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ORIGINAL ARTICLE

Comparative analysis of remission induction therapy for high-risk MDS and AML progressed from MDS in the MDS200 study of Japan Adult Leukemia Study Group

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Abstract A total of 120 patients with high-risk myelodysplastic syndrome (MDS) and AML progressed from MDS (MDS–AML) were registered in a randomized controlled study of the Japan Adult Leukemia Study Group (JALSG). Untreated adult patients with high-risk MDS and MDS–AML were randomly assigned to receive either idarubicin and cytosine arabinoside (IDR/Ara-C) (Group A) or low-dose cytosine arabinoside and aclarubicin (CA) (Group B). The remission rates were 64.7% for Group A (33 of 51 evaluable cases) and 43.9% for Group B (29 out of 66 evaluable cases). The 2-year overall survival rates and disease-free survival rates were 28.1 and 26.0% for Group A, and 32.1 and 24.8% for Group B, respectively. The duration of CR was 320.6 days for Group A and 378.7 days for Group B. There were 15 patients who lived longer than 1,000 days after diagnosis: 6 and 9 patients in Groups A and B, respectively. However, among patients enrolled in this trial, intensive chemotherapy did not produce better survival than low-dose chemotherapy. In conclusion, it is necessary to introduce the first line therapy excluding the chemotherapy that can prolong survival in patients with high-risk MDS and MDS–AML.

For the Japan Adult Leukemia Study Group.

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1 Introduction

Myelodysplastic syndrome (MDS) is a group of disorders in which abnormalities occur at the level of hematopoietic stem cells [1], leading to disturbance in the production of blood cells characterized by ineffective hematopoiesis [2], decrease in the number of peripheral blood cells and morphological/functional abnormalities in blood cells [3]. Allogeneic hematopoietic cell transplantation (allo-HCT) is the most effective curative therapy for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [4]. However, for patients with high-risk MDS (those with refractory anemia with excess of blasts in transformation (RAEB)-t and some patients with RAEB) and patients with acute myeloid leukemia progressed from MDS (MDS–AML), chemotherapy aimed at remission is being used. The reasons for this are that MDS often affects elderly people [5], suitable donors are not always available at the time of disease onset, the necessity of pretransplant conditioning chemotherapy is controversial [6, 7] with a lack of sufficient evidence, and the optimal timing for transplantation varies widely depending on disease type [8].

On the other hand, reduced-intensity conditioning has extended the use of allo-HSCT to patients otherwise not eligible for this treatment due to older age or frailty [9]. However, allo-HSCT using traditional myeloablative preparative regimens is not easily tolerated by the elderly or frailer patient, and may lead to prohibitive treatment-related mortality rates. Most patients treated in the past were younger and devoid of comorbid clinical conditions. Novel reduced-intensity regimens have recently made allogeneic transplants applicable to the elderly, providing the benefit of the graft-versus-leukemia effect to a larger number of patients in need [10].

Low-dose chemotherapy, which has been used in clinical practice for 20 years, reduces the number of myeloblasts, improves pancytopenia and induces remission not only in MDS patients but also in some MDS–AML patients [11]. Common antineoplastic agents used in low-dose chemotherapy include cytosine arabinoside (Ara-C), aclarubicin (ACR), melphalan and etoposide. Nevertheless, despite improved Ara-C and regimens, the prognosis of AML in patients beyond 60 years of age remains dismal [4]. Low-dose antineoplastic drug therapy is still being used in some patients with MDS, which is common in elderly people, especially when the patient is at risk due to poor general condition or organ disorder [12].

The Japan Adult Leukemia Study Group (JALSG) previously conducted a pilot study for the treatment of high-risk MDS and MDS–AML to compare low-dose monotherapy with low-dose Ara-C plus granulocyte colony-stimulating factor (G-CSF) and multiple drug therapy with Ara-C plus Mitoxantrone plus VP-16. Later, JALSG conducted studies using a single protocol (JALSG MDS96) in 1996, in which remission induction and post-remission therapies using Ara-C and IDR in patients with high-risk MDS (RAEB-t) and in those with MDS–AML were performed, after which the efficacy and safety of these therapies were evaluated [13]. Furthermore, a randomized controlled study (JALSG MDS200) of intensive chemotherapy (IDR/Ara-C) or low-dose chemotherapy (CA) for high-risk MDS was also performed by JALSG.

Here, we present and analyze the results of the JALSG MDS200 study to assess and evaluate the validity of the MDS200 protocol for MDS treatment.

2 Patients and methods

2.1 Patient eligibility

A total of 120 patients were initially registered into the JALSG MDS200 study between June 2000 and March 2005. They were assigned into two groups, namely, Groups A and B (Table 1). Patients aged 15 years or more and diagnosed as having high-risk RAEB with high International Prognostic Scoring System score [14], RAEB-t or MDS-AML were eligible for this study. MDS–AML denotes secondary AML transformed from MDS.

Other eligibility criteria were as follows: patients with a performance status (PS) of 0–2 (ECOG); patients whose key organs other than the bone marrow retain intact function; patients who have not undergone any chemotherapy, except for pretreatment that does not affect the outcome of the main therapy; and patients who have given informed consent. Informed consent was obtained after carefully explaining the protocol and before registration.

2.2 Study protocol

The MDS200 protocol (Fig. 1) was designed based on the results of MDS96, and involved a dose-attenuation plan and allowed a wider range of chemotherapy. Patients were randomly assigned to either Group A or B.

In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age ($\geq$60 years), (2) hypoplastic bone marrow and (3) PS $\geq$ 2. Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS96). In therapy B, the use of

Table 1 Characteristics of patients

Group	A ($n = 53$)	B ($n = 67$)	P value (A vs. B)
Age (range)	63 (23–77)	61 (32–81)	0.505
Gender			
Male	37	52	0.332
Female	16	15	
Disease type			
HR-RAEB	4	11	0.269
RAEB-T	22	29	
MDS–AML	27	27	
Infection			
Presence	10	11	0.726
None	43	56	
Karyotype[a]			
Good	23 (44.2%) $n = 52$	21 (33.9%) $n = 62$	0.524
Int	11 (21.2%)	15 (24.2%)	
Poor	18 (34.6%)	26 (41.9%)	
PB (range)			
WBC (/μL)	2,500 (700–64,240)	2,720 (600–43,700)	0.665
Hb (g/dL)	8 (4.7–12.6)	7.9 (4.4–12.7) $n = 66$	0.562
Plt (/μL)	5.8 (0.2–31.4)	5.9 (0.5–36.7)	0.363
BM (range)			
Blast (%)	30 (4–95) $n = 51$	24.2 (1.9–96) $n = 66$	0.171
Biochemical data (range)			
LDH (IU/L)	296 (132–882)	303.5 (111–906) $n = 66$	0.998
CRP (mg/dL)	0.5 (0–20.2)	0.35 (0–11.7) $n = 66$	0.292

Patients who met all of the inclusion criteria and did not meet any of the stated exclusion criteria were included the study. The disease types were classified by FAB classification

Statistical analysis between Group A and Group B was done using χ^2 test or Mann–Whitney U-test

MDS myelodysplastic syndrome, *HR-RAEB* high risk-refractory anemia excess of blasts with high International Prognostic Scoring System Score, *RAEB-T* refractory anemia excess of blasts in transformation, *MDS–AML* MDS overt leukemia, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *PB* peripheral blood, *BM* bone marrow

[a] Shows IPSS risk

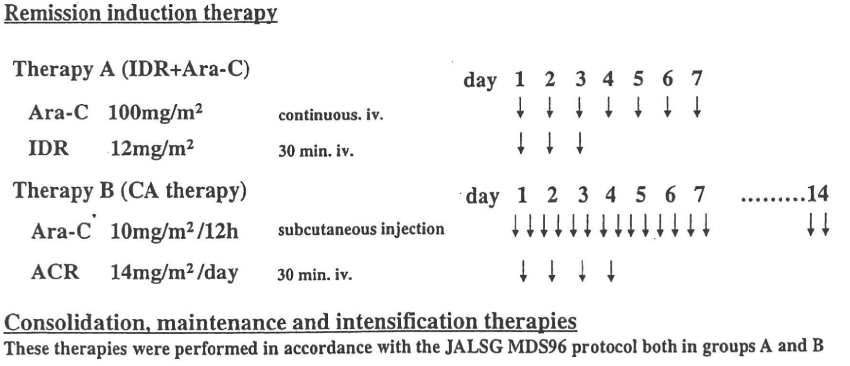

Fig. 1 Japan Adult Leukemia Study Group—myelodysplastic syndrome (JALSG MDS200 Protocol). In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age (≥60 years), (2) hypoplastic bone marrow and (3) PS ≥ 2. Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose, and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS-96). In therapy B, the use of CAG therapy involving co-administration of G-CSF was allowed. *IDR* idarubicin, *Ara-C* cytosine arabinoside, *ACR* aclarubicin, *G-CSF* granulocyte colony-stimulating factor, *iv* intravenous injection, *min* minutes

CAG therapy involving the co-administration of granulocyte colony-stimulating factor (G-CSF) was allowed.

Untreated adult patients (≥15 years) with MDS (RAEB, RAEB-t or MDS–AML) were randomly assigned to receive either IDR/Ara-C (Group A) or CA (Group B) [15]. Complete remission (CR) rate, CR duration, overall survival (OS) rate and disease-/relapse-free survival (DFS/RFS) rate were compared between the two groups.

Consolidation therapy and maintenance therapy were performed in accordance with JALSG MDS96 [13].

2.3 Evaluation of response

Response to treatment was evaluated in accordance with JALSG criteria [13]. CR was considered achieved when the following conditions remained for at least 4 weeks. For the bone marrow: blasts accounting for ≤5% of all cells; absence of blasts with Auer body; and presence of normal erythroblasts, granulocytes and megakaryocytes. For peripheral blood: absence of blasts; neutrophils ≥ 1,000/ml; platelets ≥ 100,000/μL; and no evidence of extramedullary leukemia. CR duration was defined as the duration from the day when CR is achieved to the day of relapse or death, OS or DFS as the duration from the day of initiation of treatment to the day of death and DFS as the duration in which CR patients survived without relapse. Patients who were treated with HCST were not censored at the date of transplantation. All toxicity was graded using the World Health Organization criteria [16].

2.4 Statistical analysis

The primary endpoint of this study is DFS. Assuming a 1-year DFS rate of 60% in the Group A and 40% in the Group B, this design required the randomization of 200 patients. Eligible patients were randomized according to age, sex and disease type. Differences in background factors (e.g., age, gender and disease type) between Groups A and B were statistically analyzed using the χ^2 test or Mann–Whitney *U*-test. Probability of OS and DFS were estimated according to the method of Kaplan and Meier.

3 Results

3.1 Recruitment of patients and suspension of the study

The initially registered 120 patients were assigned into two groups, namely, Groups A and B. The clinical characteristics of the registered patients are shown in Table 1. The present protocol was originally planned to recruit 200 patients for Groups A and B within 3 years. However, the recruitment pace was slower than expected and thus the study period was extended from 3 years to 4.5 years. At the end of 2004, that is, after 4.5 years from the start of the study, the number of registered patients was only 113 in Groups A and B, which was 56.5% of the target number. At that point, the committee members discussed the progress of the MDS200 study and decided to suspend it at the end of March 2005. Since the final total number of patients did not reach the target number, we did not statistically compare DFS between Groups A and B, which was the primary endpoint of this study.

3.2 Characteristics of patients

There were no clear differences in the clinical characteristics of the patients between Groups A and B, such as FAB subtype, initial blood cell count, presence of infection, distribution in the karyotype group and biochemical data, as well as sex distribution (male/female ratio, 37/16 = 2.315 in Group A, and 52/15 = 3.467 in Group B).

3.3 Treatment outcome

The remission rates were 64.7% in Group A (33 out of 51 evaluable cases) and 43.9% in Group B (29 out of 66 evaluable cases). The 2-year overall survival (OS) rates were 28.1% in Group A and 32.1% in Group B, and the 2-year DFS rates were 26.0% in Group A and 24.8% in Group B. The mean duration of CR was 320.6 days (median: 213 days) in Group A and 378.7 days (median: 273 days) in Group B (Table 2). Reflecting the intensity of the remission induction chemotherapy, the period of WBC (<1,000/μL) after the therapy was longer in Group A than in Group B (19 days and 4 days, respectively). There were more grade 3 or 4 adverse events during the remission induction therapy in Group A (19 out of 53 evaluable patients) than in Group B (13 out of 67 evaluable patients). This difference was mostly attributable to infectious episodes (17 patients in Group A and 4 patients in Group B). In terms of bleeding episodes, 1 patient in Group A and 2 in Group B had grade 3/4 adverse events. The numbers of

Table 2 Treatment outcome (Group A vs. B)

	Group A (n = 53)	Group B (n = 67)
Remission rate (%)	64.7	43.9
Mean duration of remission (days)	320.6 (median: 213)	378.7 (median: 273)
2-Year survival rate (%)	28.1	32.1
2-Year disease-free survival rate (%)	26.0	24.8

The remission rates, 2-year overall survival (OS) rates and 2-year disease-free survival (DFS) rates are shown as percentages

early death in remission induction chemotherapy (death within 30 days) were 1 patient in Group A and 3 patients in Group B (Table 3). The cause of death in each group was infection or tumor progression. The completion rate of consolidation therapies were 37.3% in Group A (12 out of 33 evaluable cases), 37.9% in Group B (11 out of 29 evaluable cases). On the other hand, the maintenance therapies were completed 21.2% in Group A (7 out of 33 evaluable cases), and 15.2% in Group B (5 out of 33 evaluable cases). The numbers of dose attenuation in Group A were 30 patients of 100% dose, 21 patients of 80% or 60% dose and 2 patients of unknown.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was performed in 11 out of 50 patients (22%) in Group A and 19 out of 66 patients (28.8%) in Group B. Among those who received allo-HSCT, the transplantation was performed during the first remission in 40%, 21% of patients in Groups A, B, respectively.

There were 15 patients who lived longer than 1,000 days after diagnosis: 6, 9 patients in Groups A, B, respectively. Regarding the transplantation among long-term survivors, 3 out of 6 patients were transplanted in Group A, 6 out of 9 in Group B. Comparing the achievement of CR among these patients in Groups A and B, all 6 patients in Group A achieved CR, but only 4 out of 9 patients in Group B achieved CR.

Table 3 Toxicity of the induction therapy

	A (n = 53) (range)	B (n = 67) (range)	P value (A vs. B)
Period of WBC <1,000 (day)	19 (0–44) n = 49	4 (0–50) n = 63	<0.0001
Toxicity (grade 3/4)			
Presence	19	13	0.427
Bleeding	2	1	ND
Infection	17	11	0.04
Others	2	2	ND
Early death (<30 days)	1	3	ND

Statistical analysis between Groups A and B was performed using the χ^2 test or Mann–Whitney U-test

ND not done

4 Discussion

In this MDS200 study, patients with high-risk MDS and AML transformed from MDS (MDS–AML) were treated with either intensive or low-dose remission induction therapy, followed by intensive post-remission therapy that was the same as in the JALSG MDS96 study [13].

Although we did not perform statistical comparison of DFS or OS between these two treatment groups due to the insufficient number of patients enrolled, the results suggest that there was no significant difference, that is, survival curves were superimposable (Figs. 2, 3). Intensive chemotherapy similar to that for AML can produce a CR rate of 64.7% for high-risk MDS and MDS–AML patients, whereas low-dose induction therapy can result in a CR rate of 43.9%. However, among the patients enrolled in this trial, the difference in CR rate did not lead to better survival as described above. In terms of adverse events, patients who received intensive treatment had more grade 3 or 4 adverse events, particularly infectious events with a longer period of leukopenia. There was no increase in the number of patients succumbing to early death (death within 30 days after the

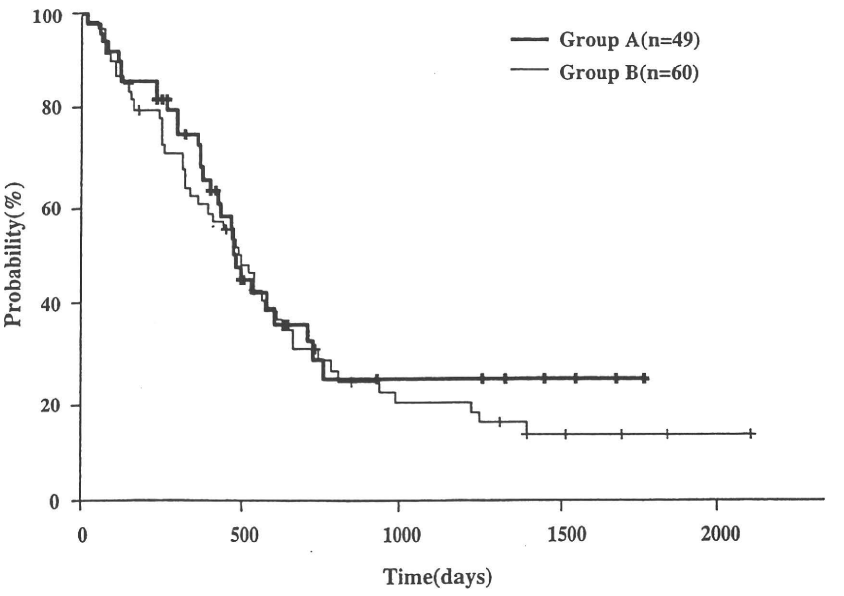

Fig. 2 Overall survival. Survival was calculated from the date of the start of treatment to the date of death due to any cause or to the date of the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR or not, but known to be alive or not

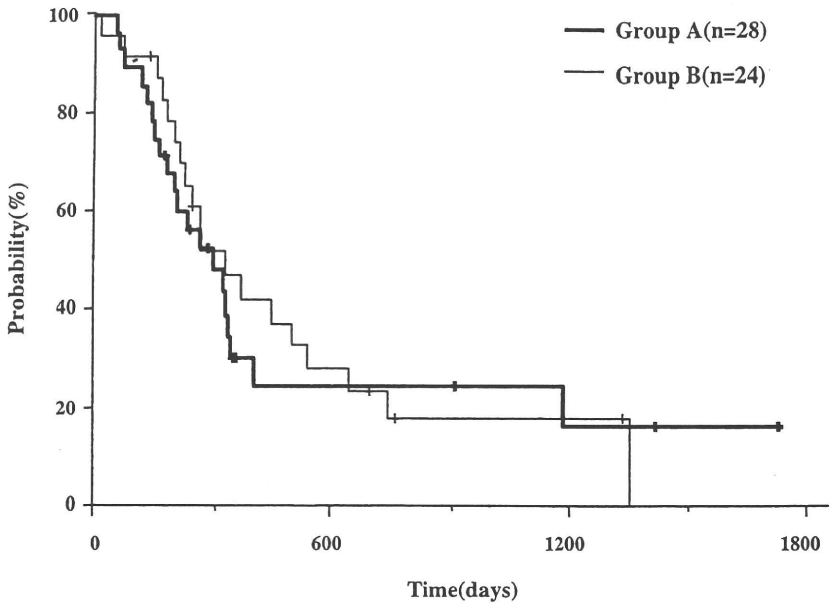

Fig. 3 Disease-/relapse-free survival. RFS was calculated from the date of achieving complete remission to the date of relapse, death or the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR state or relapse, but known to be alive or not

start of treatment) in Group A, suggesting that intensive treatment produced higher CR rate, and higher toxicity resulted in a similar survival rate with low-dose induction therapy at least during the early phase of treatment.

There are several reasons that could explain why no difference in survival rate was observed regardless of the difference in CR rate. One could be the similar post-remission therapy between Groups A and B, as demonstrated by the almost similar DFS curves among the two groups. Another reason could be the disease status at the time of transplantation for patients in the two groups. In Group A, 60% of the transplantation was performed during the period other than that covering the first CR; this was 79% in Group B. Allo-HSCT has been shown to have the strongest antileukemia effect, and this was also found in the current study in which 6 out of 15 long-term survivors received allo-HSCT in Groups A and B. From the viewpoint of transplantation, intensive treatment merely selected cases that were suitable for transplantation, as observed in the case of transplantation for relapsed AML patients [17]. There are arguments against remission induction therapy for MDS patients in that it does not affect post-transplant prognosis [6, 18]. In the results of JSHCT, the chemotherapy before undergoing allo-SCT is not necessary in patients with MDS [6]. A group from the Institute of Medical Science of Tokyo University performed umbilical cord blood stem cell transplantation without remission induction therapy in high-risk MDS patients aged not more than 55 years and obtained favorable results with reduced time from diagnosis to transplantation [19]. It is important to perform clinical studies based on the concept that HCST should be performed immediately after diagnosis without remission induction, and determine the types of patients who would benefit from remission induction therapy prior to transplantation in terms of prognosis. In the present study, although suspended because of the insufficient number of patients enrolled, it appears that remission induction therapy with IDR and Ara-C did not produce better survival than that with low-dose chemotherapy despite higher CR rate. Therefore, it is suggested that CR rate is not a suitable surrogate marker for the evaluation of the outcome of chemotherapy for high-risk MDS and MDS–AML. In the latest reports, induction chemotherapy for patients with high-risk MDS and MDS–AML also provide no survival advantage [20, 21]. Considering the low survival rate of patients in this category, it is clearly necessary to introduce new strategies for the treatment of high-risk MDS and MDS–AML, such as molecular targeting agents and allo-HSCT with reduced-intensity conditioning regimens.

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ORIGINAL ARTICLE

Management of infection in patients with acute leukemia during chemotherapy in Japan: questionnaire analysis by the Japan Adult Leukemia Study Group

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Abstract Guidelines for the management of febrile neutropenia (FN), deep fungal infection or use of granulocyte colony-stimulating factor (G-CSF) published in the US and Europe cannot be directly applied in other countries. In this study, we undertook a questionnaire survey of member institutions of the Japan Adult Leukemia Study Group to investigate the status of, and problems with, the management of infectious complications in patients with acute leukemia. The questionnaire consisted of 52 multiple-choice questions covering therapeutic environment, antibacterial, and antifungal prophylaxis, empirical therapy (ET) for FN, and use of G-CSF. The results were compared to a previous survey performed in 2001. Usable responses were received from 134 of 184 (71.7%) institutions. With regard to antibacterial prophylaxis, fluoroquinolones and sulfamethoxazole-trimethoprim were most commonly used. Regarding antifungal prophylaxis, the most frequently used agent was fluconazole, followed by itraconazole. In ET for FN, monotherapy with cephems or carbapenems accounted for almost all of the responses. Most respondents indicated that they used micafungin (MCFG) in ET. Prophylactic use of G-CSF during remission induction therapy in acute myeloid leukemia was reported by only 4% of respondents. Strategies for antibacterial and antifungal prophylaxis or treatment of FN should be reviewed and updated as needed.

Keywords JALSG · Febrile neutropenia · Prophylaxis · G-CSF · Leukemia

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1 Introduction

Recent advances in chemotherapy and transplantation have improved the treatment of adult acute leukemia. The major complication during chemotherapy for acute leukemia is infection, such as sepsis and pneumonia, highlighting the clinical importance of preventing and treating infection in these patients. Guidelines for the management of febrile neutropenia (FN) or deep fungal infection or use of colony-stimulating factors (CSFs) published in the US and Europe cannot necessarily be applied in other countries due to differences in national health systems and in climate, especially humidity. For this reason, a guideline specific to Japanese settings was released in 1998 [1], and subsequently revised in 2004 [2]. A barrier to the development of such guidelines is the lack of domestic information on the actual management of infectious complications. The Japan Adult Leukemia Study Group (JALSG) was established in 1987 and is the largest adult leukemia study group in Japan. Although the identical chemotherapeutic regimen is administered by all JALSG-member institutions, supportive care is decided by each institution. A fact-finding questionnaire on the management of infectious complications in patients with acute leukemia, developed by the Supportive Care Committee of the JALSG, was distributed to all 196 and 187 member institutions in 2001 and 2007, respectively. In this report, we evaluate the results of the follow-up 2007 questionnaire. In particular we investigated

the current status of, and problems with, the management of infectious complications according to members of the JALSG, especially focusing on current antibiotic regimens. The results were compared to the previous analysis performed in 2001 [3, 4].

2 Methods

A questionnaire on infectious complications in patients with acute leukemia was mailed to all members of the JALSG in July 2007, and the results were collected by the end of September 2007 and analyzed. The questionnaire consisted of 52 multiple-choice questions covering the therapeutic environment, antibacterial prophylaxis, antifungal prophylaxis, empirical therapy (ET) for FN and treatment in patients unresponsive to ET, therapy for deep fungal infection, the use of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) drugs, and the use of granulocyte colony-stimulating factor (G-CSF). Responses were calculated as percentage of respondents. For all questions, multiple answers were allowed giving the possibility of scores greater than 100%. Respondents were instructed to complete the questionnaire as follows: in principle, they should assume the development of neutropenia during remission induction therapy for acute myeloid leukemia (AML), as it is a frequent complication of infection; answers should reflect actual practice rather than literature knowledge or goals; each JALSG institution should answer according to its own practice, and whenever possible the questionnaire should be completed by a physician involved in daily practice. Approval for the study was obtained from the JALSG. The results of the current survey were compared with those from the 2001 survey.

3 Results

Usable responses were received from 134 out of 184 institutions (Appendix 1), giving a response rate of 71.7% for the 2007 survey (compared with a response rate of 63.8% for the 2001 survey). Answers to the main questions are summarized below.

3.1 Therapeutic environment

Overall, 98% of respondents indicated that their institution had a single- or multi-patient rooms with high efficiency particulate air filtration and laminar airflow (HEPA/LAF) units, and 76% said that a HEPA/LAF unit room was often used during AML remission therapy (vs. 37% in the survey of 2001). Given the fact that the same facility may use a variety of different ward settings for the management of neutropenia during AML remission induction, the respondents were allowed to select two alternative settings used at their institution, if applicable. On this basis, it was found that 66% used a single-patient room with HEPA/LAF unit, 27% used a multi-patient room with HEPA/LAF unit, 44% used a single-patient conventional isolation room with a portable HEPA/LAF system, and 19% used a multi-patient conventional isolation room with a portable HEPA/LAF system.

3.2 Prophylaxis

Oral drugs commonly used for antibacterial and antifungal prophylaxis are listed in Table 1. With regard to antibacterial prophylaxis, fluoroquinolones (58%), sulfamethoxazole-trimethoprim (ST, 37%) and polymixin-B (26%) were most commonly used, while 13% of respondents indicated that they did not use prophylaxis against bacterial infection. In contrast, in the 2001 survey the three most frequently used prophylactic drugs for bacterial infection

Table 1 Comparison of survey results regarding the drugs used for antibacterial and antifungal prophylaxis in Japanese hospitals in 2007 (134 institutions) and 2001 (125 institutions)

	2007 (%)	2001 (%)
Antibacterial agent		
1. PL-B	26	42
2. ST	37	30
3. Fluoroquinolones	58	52
4. Combinations of 1,2 and/or 3 above	3	5
5. Did not respond	3	2
6. No approved policy[a]	7	4
7. Did not use prophylaxis	13	6
Antibacterial agent		
1. FLCZ 100-200 mg	64	47
2. FLCZ 400 mg	1	3
3. MCFG 50 mg	1	NA
4. MCFG 75 mg	0	NA
5. ITCZ cap/os	25	14[b]
6. AMPH-B syrup	5	50[c]
7. Combination of AMPH-B and FLCZ	2	0
8. Did not respond	1	4
9. Did not use prophylaxis	8	3

Percentages exceed 100% since respondents could tick more than one box if applicable

NA not available in 2001, *PL-B* polymixin-B, *ST* sulfamethoxazole-trimethoprim, *MCFG* micafungin, *FLCZ* fluconazole, *ITCZ* itraconazole, *cap* capsule, *os* oral solution, *AMPH-B* amphotericin B

[a] Treatment policy not finalized by the hospital and thus no approved recommendation available, and decision was on a case by case basis

[b] Only capsules (200 mg/day) were available in 2001

[c] 300–2,400 mg/kg (2001)

were fluoroquinolones (52%), polymixin-B (42%) and ST (30%), and 6% of respondents gave no prophylaxis. Regarding antifungal prophylaxis, the most frequently used agent was fluconazole (FLCZ, alone or in combination) (67%) followed by itraconazole (ITCZ) (oral solution and capsule, 25%), and amphotericin B (AMPH-B, alone or in combination) syrup (7%), compared with FLCZ (50%), AMPH-B syrup (50%), and ITCZ (14%) in 2001. No prophylaxis for fungal infection was given by 8% of respondents (3% in 2001 survey). ST was used in remission induction therapy (32%) and consolidation therapy (32%) in patients with AML, and also in remission induction therapy (63%) and consolidation therapy (59%) in acute lymphoblastic leukemia (ALL).

3.3 Empirical antibiotic therapy

With regard to FN, intravenous treatment with antibiotics in patients with FN was given prophylactically before the onset of fever by 3% of respondents, and after the onset of fever at ≥37, ≥37.5, and ≥38°C by 4, 37, and 53%, respectively. Drugs used in ET for FN are listed in Table 2. Monotherapy with either cephems (77%) or carbapenems (31%) accounted for the majority of responses. Dual therapy which included aminoglycosides was used by 31%, including combinations with cephems (20%) or carbapenems (5%). Results from the 2001 survey showed that cephems plus aminoglycoside were used by 37% of respondents, cephems alone by 29%, and carbapenems alone by 21%.

The timing of anti-MRSA drug administration was investigated, and the results showed that administration was started by 12% of respondents if the first-line antibacterial therapy was ineffective, and by 41% if second-line or subsequent therapy was ineffective. Thirty-four percent of respondents started administration of anti-MRSA drugs if any gram-positive strain was detected in culture, while 7% said it was not used until a definite diagnosis of MRSA infection was made, and 12% said it was used as first-line therapy if the patient was at a high risk according to the US guidelines. Among anti-MRSA drugs, the initial drug prescribed was most frequently vancomycin (80%), followed by teicoplanin (13%), arbekacin (5%), or linezolid (2%).

Table 2 Comparison of survey results regarding empirical antibacterial therapy for febrile neutropenia in Japanese hospitals in 2007 (134 institutions) and 2001 (125 institutions)

Antibacterial agent	2007 (%)	2001 (%)
1. Cephems	77	29
2. Cephems + AG	20	37
3. Carbapenems	31	21
4. Carbapenems + AG	5	16
5. Antipseudomonal penicillins	5	1
6. Antipseudomonal penicillins + AG	5	14
7. Cephems + Antipseudomonal penicillin	0	4
8. Cephems + Antipseudomonal penicillin + AG	0	1
9. AntiMRSA[a] + Cephems	1	1
10. AntiMRSA[a] + Carbapenems	0	2
11. AntiMRSA[a] + Cephems + AG	0	0
12. AntiMRSA[a] + Carbapenems + AG	0	0
13. Others	1	10
14. None	0	0

Percentages exceed 100% since respondents could tick more than one box if applicable

AG aminoglycoside

[a] With regard to anti-MRSA, the 2001 questionnaire only enquired about vancomycin

3.4 Empirical and targeted antifungal therapy

With regard to antifungal therapy, standard ET and a preemptive/presumptive approach using β-glucan/galactomannan or CT-scan was adopted by 54 and 42% of respondents, respectively. The timing of initiation of antifungal therapy was selected empirically by 54% of respondents, while 20% said it was preemptive and 22% said it was done presumptively. Among respondents who used an empirical approach for antifungal treatment, 88% prescribed micafungin (MCFG) either with or without antifungal prophylaxis. The percentage of each antifungal agent is shown in Table 3. In targeted therapy for *Candida albicans*, *Candida glablata*, and *Candida parapsilosis*, the

Table 3 Drugs used for empirical antifungal therapy in respondents of the 2007 survey of Japanese hospitals who used an empirical approach ($n = 73$)

Antifungal agent	Patients with febrile neutropenia	
	Without antifungal prophylaxis 2007 (%)	With antifungal prophylaxis 2007 (%)
1. FLCZ	4	8
2. MCFG	11	77
3. VRCZ	3	12
4. ITCZ	0	4
5. L-AMB	1	3
6. AMPH-B	1	0
7. Others	0	0

Percentages exceed 100% since respondents could tick more than one box if applicable

FLCZ fluconazole, *MCFG* micafungin, *VRCZ* voriconazole, *ITCZ* itraconazole, *L-AMB* liposomal amphotericin B, *AMPH-B* amphotericin B

three most commonly used drugs were FLCZ, MCFG, and voriconazole (VRCZ). The rates of FLCZ use for these organisms were 57, 2, and 7%, respectively; rates for MCFG use were 28, 50, and 26%, and for VRCZ were 7, 27, and 36%, respectively. In the treatment of invasive pulmonary aspergillosis (IPA), VRCZ and L-AMB were used by 69 and 21% of respondents, respectively. This was markedly different from the 2001 survey, in which the main treatments used for IPA were AMPH-B (80%) and combination treatment (18%) (Table 4).

In fungemia, susceptibility to antimycotic drugs was tested by only 28% of responders. ß-D-glucan measurement was used for monitoring or early diagnosis of fungemia by 99% of facilities, and most respondents (53%) reported that the frequency of ß-D-glucan testing was once a week. It was performed either by an outside contractor (46%, including Biochemical Seikagaku 45% and Wako 1%) or in-house using a Wako test kit (34%) or using a biochemical method such as Fungitec (9%). With regard to galactomannan antigen, 75% of facilities used this method for the purpose of diagnosis of IPA, compared with 57% in 2001. Most responders (71%) did not perform genetic diagnosis.

3.5 Use of G-CSF

Because the use of G-CSF in the treatment of AML and ALL may differ, questions were asked separately for AML remission induction therapy (disappearance of blasts in peripheral blood), AML consolidation therapy (with or without high dose cytarabine), ALL remission induction therapy, and ALL consolidation therapy. The results are shown in Tables 5 and 6. While G-CSF was used primarily in the treatment of life-threatening infections in patients with AML (25–37%; versus 28% in the 2001 survey), prophylactic use of G-CSF before the onset of fever was common in patients with ALL (63–65%), compared with the 2001 survey rates of 52–54%. Only 4% of respondents used prophylactic G-CSF during remission induction therapy in AML.

4 Discussion

This survey adds additional information to the previous 2001 survey on the treatment practices of member hospitals of the JALSG with regard to infectious complications in patients with acute leukemia during chemotherapy in Japan. This is useful because guidelines for the management of FN, deep fungal infection, or the use of G-CSF published in the US and Europe cannot necessarily be applied in other countries.

The proportion of institutions using single- or multi-patient rooms with a HEPA/LAF unit for AML remission therapy increased from 37% in 2001 to 76% in 2007. Of the member institutions of JALSG, 98% have a room with HEPA/LAF unit. The National Health Insurance reimbursement for the treatment of leukemia in a room with HEPA/LAF unit is 30,000 yen (about 300 US dollars) per

Table 4 Comparison of survey results regarding targeted antifungal therapy for fungal infection in Japanese hospitals in 2007 (134 institutions) and 2001 (125 institutions)

Antifungal agent	*Candida albicans* 2007 (%)	*Candida glablata* 2007 (%)	*Candida parapsilosis* 2007 (%)	Candidemia 2001 (%)	Invasive pulmonary aspergillosis 2007 (%)	Invasive pulmonary aspergillosis 2001 (%)
1. FLCZ	57	2	7	26	0	0
2. MCFG	28	50	26	NA	4	NA
3. VRCZ	7	27	36	NA	69	NA
4. ITCZ	1	4	9	0	1	2
5. L-AMB	3	13	16	NA	21	NA
6. AMPH-B	2	4	4	58	3	80
7. MCZ	0	0	0	3	0	0
8. FLCZ + AMPH-B	0	0	0	12	0	0
9. 5FC + AMPH-B	0	0	0	1	0	2
10. ITCZ + AMPH-B	0	0	0	1	0	16
11. MCFG + VRCZ	0	0	0	NA	1	NA
12. No approved policy[a]	1	1	2	0	1	0
13. Others	0	0	0	0	0	0

Percentages exceed 100% since respondents could tick more than one box if applicable

NA not available in 2001, *FLCZ* fluconazole, *MCFG* micafungin, *VRCZ* voriconazole, *ITCZ* itraconazole, *L-AMB* liposomal amphotericin B, *AMPH-B* amphotericin B, *MCZ* miconazole, *5FC* flucytosine

[a] Treatment policy not finalized by the hospital and thus no approved recommendation available, and decision was on a case by case basis

Table 5 Comparison of survey results regarding the use of G-CSF in AML in Japanese hospitals in 2007 (134 institutions) and 2001 (125 institutions)

Clinical status	AML remission induction 2007 (%)	AML remission induction 2001 (%)	AML consolidation high dose Ara-C regimen 2007 (%)	AML consolidation no high dose Ara-C regimen 2007 (%)	AML consolidation 2001 (%)
1. Prophylaxis for neutropenia	4	3	13	7	8
2. FN with ET	7	12	13	10	10
3. FN if refractory to ET	16	20	17	19	19
4. Clinically documented infection	26	23	24	31	21
5. Microbiologically documented infection	11	6	9	10	5
6. Life-threatening infection	37	28	25	30	28
7. Not used	10	7	6	7	7
8. No approved policy[a]	1	3	3	2	3
9. Others	1	3	4	2	2

G-CSF granuloyte-colony stimulating factor, *AML* acute myeloid leukemia, *Ara-C* cytarabine, *FN* febrile neutropenia, *ET* empiric antibiotic therapy

[a] Treatment policy not finalized by the hospital and thus no approved recommendation available, and decision was on a case by case basis

Table 6 Comparison of survey results regarding the use of G-CSF in ALL in Japanese hospitals in 2007 (134 institutions) and 2001 (125 institutions)

Clinical status	ALL remission induction 2007 (%)	ALL remission induction 2001 (%)	ALL consolidation 2007 (%)	ALL consolidation 2001 (%)
1. Prophylaxis for neutropenia	65	52	63	54
2. FN with ET	10	20	10	18
3. FN if refractory to ET	13	14	14	13
4. Clinically documented infection	6	2	6	4
5. Microbiologically documented infection	5	2	6	2
6. Life-threatening infection	6	1	5	1
7. Not used	1	0	1	1
8. No approved policy[a]	2	3	3	4
9. Others	2	5	3	4

G-CSF granulocyte-colony stimulating factor, *ALL* acute lymphoblastic leukemia, *FN* febrile neutropenia, *ET* empiric antibiotic therapy

[a] Treatment policy not finalized by the hospital and thus no approved recommendation available, and decision was on a case by case basis

patient per day in Japan. HEPA/LAF units are quite effective in the management of fungal infection, and this administrative policy did much to help increase the number of institutions in which a room with a HEPA/LAF unit is available, not only for patients receiving hematopoietic stem cell transplantation, but also for patients with leukemia.

The prophylactic use of fluoroquinolones during neutropenia after chemotherapy in patients with AML is controversial. In the current survey, 58% of respondents indicated that they used fluoroquinolones. The Infectious Disease Society of America (IDSA) guideline published in 2002 did not recommend the routine use of antibiotic prophylaxis in afebrile neutropenic patients except for ST, which was used to prevent *Pneumocystis jiroveci*. In contrast, a large-scale meta-analysis published in 2005 reported that all-cause mortality, as well as infection-related mortality, fever and the risk of documented infection, were lower in patients receiving fluoroquinolones compared with a placebo group [6]. A prospective randomized study published in the same year by Bucaneve and colleagues also concluded that fluoroquinolones were effective in the prophylactic treatment of bacterial infections, with the risk of fever, microbiologically documented infection, and bacteremia reduced to a greater extent in a levofloxacin group compared with a placebo group [7]. Since fluoroquinolone use has the potential to increase the risk of producing fluoroquinolone resistant gram-negative bacilli, this may raise some epidemiological concerns; nonetheless, fluoroquinolones can be useful for prophylaxis, particularly in patients with neutropenia of long duration, such as during the chemotherapeutic treatment of leukemia.

With regard to antifungal prophylaxis, the most frequently used agent was FLCZ, at 67%, followed by ITCZ (oral solution and capsule), at 25%. In contrast, use of amphotericin (as AMPH-B syrup) was only 7%. Compared to the previous survey, the use of AMPH-B syrup decreased markedly. Recently, a large-scale meta-analysis comparing anti-fungal prophylaxis with placebo, no treatment, or nonsystemic antifungals in cancer patients after chemotherapy was reported [8]. According to this analysis, the use of antifungal prophylaxis in patients with acute leukemia resulted in the reduction of fungal-related mortality rates and documented invasive fungal infections. There was a reduction in all-cause mortality, which did not reach statistical significance. This meta-analysis also found that ITCZ, posaconazole, and amphotericin (i.e. drugs with anti-mold activity), rather than FLCZ, reduced the risk of documented aspergillus infection and possibly had some effect on all-cause mortality [8]. The incidence of invasive fungal infection and the species of offending organisms varied widely between institutions, and the local epidemiology of fungal infections is very important in making therapeutic choices. If IPA is prevalent at institutions, agents with anti-mold activity, such as ITCZ, are more appropriate than FLCZ for antifungal prophylaxis. On the basis of this background, a nationwide epidemiological investigation of IPA is being undertaken in Japan and the results are awaited with keen interest.

Among patients with fever, treatment of those with neutropenia was primarily symptom-based, with 53% initiating treatment in those with a temperature of 38°C. With regard to empirical therapy for FN, monotherapy with cephems or carbapenems was most common, while in patients unresponsive to empirical therapy, the addition of, or change to, cephems, aminoglycosides, or carbapenems was implemented by 49% of respondents. These results might reflect the influence of reports on mono- and dual-therapy randomized controlled trials published overseas [9] or in Japan [10] since the previous survey. The incidence of infections with gram-positive cocci causing FN has increased in recent years. This change can be accounted for by a relative decrease in the incidence of infections with gram-negative bacilli because of prophylaxis with fluoroquinolones, and an increase in the incidence of intravascular catheter-related infections. The timing of initiation of anti-MRSA agents in the treatment of FN has always been debated. The National Health Insurance system of Japan supports the use of anti-MRSA drugs only for patients with documented MRSA infection. Thus, it is difficult to use anti-MRSA drugs as part of initial empirical therapy and in this survey the proportion of institutions withholding use until MRSA infection was confirmed was 7%. Compared to the results of the 2001 survey, in which the proportion was 30%, this percentage has clearly decreased. The US IDSA guideline suggests that the use of intravenous vancomycin should be limited wherever possible, on account of the potential emergence of vancomycin-resistant organisms [5]. The IDSA guideline also notes that initial empirical therapy with vancomycin is allowed only when the following clinical findings are obtained: (1) clinically suspected serious catheter-related infections, (2) known colonization with penicillin- and cephalosporin-resistant pneumococci or MRSA, (3) positive results of blood culture for gram-positive bacteria before final identification and susceptibility testing, and (4) hypotension or other evidence of cardiovascular impairment. The National Health Insurance of Japan does not cover the use of anti-MRSA agents except for the treatment of documented infections with MRSA. While patients would suffer greatly from such infections, the situation would be more serious if resistant organisms were to appear. From an epidemiological viewpoint we think that the use of anti-MRSA agents should continue to be restricted. Similar concerns have been expressed regarding the use of the carbapenems, although they are included in both the IDSA and Japanese guidelines. Interestingly, carbapenem use (with or without an aminoglycoside) remained relatively constant between the 2001 and 2007 surveys, and perhaps somewhat disappointingly the use of antipseudomonal penicillins remained at a relatively low level.

Among antifungal agents used in empirical therapy for FN, the recent launch of new products has led to marked changes in patterns of administration. MCFG, which was not marketed at the time of the previous survey, was the most frequently prescribed agent, at 88% among respondents who used an empirical approach to antifungal treatment. In contrast, the use of ITCZ and L-AMB was low (each $\leq$4%) in spite of their indication for FN. With regard to strain-specific treatment, FLCZ was most frequently prescribed (57%) for *C. albicans*, followed by MCFG (28%). Among other fungi, MCFG was most frequently prescribed for *C. glablata* (50%), followed by VRCZ (27%), L-AMB (13%), and ITCZ and AMPH-B (4%) while FLCZ was prescribed least often (2%). For *C. parapsilosis*, VRCZ was most frequently prescribed (36%), followed by MCFG (26%), L-AMB (16%), ITCZ (9%), and FLCZ (7%). Invasive aspergillosis was treated with VRCZ in 69% of respondents, probably owing to overseas evidence, particularly the Herbrecht study [11].

With regard to the determination of ß-D-glucan, only 1% failed to perform this test. Determination was contracted out by 46% of institutions, while the Wako kit was most commonly used by those hospitals performing this test in-house (34%). Based on the survey, opinion on the suitability of ß-D-glucan for use as a screening tool for fungi, especially *Candida* or *Aspergillus* appears to vary. In contrast, determination of galactomannan antigen had increased from 57 to 75% in 2007.

The criteria for starting G-CSF therapy differs between the US and Japan. According to the American Society of Clinical Oncology (ASCO) guideline published in 2006, the use of G-CSFs following initial induction therapy for AML is reasonable [12]. The ASCO guideline states that CSFs have no favorable impact on remission rate, remission duration or survival, but have beneficial effects on the incidence of severe infection. However, most Japanese hematologists prefer to use G-CSF for documented infections in patients with AML, because of the possible stimulating activity of G-CSF on AML cells. The results of the 2007 questionnaire showed little change from the 2001 survey with regard to AML induction therapy, AML consolidation therapy, ALL induction therapy, or ALL consolidation therapy.

In conclusion, comparison of the results of the present survey with those from 2001 highlights some significant changes in the use of drugs for the management of infections, including antifungal prophylaxis and empirical therapy. One reason for these changes is the introduction of a number of new antifungal agents since 2001. In addition, anti-MRSA drugs have also been launched, meaning that treatment strategies for FN will change further in the future. Guidelines for FN are currently available, and therapeutic measures should be reviewed and updated as needed, bearing in mind the changing medical environment in Japan, including technical improvements in diagnostic methods and the launch of new antibacterial and antifungal agents. This 2007 questionnaire analysis provides background information which broadens our perspective of current prophylactic practices in the treatment of acute adult leukemia in Japan, and will hopefully help establish guidelines for the management of infections in the fight against FN and leukemia.

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Appendix 1: Institutions responding to the questionnaire

Nihon University School of Medicine, Higashijujo Hospital, Kasukabe Municipal Hospital, Tokyo Metropolitan Komagome Hospital, Nagoya University Graduate School of Medicine, Daido Hospital, Yokkaichi Municipal Hospital, Aichi Cancer Center, Japanese Red Cross Nagoya First Hospital, Fujita Health University School of Medicine, Mie University Graduate School of Medicine, Suzuka Kaisei Hospital, Takeuchi Hospital, Kinki University School of Medicine, Osaka Medical Center for Cancer and Cardiovascular Diseases, Shikoku Cancer Center, Atomic Bomb Disease Institute - Nagasaki University, Graduate School of Biomedical Sciences, Sasebo City General Hospital, National Hospital Organization Nagasaki Medical Center, Kumamoto University School of Medicine, Kumamoto City Hospital, NTT West Kyushu General Hospital, Jichi Medical School, Okayama University Hospital, National Hospital Organization Minami-Okayama Medical Center, Chugoku Central Hospital of the Mutual Aid Association of Public School Teachers, National Hospital Organization Okayama Medical Center, Okayama Rosai Hospital, Kagawa Rosai Hospital, Gunma University Graduate School of Medicine, National Hospital Organization Nishigunma National Hospital, Fukaya Red Cross Hospital, University of Fukui, Kurashiki Central Hospital, Kanazawa Medical Center, Shimada Municipal Hospital, National Cancer Center Hospital, International Medical Center, Saitama Medical University, Hyogo College of Medicine, National Hospital Organization Osaka National Hospital Internal Medicine, Takarazuka Municipal Hospital, Uegahara Hospital, Kawasaki Medical School, Chiba University Hospital, Chiba Aoba Municipal Hospital, Social Insurance Funabashi Central Hospital, Nara Medical University, Jikei University School of Medicine, Dokkyo University School of Medicine, National Hospital Organization Nagoya Medical Center, Kochi Medical School - Kochi University, Shiga University of Medical Science, National Cancer Center East, Anjo Kosei Hospital, St. Marianna University School of Medicine, Yokohama Seibu Hospital, Shinshu University School of Medicine, Nagano Red Cross Hospital, Tokyo Women's Medical University, Tama-Hokubu Medical Center, Hamamatsu University School of Medicine, Fujieda Municipal General Hospital, Kagoshima University Hospital, Tochigi Cancer Center, Kanazawa University Graduate School of Medical Science, Toyama City Hospital, Seirei Numazu Hospital, Hematology, Tokyo Medical, University, Kyorin University School of Medicine, Hokkaido University Graduate School of Medicine, Sapporo Kousei Hospital, Hakodate Central Hospital, Saiseikai Maebashi Hospital, Higashi Municipal Hospital of Nagoya, Tokai University School of Medicine, Yamaguchi University School of Medicine, Yamaguchi Prefecture Central Hospital, Osaka City University, University of Tokyo, Niigata University, Medical and Dental Hospital, Oita University Faculty of Medicine, Oita Prefectural Hospital, Kouseiren Tsurumi Hospital, National Kyushu Cancer Center, National Hospital Organization Kyushu Medical Center, Fukuoka Postal Services Agency Hospital, Aso Iizuka Hospital, Teikyo University School of Medicine, Teikyo University Mizonokuchi Hospital, Sapporo Hokuyu Hospital, Aichi Medical University, Yamagata University Faculty of Medicine, Aomori Prefectural Central Hospital, Hyogo Cancer Center, Kyoto Prefectural University of Medicine, Social Insurance Kyoto Hospital, Social Insurance Kobe Central Hospital, National Hospital Organization Shiga Hospital, National Defense Medical College,

Akita University School of Medicine, NTT Kanto Medical Center, Yokohama City University Hospital, Yokohama City University Medical Center, Kanagawa Cancer Center, Fujisawa City Hospital, Shizuoka Red Cross Hospital, Tohoku University School of Medicine, Osaki Citizen Hospital, Hiroshima University, Kagawa University, Kagawa Prefectural Central Hospital, Sakaide City Hospital, Juntendo University School of Medicine, Kanazawa Medical University, Kobe University Graduate School of Medicine, Jiaikai Imamura Bun-in Hospital, Ehime University Graduate School of Medicine, Metropolitan Bokuto Hospital, Otsu Red Cross Hospital, Yokohama City Minato Red Cross Hospital, Saitama Medical Center Jichi Medical University, Ehime Prefectural Central Hospital, International Medical Center of Japan, National Hospital Organization Kure Medical Center, Nagoya Daini Red Cross Hospital, University of Yamanashi Hospital, Heartlife Hospital, Musashino Red Cross Hospital, Saitama Medical Center, PL Hospital, Toyama Prefectural Central Hospital, Shimane Prefectural Central Hospital, Miyagi Cancer Center.

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bjh research paper

Prospective monitoring of *BCR-ABL1* transcript levels in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia undergoing imatinib-combined chemotherapy

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Summary

The clinical significance of minimal residual disease (MRD) is uncertain in patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL) treated with imatinib-combined chemotherapy. Here we report the results of prospective MRD monitoring in 100 adult patients. Three hundred and sixty-seven follow-up bone marrow samples, collected at predefined time points during a uniform treatment protocol, were analysed for *BCR-ABL1* transcripts by quantitative reverse transcription polymerase chain reaction. Ninety-seven patients (97%) achieved complete remission (CR), and the relapse-free survival (RFS) rate was 46% at 3 years. Negative MRD at the end of induction therapy was not associated with longer RFS or a lower relapse rate ($P = 0{\cdot}800$ and $P = 0{\cdot}964$ respectively). Twenty-nine patients showed MRD elevation during haematological CR. Of these, 10 of the 16 who had undergone allogeneic haematopoietic stem cell transplantation (HSCT) in first CR were alive without relapse at a median of 2·9 years after transplantation, whereas 12 of the 13 who had not undergone allogeneic HSCT experienced a relapse. These results demonstrate that, in Ph+ ALL patients treated with imatinib-combined chemotherapy, rapid molecular response is not associated with a favourable prognosis, and that a single observation of elevated MRD is predictive of subsequent relapse, but allogeneic HSCT can override its adverse effect.

Keywords: acute lymphoblastic leukaemia, Philadelphia chromosome, *BCR-ABL1*, imatinib, minimal residual disease.

The recent development of imatinib-combined chemotherapy has drastically improved overall treatment results in Philadelphia chromosome-positive acute lymphoblastic leukaemia (Ph+ ALL) (Ottmann & Wassmann, 2005; Yanada & Naoe, 2006; Thomas, 2007). Nearly 95% of newly diagnosed patients now achieve complete remission (CR) (Thomas *et al*, 2004;

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Lee *et al*, 2005; Wassmann *et al*, 2006; Yanada *et al*, 2006). However, outcome after CR depends on the individual patient and is not predictable. Young patients generally undergo allogeneic haematopoietic stem cell transplantation (HSCT) after achieving CR if a suitable donor is available, based on the concept that it is the established treatment with curative potential for this disease (Cornelissen *et al*, 2001; Dombret *et al*, 2002; Stirewalt *et al*, 2003; Yanada *et al*, 2005). Nevertheless, a fraction of patients experience a relapse even prior to transplantation, whereas some remain alive in remission for years without undergoing HSCT.

Minimal residual disease (MRD), as measured by reverse transcription-polymerase chain reaction (RT-PCR) or flow cytometry, has been shown to be useful for predicting prognosis in paediatric (Brisco *et al*, 1994; Cave *et al*, 1998; Coustan-Smith *et al*, 1998; van Dongen *et al*, 1998; Dworzak *et al*, 2002; Nyvold *et al*, 2002; Zhou *et al*, 2007) and adult ALL patients (Brisco *et al*, 1996; Mortuza *et al*, 2002; Vidriales *et al*, 2003; Bruggemann *et al*, 2006; Raff *et al*, 2007). However, the utility of MRD as a prognostic indicator has been established on the basis of data from patients treated with chemotherapy alone, and it remains to be determined whether it is useful in patients treated with chemotherapy in combination with imatinib. The Japan Adult Leukemia Study Group (JALSG) recently conducted a phase II trial of imatinib-combined chemotherapy in newly diagnosed Ph+ ALL patients (Towatari *et al*, 2004; Yanada *et al*, 2006, 2008). In that trial, *BCR-ABL1* transcript levels in bone marrow were prospectively monitored at predetermined time points using quantitative real-time RT-PCR (RQ-PCR). The results are presented here, with particular emphasis on the prognostic significance of rapid MRD clearance and MRD kinetics.

Patients and methods

Patients

The patient eligibility requirements of the phase II trial were as follows: newly diagnosed with Ph+ ALL, aged 15–64 years, an Eastern Cooperative Oncology Group performance status of 0–3, and adequate liver, kidney and heart function. Written informed consent was obtained from all patients prior to registration. The protocol was reviewed and approved by the institutional review boards of all of participating centres and was conducted in accordance with the Declaration of Helsinki. This trial was registered at http://www.clinicaltrials.gov as #NCT00130195.

The treatment schedule is summarized in Table I. Allogenic HSCT was allowed after achieving CR if the patient had a suitable donor. The original target sample size was 77 patients (Yanada *et al*, 2006), with the CR rate defined as the primary endpoint. Eighty patients had been enrolled by January 2005, when enrolment was extended to 100 patients to attain a more precise point estimate of the overall survival (OS) rate. This sample size enabled the lower limit of the 95% confidence interval (CI) of OS rate (expected to be 70% at 1 year) to be higher than 60%.

MRD evaluation

Molecular monitoring was performed with use of the RQ-PCR assay in a single independent laboratory. Bone marrow samples were collected at diagnosis; at days 28 and 63 of the induction course; after the first, second, fifth and sixth consolidation courses; after 1 year of treatment; and at the end of therapy (2 years from the date of CR).

Total RNA was extracted from mononuclear cells using the QIAamp RNA blood mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. The concentration and purity of RNA were measured by spectrophotometric determination of the A260/A280 ratio. Total RNA (1·5 μg) was transcribed to cDNA in a 22·5-μl reaction mixture containing 500 ng of random hexamer (Invitrogen, Carlsbad, CA, USA), 50 units of reverse transcriptase (Invitrogen), 40 units of RNase inhibitor (Invitrogen) and 500 μmol/l dNTP. The reaction mixture (total volume: 50 μl) contained 7·5 μl of a 22·5-μl RNA mixture (corresponding to 500 ng of RNA), 15 pmol of forward and reverse primers, 10 pmol of TaqMan probe, and 25 μl of 2× TaqMan universal PCR master mix (Applied Biosystems, Foster City, CA, USA). The primer and probe sequences have been described elsewhere (Towatari *et al*, 2004). Amplification was carried out with an initial activation of the polymerase at 50°C for 2 min and 95°C for 10 min, followed by 50 cycles consisting of two steps: 95°C for 15 s and 60°C for 1 min. Fluorescent emission spectra were monitored every 7 s and analysed using the PRISM 7700 system with SEQUENCE DETECTION SYSTEM software (version 1.7; Applied Biosystems). Amplified cDNA fragments were cloned into the pCRII vector (Invitrogen) and used as the reference standard. The copy number of each plasmid was calculated from the DNA concentration (determined by measuring A260) and the molecular weight of the plasmid. The copy number of the *BCR-ABL1* transcripts was calculated by comparing the C_t values of samples with those of the standard and converted to molecules per microgram RNA after being normalized by means of *GAPDH*. The threshold for quantification was 50 copies/μg RNA, which corresponded to a minimal sensitivity of 10^{-5}. Detectable MRD levels below this threshold were referred to as '<50 copies/μg' to distinguish from undetectable MRD. Nested PCR was not performed in this study. Samples with *GAPDH* levels below $5{\cdot}7 \times 10^5$ copies/μg RNA were not eligible for MRD evaluation.

Statistical analysis

Relapse-free survival (RFS) was defined as the time from CR to relapse, death, or last follow-up, and OS was defined as the time from registration to death or last follow-up. A Kaplan–Meier survival analysis was performed to estimate the probabilities of RFS and OS, with differences between the curves

Table I. Treatment schedule.

Drug	Dose	Route	Days
Induction			
Cyclophosphamide	1200 mg/m^2 (800 mg/m^2)*	IV (3 h)	1
Daunorubicin	60 mg/m^2 (30 mg/m^2)*	IV (1 h)	1–3
Vincristine	1·3 mg/m^2†	IV (bolus)	1, 8, 15, 22
Prednisolone	60 mg/m^2	PO	1–21 (1–7)*
Imatinib	600 mg	PO	8–63
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	29
Consolidation #1			
Methotrexate	1 g/m^2	IV (24 h)	1
Cytarabine	2 g/m^2 (1 g/m^2)* twice a day	IV (3 h)	2, 3
Methylprednisolone	50 mg twice a day	IV (bolus)	1–3
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	1
Consolidation #2			
Imatinib	600 mg	PO	1–28
Methotrexate, cytarabine, dexamethasone	15 mg, 40 mg, 4 mg	IT	1
Consolidation #3	Repeat #1		
Consolidation #4	Repeat #2		
Consolidation #5	Repeat #1		
Consolidation #6	Repeat #2		
Consolidation #7	Repeat #1		
Consolidation #8	Repeat #2		
Maintenance‡			
Vincristine	1·3 mg/m^2†	IV (bolus)	1
Prednisolone	60 mg/m^2	PO	1–5
Imatinib	600 mg	PO	1–28

IV, intravenously; PO, orally; IT, intrathecally.
*For patients aged 60 and older.
†Maximum 2·0 mg.
‡Repeated every 4 weeks up to 2 years from the date of complete remission.

qualified with the log-rank test. The cumulative incidence of relapse was calculated with death during CR considered as a competing risk, and differences between the curves were qualified with Gray's test. STATA version 8 software (StataCorp, College Station, TX, USA) and R software version 2.4.0 (The R Foundation for Statistical Computing, http://www.r-project.org) were used for statistical analyses. P values ≤0·05 were considered to be statistically significant.

Results

Patients and treatment results

The median patient age was 45 years (range 15–64 years); 55 were male and 45 were female. Twenty-five patients were positive for major *BCR-ABL1*, and 75 for minor *BCR-ABL1*. Ninety-seven patients (97%) achieved CR. The median and maximum follow-up periods were 3·2 and 5·1 years respectively. The outcomes of 100 patients are detailed in Fig 1. Relapse occurred in 38 patients after a median CR duration of 7·3 months (range 2·1–37·4). Allogeneic HSCT was performed in 60 patients during first CR, and in 19 patients beyond first CR. For patients allografted in first CR, the median time to HSCT was 5·3 months (range 2·2–17·1). No patient underwent autologous HSCT. The probability of OS for the entire cohort was 55% at 3 years. The 1-year OS rate, the endpoint for the study extension, was 83% (95% CI 74–89%). Among the 97 patients who achieved CR, the probability of RFS was 46% at 3 years. Neither transcript types nor copy numbers at diagnosis were associated with RFS (P = 0·709 and P = 0·851 respectively).

MRD kinetics

The number of patients who underwent MRD monitoring decreased with time because of prior relapse, death, or transfer to allogeneic HSCT. Thus, the total number of follow-up samples was 367 (77% of all possible samples at all time points): 86 of 98 (88%) at day 28, 85 of 97 (88%) at day 63, 75 of 90 (83%) after the first consolidation (C#1), 55 of 73 (75%) after C#2, 31 of 38 (82%) after C#5, 22 of 32 (69%) after C#6, 11 of 15 (73%) at 1 year, and 2 of 9 (22%) at 2 years.

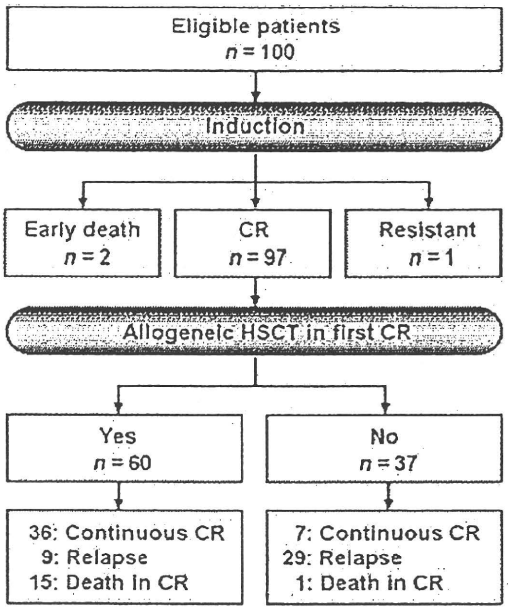

Fig 1. Flow diagram showing patient outcomes. CR, complete remission; HSCT, haematopoietic stem cell transplantation.

Figure 2 shows the percentages of patients with negative and low (<50 copies/μg) MRD levels at each time point. There was a progressive increase in the percentage of patients with negative MRD during the early treatment courses, with 24% at day 28, 48% at day 63, 68% after C#1, and 67% after C#2. Nearly all samples measured at 1 year and at 2 years were negative for MRD, although only a small number of samples were analysed at these time points. The only patient whose MRD was positive (87 copies/μg) at 1 year experienced a relapse 8 months later. All of the three patients who experienced a relapse during maintenance therapy had showed MRD elevation prior to haematological relapse.

Rapid MRD clearance and outcome

RQ-PCR results at the end of induction therapy (day 63) were available for 85 patients. One patient with a *BCR-ABL1* level of 160 000 copies/μg failed to achieve CR. Figure 3 shows the RFS rates and cumulative incidences of relapse in 84 CR patients according to MRD detection at day 63. PCR negativity was not associated with a higher RFS rate (46% vs. 42% at 3 years, $P = 0{\cdot}800$; Fig 3A) or a lower relapse rate (40% vs. 41% at 3 years, $P = 0{\cdot}964$; Fig 3B). A relatively small number of patients ($n = 11$) whose MRD levels exceeded 1000 copies/μg at day 63 had trends toward lower RFS ($P = 0{\cdot}092$, Fig 4A) and higher relapse rate ($P = 0{\cdot}070$, Fig 4B). Neither PCR negativity at day 28 nor after C#1 was associated with higher RFS ($P = 0{\cdot}867$ and $P = 0{\cdot}549$) or lower relapse rates ($P = 0{\cdot}796$ and $P = 0{\cdot}667$).

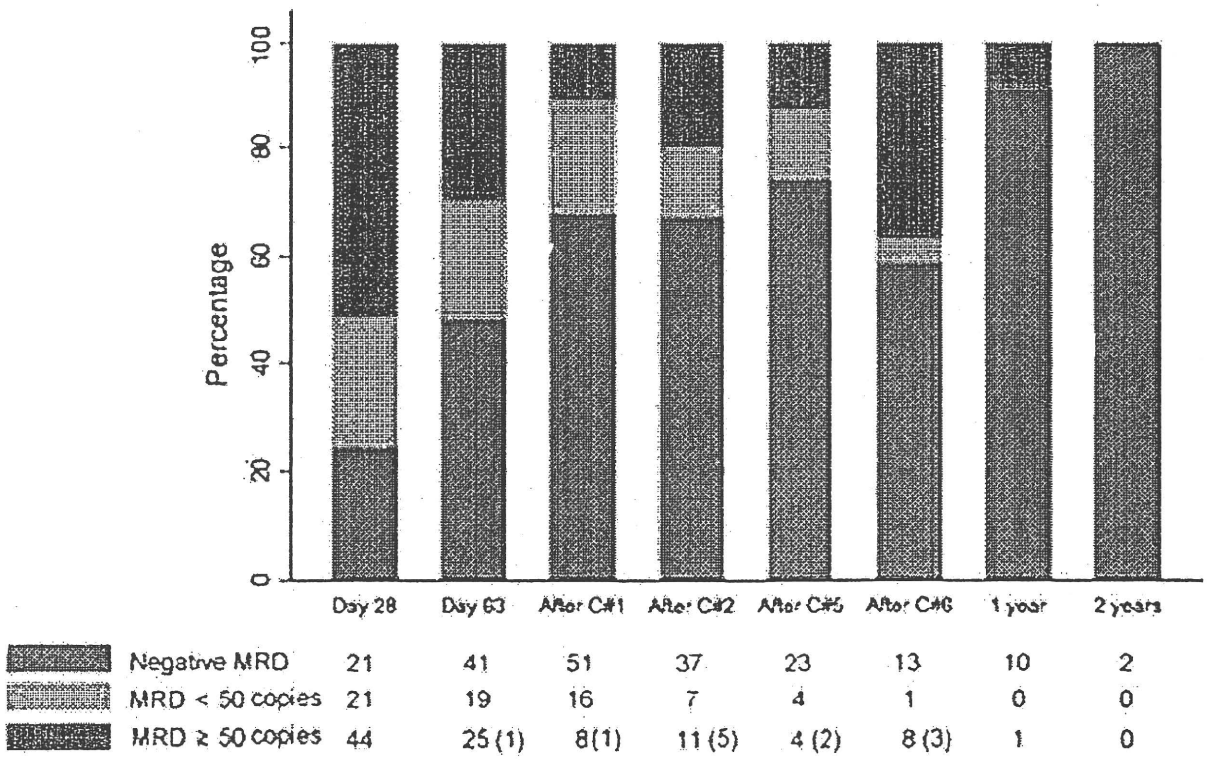

Fig 2. Frequencies of negative and low (<50 copies/μg RNA) *BCR-ABL1* transcript levels at each time point. Figures in parentheses represent the number of patients who developed haematological relapse at that time point.