, et al. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J Immunol Methods. 2004;289:1–16.
32. Kuter DJ, Bussel JB, Newland A, Wasser JS, Lyons RM, George JN, et al. Long-term efficacy and safety of romiplostim treatment of adult patients with chronic immune thrombocytopenia (ITP): final report from an open-label extension study. ASH Annu Meet Abstr. 2010;116:68.
33. Bussel JB, Kuter DJ, Pullarkat V, Lyons RM, Guo M, Nichol JL. Safety and efficacy of long-term treatment with romiplostim in thrombocytopenic patients with chronic ITP. Blood. 2009;113: 161–71.
34. Kuter DJ, Rummel M, Boccia R, Macik BG, Pabinger I, Selleslag D, et al. Romiplostim or standard of care in patients with immune thrombocytopenia. N Engl J Med. 2010;363:1889–99.

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ORIGINAL ARTICLE

C-terminal mutation of *RUNX1* attenuates the DNA-damage repair response in hematopoietic stem cells

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Loss-of-function mutations of *RUNX1* have been found in acute myeloid leukemia (AML) and myelodysplastic syndromes (MDSs). Although several reports have suggested roles for RUNX1 as a tumor suppressor, its precise function remains unknown. Because gene alterations of *RUNX1* by themselves do not lead to the development of leukemia in mouse models, additional mutation(s) would be required for leukemia development. Here, we report that the C-terminal deletion mutant of *RUNX1*, RUNX1dC, attenuates DNA-damage repair responses in hematopoietic stem/progenitor cells. γH2AX foci, which indicate the presence of DNA double-strand breaks, were more abundantly accumulated in RUNX1dC-transduced lineage$^-$ Sca1$^+$c-kit$^+$ (LSK) cells than in mock-transduced LSK cells both in a steady state and after γ-ray treatment. Expression profiling by real-time -PCR array revealed RUNX1dC represses the expression of *Gadd45a*, a sensor of DNA stress. Furthermore, bone marrow cells from MDS/AML patients harboring the *RUNX1*-C-terminal mutation showed significantly lower levels of *GADD45A* expression compared with those from MDS/AML patients with wild-type *RUNX1*. As for this mechanism, we found that RUNX1 directly regulates the transcription of *GADD45A* and that RUNX1 and p53 synergistically activate the *GADD45A* transcription. Together, these results suggest Gadd45a dysfunction due to *RUNX1* mutations can cause additional mutation(s) required for multi-step leukemogenesis.
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Keywords: RUNX1; DNA damage; GADD45A; acute myeloid leukemia; myelodysplastic syndromes

Introduction

Myelodysplastic syndromes (MDSs) are clonal hematological disorders derived from gene alterations at the level of hematopoietic stem cells, which are characterized by ineffective hematopoiesis, dysplastic morphology of blood cells and a high possibility of transition to acute myeloid leukemia (AML). A number of genetic or epigenetic alterations involved in the pathogenesis of MDS have been identified: activating point mutations of signaling molecules such as N-RAS and FLT3;[1] deletion, point mutations and/or silencing of cell cycle inhibitory molecules such as p15 and p53;[2,3] deletion, point mutations and generation of chimeric genes from transcriptional factors such as EVI-1 and RUNX1;[4,5] and point mutations of nuclear proteins such as nucleophosmin and TET2.[6,7] Among these changes, point mutations of *RUNX1* have been detected in about 10–20% of patients classified as MDS/AML (high-risk MDS and AML following MDS).[5] The transcription factor RUNX1 and its heterodimeric partner core-binding factor (CBF)β (also known as phosphatidylethanolamine-binding protein2β) comprise CBFs. CBFs are the most frequent targets of gene rearrangement and mutation in human leukemias; leukemias harboring mutations in either subunit of a CBF are commonly called CBF leukemias.[8] Recently, Tang *et al.*[9] reported *RUNX1* mutations were detected in 13.2% of 470 adult patients with *de novo* AML. In addition, hereditary loss-of-function mutations of *RUNX1* cause familial platelet disorder with predisposition to AML, which is characterized by decreased platelet count and propensity to develop AML.[10] These findings suggest RUNX1 works as a tumor suppressor and impaired RUNX1 function promotes leukemia development. Nonetheless, *RUNX1* deletion or dominant-negative inhibition of RUNX1 by itself is not sufficient for leukemia development in several mouse models,[11,12] indicating that additional cooperating events are required. However, the mechanisms by which impaired RUNX1 functions lead to subsequent genetic alterations are not fully understood.

Cells in the human body are always exposed to DNA stresses, which induce damages to chromosomal DNA.[13] Physiological stresses such as hydrolytic reactions, non-enzymatic methylations and oxygen radicals generate DNA-base lesions.[14] Environmental agents such as ultraviolet (UV), ionizing radiation and a lot of genotoxic chemicals also induce DNA damages including single- and double-strand breaks (DSBs). These DNA lesions are repaired through damage-specific repair pathways. However, if these lesions are left unrepaired, these cells alone and/or in combination with additional mutations would have a higher risk of tumor development.[15] In a previous study, it was reported that *Runx1*-deficient mice had an increased incidence of hematological malignancy compared with wild-type (WT) mice after treatment with the mutagen, *N*-ethyl-*N*-nitrosourea.[16]

We speculated RUNX1 might have a role in the DNA-damage repair (DDR) response. Here, we show that a C-terminal deletion mutant of *RUNX1*, RUNX1dC, enhances DNA-damage accumulation in hematopoietic stem cell-enriched lineage$^-$Sca1$^+$c-kit$^+$ (LSK) cells. Furthermore, we found RUNX1dC attenuates the DDR response after exposure to DNA-damage agents. As for this mechanism, we found that RUNX1dC suppresses the transcription of a sensor of DNA stress, *Gadd45a*.[17] Moreover, bone marrow (BM) cells from MDS/AML patients harboring a *RUNX1*-C-terminal mutation showed significantly lower *GADD45A* expression than those from MDS/AML patients with WT *RUNX1*. These results suggest RUNX1

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regulates *Gadd45a* transcription and impaired RUNX1 function can cause additional mutation(s) required for multi-step leukemogenesis.

Materials and methods

Real-time (RT)-PCR array

Total cellular RNA was extracted from 4×10^6 cells using the RNeasy Mini Kit (Qiagen, Tokyo, Japan). A total of 4 μg of RNA was reverse-transcribed into cDNA using the RT^2 First Strand Kit (SABiosciences, Frederick, MD, USA) and subjected to RT-PCR array analysis (RT^2Profiler PCR Array: Mouse DNA Damage Signaling, SABiosciences). Gene expression profiles of 32D-neo and 32D-RUNX1dC cells were analyzed by the ΔΔCt method.

Colony-forming assay

32D-neo and 32D-RUNX1dC cells (1×10^5 cells) were suspended in 500 μl of phosphate buffered saline and exposed to UV-B (800 or 1600 J/m^2). Then, cells were washed twice with PBS containing 2% FBS, plated into dishes (1×10^3 cells/dish) and cultured in the methylcellulose media M3231 (Stem Cell Technologies, Vancouver, British Colombia, Canada) containing mouse interleukin-3 (mIL-3). Colony numbers were counted after 7 days of culture. Also, retrovirus-transfected LSK cells were exposed to UV-B (2400 J/m^2), plated into dishes (2×10^3 cells/dish) and cultured in methylcellulose media M3434 (Stem Cell Technologies) containing mSCF, mIL-3, human IL-6 (hIL-6) and human erythropoietin. Colony numbers were counted after 10 days. All experiments were carried out in triplicate. DNA repair activities of the test cells are represented relative to those of untreated cells.

Quantitative PCR assays for the expression of GADD45A in MDS/AML patients

Total cellular RNA was extracted from BM mononuclear cells of 23 MDS/AML patients, 5 healthy donors and 5 non-Hodgkin

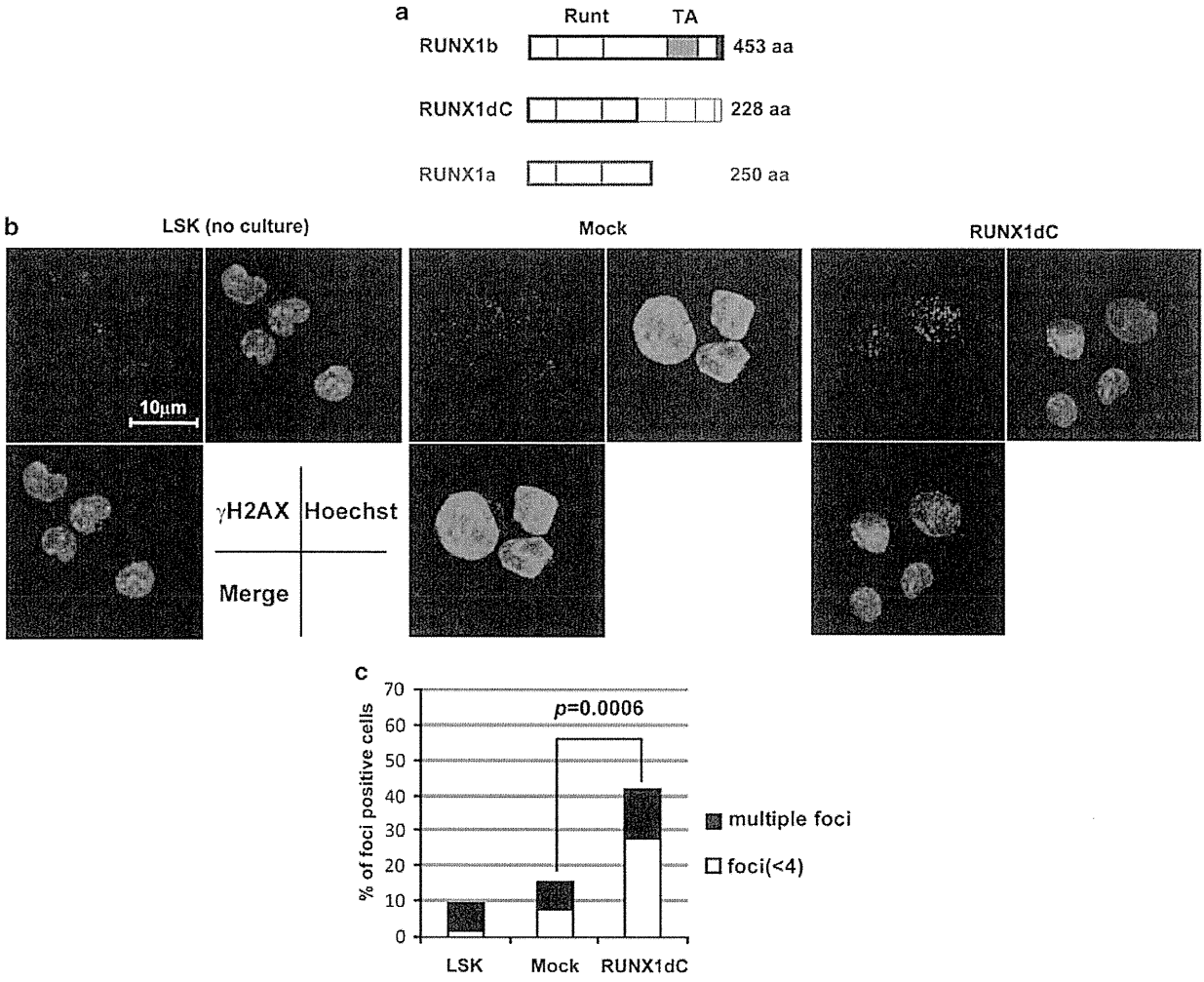

Figure 1 RUNX1dC induces DNA-damage accumulation in LSK cells. (**a**) Structures of WT RUNX1 (RUNX1b), RUNX1dC and RUNX1a. RUNX1dC lacks 225 C-terminal amino acids because of the insertion of ACCGT at 669–670 bp, which causes a frameshift mutation. (**b**) Accumulations of DSBs in Mock- and RUNX1dC-transduced LSK cells were detected by γH2AX antibody (2 days after gene transduction). LSK refers to LSK cells just after FACS sorting. Hoechst refers to Hoechst 33342. The scale bar (10 μm) applies to all images. (**c**) The percentage of γH2AX foci-positive cells in Mock- and RUNX1dC-transduced LSK cells. Results shown are the average of three experiments. Open square indicates % of γH2AX foci-positive cells (with fewer than four foci per cell), and closed square indicates % of multiple foci-positive cells (four or more foci per cell).