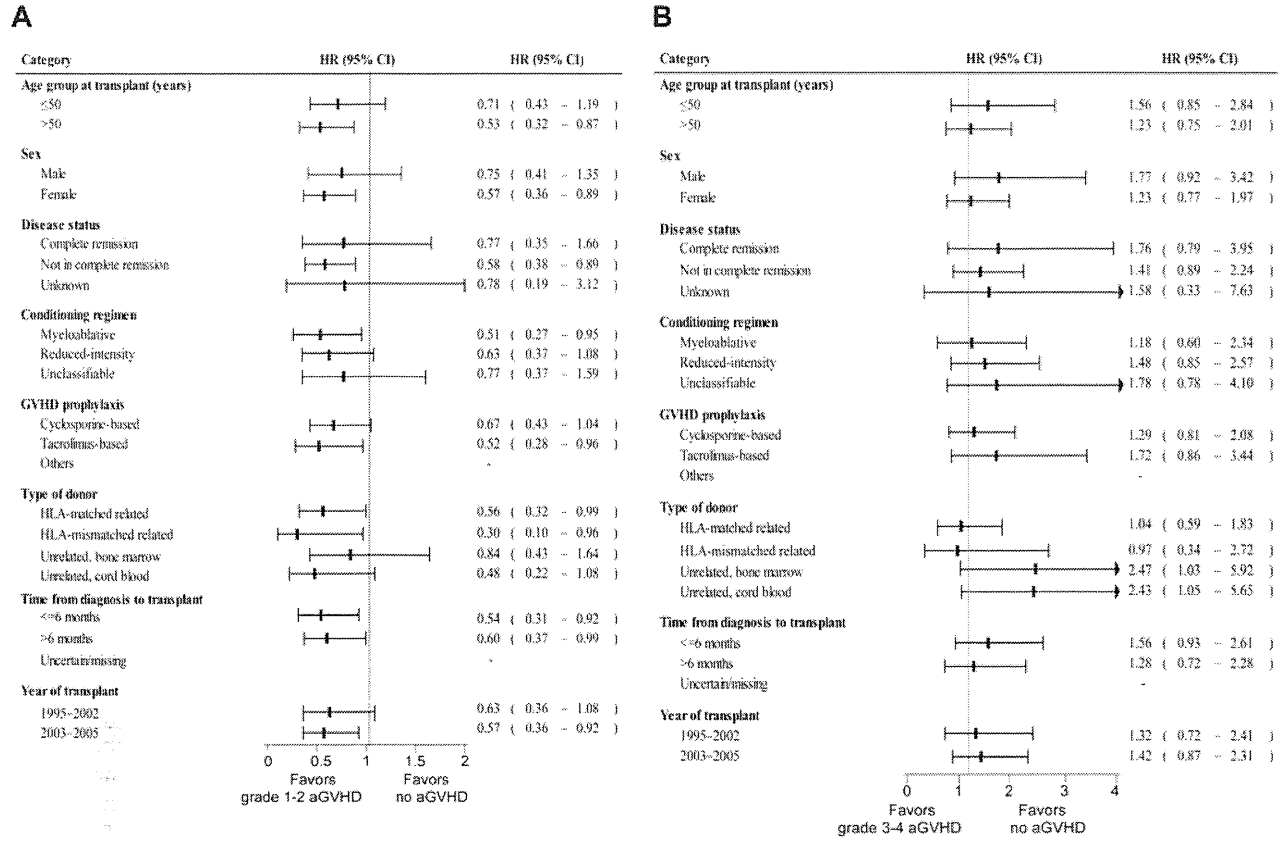

Figure 2. Impact of the grade of acute GVHD on overall survival in each stratified category. Effects of grade 1-2 (A) and grade 3-4 acute GVHD (B) on overall survival are shown as forest plots. Square boxes on lines indicate hazard ratios compared with "no acute GVHD group," and horizontal lines represent the corresponding 95% CI. Abbreviations used are the same as described in the footnotes to Tables 1 and 2.

outcomes including overall survival, disease-associated mortality, and treatment-related mortality after allogeneic HCT for ATL. In the present study, the occurrence of both grade 1-2 and grade 3-4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD. However, positive effect of GVHD on reduced disease-associated mortality was counterbalanced by increased treatment-related mortality among patients who developed severe acute GVHD, and an overall beneficial effect on survival was observed only with the development of mild-to-moderate acute GVHD. In contrast to acute GVHD, no beneficial effect was observed in association with the development of chronic GVHD, although the point estimate of the HR comparing limited chronic GVHD versus the absence of chronic GVHD

Table 2. Effect of acute GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Overall survival*				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.60 (0.42-0.85)	.004	0.65 (0.45-0.93)	.018
Grade 3 or 4 acute GVHD vs no acute GVHD	1.38 (0.94-2.01)	.099	1.64 (1.10-2.42)	.014
Disease-associated mortality†				
Grade 1 or 2 acute GVHD vs no acute GVHD	0.47 (0.28-0.79)	.005	0.54 (0.32-0.92)	.023
Grade 3 or 4 acute GVHD vs no acute GVHD	0.41 (0.21-0.81)	.010	0.44 (0.22-0.90)	.024
Treatment-related mortality‡				
Grade 1 or 2 acute GVHD vs no acute GVHD	1.13 (0.67-1.89)	.649	1.22 (0.72-2.07)	.461
Grade 3 or 4 acute GVHD vs no acute GVHD	3.34 (1.94-5.74)	< .001	3.50 (2.01-6.11)	< .001

*Other significant variables were sex of recipient, female (reference, 1.00) and male (HR, 1.70; 95% CI, 1.24-2.32; $P = .001$); achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.05; 95% CI, 1.44-2.92; $P < .001$), and status not known (HR, 2.21; 95% CI, 1.15-4.22; $P = .017$); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 1.71; 95% CI, 1.04-2.84; $P = .036$), unrelated donor of bone marrow (HR, 1.39; 95% CI, 0.94-2.06; $P = .096$), and unrelated cord blood (HR, 1.86; 95% CI, 1.22-2.83; $P = .004$).

†Other significant variables were achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.98; 95% CI, 1.62-5.47; $P < .001$), and status not known (HR, 0.96; 95% CI, 0.21-4.49; $P = .963$); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 2.14; 95% CI, 1.00-4.55; $P = .049$), unrelated donor of bone marrow (HR, 1.45; 95% CI, 0.81-2.61; $P = .214$), and unrelated cord blood (HR, 1.25; 95% CI, 0.63-2.49; $P = .517$).

‡Another significant variable was achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 1.17; 95% CI, 0.74-1.84; $P = .498$) and status not known (HR, 2.31; 95% CI, 1.04-5.15; $P = .040$).

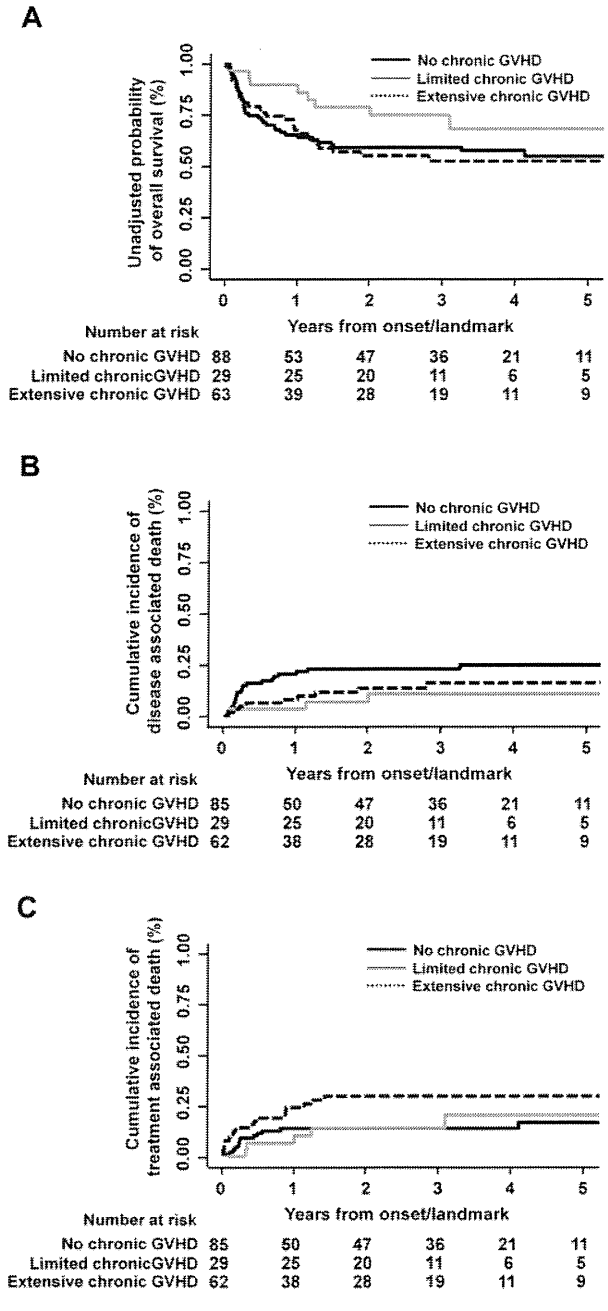

Figure 3. Semi-landmark plots for impact of chronic GVHD. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

suggested the trend toward a reduced risk of disease-associated deaths in the limited chronic GVHD group.

Our present findings are in contrast to the previous reports showing the beneficial effects of chronic GVHD rather than acute GVHD on the prevention of disease recurrence after allogeneic HCT. It is less likely that the particular characteristics of chronic GVHD in patients with ATL biased the results, because the incidence rate and median onset day of chronic GVHD in our cohort were similar to those reported in previous studies evaluating the incidence of chronic GVHD among Japanese patients, most of whom had received allogeneic HCT for myeloid neoplasms or acute lymphoblastic leukemia.[30-32] Conceivably, the rapid tempo of disease recurrence of ATL might be such that chronic GVHD is less potent in terms of harnessing clinically relevant graft-versus-leukemia responses compared with acute GVHD. However, the results of our analysis regarding the effect of chronic GVHD should be interpreted with caution because the number of patients evaluable for chronic GVHD was relatively small in our study for providing sufficient statistical power. The effect of chronic GVHD on outcomes after HCT for ATL should be further explored in a larger cohort.

The occurrence of GVHD has been shown to exert a potent graft-versus-leukemia effect in terms of reducing relapse incidence in acute leukemia or chronic myeloid leukemia.[33,34] In contrast, multiple studies have documented a correlation between GVHD in its acute or chronic form and treatment-related mortality. In a study of patients undergoing HLA-identical sibling HCT for chronic myeloid leukemia, the overall beneficial effect on long-term survival was demonstrated only in a group of patients who developed grade 1 acute GVHD or limited chronic GVHD.[33] In another study of HLA-identical sibling HCT for leukemia using cyclosporine and methotrexate as GVHD prophylaxis, a benefit of mild GVHD was only seen in high-risk patients but not in standard-risk patients. Therefore, the therapeutic window between decreased relapse incidence and increased transplant-related mortality in association with the development of GVHD has been considered to be very narrow.[34]

With regard to the effectiveness of allogeneic HCT for ATL, it is also of note here that posttransplant eradication of ATL cells can be achieved without the use of high-dose chemoradiotherapy: patients who received a transplant with reduced intensity conditioning had survival outcomes similar to those who received a transplant with myeloablative conditioning in our study. Intriguingly, several small cohort studies exhibited that abrupt discontinuation of immunosuppressive agents resulted in disappearance or reduction in the tumor burden in allografted patients with ATL. In some cases, remission of ATL was observed along with the development of GVHD.[19,20,22] Taken together with the findings of this study, it is suggested that ATL is particularly susceptible to immune modulation following allogeneic HCT. To clarify the presence of such "graft-versus-ATL" effect, further investigations are needed to assess the efficacy of donor lymphocyte infusion or withdrawal of immunosuppressive agents on relapse after transplantation.

Of the HTLV-I gene products, Tax is a dominant target of HTLV-I–specific cytotoxic T lymphocytes. The vigorous Tax-specific cytotoxic T-cell responses were demonstrated in recipients who obtained complete remission after allogeneic HCT for ATL, suggesting that "graft-versus-HTLV-I" responses might contribute to the eradication of ATL cells.[35,36] However, Tax is generally undetectable or present in very low levels in primary ATL cells.[37,38] In addition, small amounts of HTLV-I provirus can be detected in peripheral blood of recipients who attained long-term remission of ATL, even after HCT from HTLV-I–negative donors.[39,40] These findings suggest that "graft-versus-ATL" effect can be harnessed without complete elimination of HTLV-I. It is also important to note that allogeneic HCT is emerging as an effective treatment option for other mature T-cell neoplasms not related to HTLV-I, such as mycosis fungoides/Sézary syndrome and various types of aggressive peripheral T-cell lymphomas.[41,42] These observations raised the possibility that the common targets for alloimmune responses might exist across a spectrum of malignant T-cell neoplasms, including ATL. The minor histocompatibility antigens or tumor-specific antigens can be other targets of alloimmune anti-ATL effect.[43-45] Therefore, the elucidation of the mechanism underlying an immunologic eradication of primary ATL cells may

Table 3. Effect of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

	Univariate analysis		Multivariate analysis	
Outcome	HR (95% CI)	*P*	HR (95% CI)	*P*
Overall survival*				
Limited chronic GVHD vs no chronic GVHD	0.71 (0.34-1.47)	.353	0.72 (0.35-1.50)	.385
Extensive chronic GVHD vs no chronic GVHD	1.45 (0.90-2.35)	.131	1.40 (0.86-2.30)	.176
Disease-associated mortality†				
Limited chronic GVHD vs no chronic GVHD	0.45 (0.14-1.46)	.183	0.45 (0.14-1.44)	.178
Extensive chronic GVHD vs no chronic GVHD	0.81 (0.39-1.67)	.563	0.80 (0.39-1.64)	.536
Treatment-related mortality‡				
Limited chronic GVHD vs no chronic GVHD	1.59 (0.64-3.95)	.316	1.56 (0.63-3.87)	.342
Extensive chronic GVHD vs no chronic GVHD	2.85 (1.41-5.77)	.004	2.75 (1.34-5.63)	.006

*There was no significant variable.
†There was no significant variable.
‡There was no other significant variable.

lead to a new strategy for improving outcomes of allogeneic HCT not only for ATL but also for other intractable T-cell neoplasms.

This study has several limitations. First, acute GVHD might be intentionally induced for some patients considered at high risk of relapse by treating clinicians. Second, the information on the day when each grade of GVHD occurred was not available. Therefore, we treated the development of acute and chronic GVHD in their worst severity as a time-varying covariate. To validate the results, we also performed the landmark analysis and obtained consistent results. Third, the relatively small number of patients with chronic GVHD might mask or bias the effect of chronic GVHD on outcomes. Last, the effect of multiple testing should be taken into account for the interpretation of the secondary end points.

In conclusion, the development of acute GVHD was associated with lower disease-associated mortality after allogeneic HCT for ATL compared with the absence of acute GVHD. However, improved survival can be expected only among a group of patients who developed mild-to-moderate acute GVHD because those who developed severe acute GVHD were at high risk of treatment-related mortality. New strategies that enhance the allogeneic anti-ATL effect without exacerbating GVHD are required to improve the outcomes of patients undergoing allogeneic HCT for ATL.

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The views expressed in this report are those of authors and do not indicate the views of the JSHCT, JMDP, or JCBBN.

This work is in memory of T.U., who died during the preparation of this manuscript.

Authorship

Contribution: T.I. and T.U. designed the research and organized the project; M. Hishizawa, J.K., T.I., and T.U. reviewed and analyzed data and wrote the paper; J.K., T.I., and K.M. performed statistical analysis; Y.A., R.S., and H.S. collected data from JSHCT; T.K. and Y. Morishima collected data from JMDP; T.N.-I., and S. Kato collected data from JCBBN; and A.U., S.T., T.E., Y. Moriuchi, R.T., F.K., Y. Miyazaki, M.M., K.N., M. Hara, M.T., S. Kai, and J.O. interpreted data and reviewed and approved the final manuscript.

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A list of other members who contributed data on allogeneic HSCT for ATL to JSHCT, JMDP, and JCBBN appears in the online supplemental Appendix.

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ORIGINAL ARTICLE

Randomized comparison of fixed-schedule versus response-oriented individualized induction therapy and use of ubenimex during and after consolidation therapy for elderly patients with acute myeloid leukemia: the JALSG GML200 Study

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Abstract We conducted a multicenter prospective randomized study to compare a fixed-scheduled induction therapy with a response-oriented individualized induction therapy for elderly patients with acute myeloid leukemia (AML). Newly diagnosed AML patients, aged between 65 and 80, were randomly assigned to receive fixed or individualized induction. Both groups received daunorubicin (DNR) 40 mg/m^2 for 3 days and behenoyl cytarabine (BHAC) 200 mg/m^2 for 8 days. In the individualized group, bone marrow biopsy was done on days 8 and 10, and according to the cellularity and blast ratio, the patients received additional DNR and BHAC for two to four more days. All patients achieving complete remission (CR) were randomized a second time to determine whether they would receive ubenimex. CR was obtained in 60.1 % of the fixed group and 63.6 % of the individualized group.

For Japan Adult Leukemia Study Group (JALSG).

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Predicted 4-year relapse-free survival (RFS) was 9 % for the fixed group and 18 % for the individualized group. There were no statistically significant differences in CR and RFS between the fixed and individualized groups. In the ubenimex group, prolonged RFS was observed. Notably, gender was a prognostic factor in this study, as 102 female patients had a significantly higher CR rate (72.5 vs. 54.3 %, $p = 0.0048$) and better OS (24 vs. 14 % at 4 years, $p = 0.018$), compared with 140 male patients.

Keywords Acute myeloid leukemia · Elderly · Response-oriented individualized induction therapy · Daunorubicin · Behenoyl cytarabine (enocitabine, BHAC)

Introduction

With the extension of life-span, elderly patients with acute myeloid leukemia (AML) are increasing in number, and the median age of AML is presently around 65–70. Prognosis of these patients is poorer, compared with younger patients, as their complete remission (CR) rate is around 50 % and overall survival (OS) is <20 % at 5 years, showing no remarkable progress during the past decades, despite every possible effort by many investigators. Regrettably, there is no recommendable standard regimen effective enough for the treatment of elderly AML [1–6].

In Japan, a response-oriented individualized induction therapy has been employed for AML since the DCMP two-step therapy, using daunorubicin (DNR), cytarabine (Ara-C), 6-mercaptopurine (6MP) and prednisolone (PSL) by Uzuka et al. in the mid 1970s, reporting more than 80 % CR rate, which is not surprisingly high today but was remarkable at that time even for a single institutional study [7]. Subsequently, a response-oriented individualized BHAC-DMP induction therapy, using behenoyl Ara-C (BHAC, enocitabine), DNR, 6MP and PSL, was developed by Ohno et al. [8], reporting more than 80 % CR in adult AML by a single institutional study. A multi-institutional AML87 study, conducted by the Japan Adult Leukemia Study Group (JALSG), confirmed the high CR rate of BHAC-DMP therapy for adult AML, resulting in 80 % CR rate [9]. Succeeding JALSG studies, AML89 [10] and AML92 [11] also employed the response-oriented individualized induction therapy and reported 81 and 77 % CR rates, respectively, for younger adult patients with non-M3 type AML. These CR rates were around 10 % higher than those reported from cooperative study groups in the USA and Europe, where fixed-scheduled induction therapies were employed [3, 12–14].

However, after clinical introduction of idarubicin (IDR), a more potent derivative of DNR, the JALSG AML95 study which prospectively compared the two treatment schedules, using Ara-C and IDR instead of DNR, could not demonstrate any advantage of the response-oriented individualized induction therapy over the fixed-scheduled induction therapy for younger patients with AML of age <65 [15].

In the present study, with elderly AML patients of age from 65 to 80, we compared a response-oriented individualized induction therapy with a fixed-scheduled induction therapy using BHAC and DNR. Additionally, we randomly compared the effectiveness of ubenimex among patients who had achieved CR by these two induction regimens. Ubenimex, a dipeptide immunostimulator, reportedly prolonged OS and disease-free survival in adult AML patients when used during and after consolidation therapy [16–18].

Materials and methods

Patients

From August 2000 to December 2005, all newly diagnosed elderly patients with AML were consecutively registered from 55 institutions which participated in this study. Informed consent was obtained from all the patients before registration in accordance with the Declaration of Helsinki. AML was first diagnosed by the French–American–British (FAB) classification at each institution. Peripheral blood and bone marrow smears from all registered patients were sent to Nagasaki University, and examined with May-Giemsa, peroxidase and esterase staining. Then, diagnosis was reevaluated by the central review committee. Eligibility criteria for the randomization study included age from 65 to 80 years, AML by FAB classification except M3, adequate functioning of the liver (serum bilirubin level <2.0 mg/dL), kidney (serum creatinine <2.0 mg/dL), heart (ejection fraction >50 %) and lungs, an Eastern Cooperative Oncology Group performance status between 0 and 2,

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and written informed consent for the randomized study. Patients were not eligible if they had pre-diagnosed myelodysplastic syndromes (MDS), but were eligible if they had no definite diagnosis of MDS, even when they had previous history of hematological abnormality. Patients with ill-controlled diabetes mellitus, angina pectoris, infectious episodes and liver cirrhosis were not eligible, as well as those with positive HIV antibody, HCV antibody and HB antigen. Patients who did not meet the eligibility criteria or did not agree to the randomization study were included also for the initial evaluation and survival. Cytogenetic analysis was performed by standard methods of G-banding, and abnormalities were grouped according to the MRC classification [19]. The protocol was approved by the institutional review board of each hospital.

Treatment regimens

Eligible patients who had given their informed consent for the randomized study were assigned to receive either a fixed-scheduled induction therapy or a response-oriented individualized induction therapy through a centralized computer system. All assigned patients received DNR 40 mg/m^2/day by 30-min infusion on days 1–3 and BHAC 200 mg/m^2/day by 3-h infusion on days 1–8. For patients of age 70 or older, the dose of DNR was reduced to 30 mg/m^2/day. In the individualized group, bone marrow aspiration was performed on day 8, and if the marrow was not severely hypoplastic and had more than 20 % blasts, additional BHAC was given on days 9 and 10. If 20–50 % of blasts remained, DNR was added on day 8, and if more than 50 % of blasts remained, DNR was added on days 8 and 9. Another bone marrow aspiration was performed on day 10, and if the marrow was not severely hypoplastic and had more than 20 % blasts, additional BHAC was given on days 11 and 12. If 20–50 % of blasts remained, DNR was added on day 11, and if more than 50 % of blasts remained, DNR was added on days 11 and 12 (Fig. 1). If patients had documented infection or other complications on day 8 or day 11, cancellation of additional chemotherapy was permitted by the attending physician's judgment. In the fixed-scheduled group, patients did not receive additional doses, regardless of their marrow status at day 8. If patients did not achieve CR by the first course, the same induction therapy was repeated at approximately 3- to 4-week interval. If patients did not achieve CR with two courses, these cases were judged as failure.

All patients who had achieved CR received 3 courses of consolidation therapy, and were randomly assigned either to receive daily 30 mg of ubenimex (Bestatin, Nippon Kayaku, Tokyo, Japan) or not, concomitantly during the consolidation therapy. The first course of consolidation consisted of BHAC (200 mg/m^2 by 3-h infusion on days 1–5) and mitoxantrone (MIT, 7 mg/m^2 by 30-min infusion on days 1–3). The second consisted of BHAC (200 mg/m^2 on days 1–7), DNR (30 mg/m^2 by 30-min infusion on days 1–2) and etoposide (ETP; 100 mg/m^2 by 1-h infusion on days 1–3). The third consisted of BHAC (200 mg/m^2 on

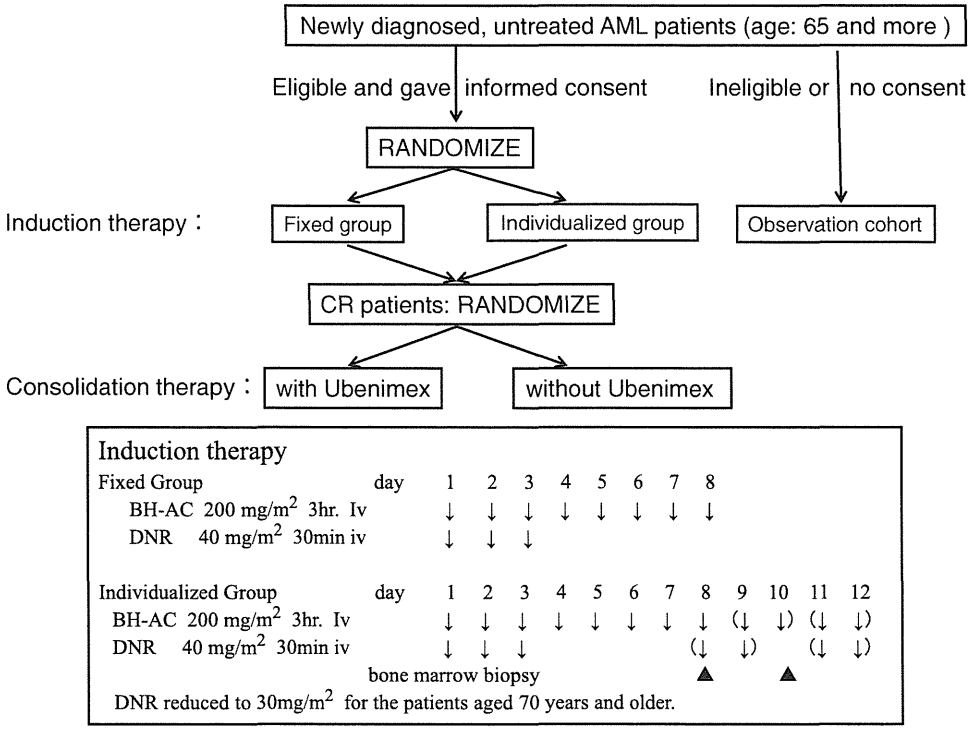

Fig. 1 Consort diagram and treatment schedule of induction therapy. Eligible patients were randomized to fixed group or individualized group. Patients achieved complete remission were done second randomization to with ubenimex or without ubenimex. Induction therapy in individualized group, BHAC dosage should be escalated up to twelve doses and up to seven doses for daunorubicin according to the bone marrow state

days 1–5) and aclarubicin (ACR; 14 mg/m^2 by 30-min infusion on days 1–5). For patients of age 70 or more, the dose of MIT, DNR, ETP and ACR was reduced to 5, 25, 75 and 10 mg/m^2, respectively. Each consolidation course was given as soon as possible after the leukocyte and platelet counts had recovered to more than 3,000 and 100,000/μL, respectively. Intrathecal methotrexate (15 mg), Ara-C (40 mg) and PSL (10 mg) were given after the third consolidation therapy for the prophylaxis of central nervous system leukemia. Patients assigned to be given ubenimex received it for 3 more months after the completion of consolidation therapy, but no further chemotherapy was given to either group. For non-eligible patients or for those who did not give informed consent for the randomized study, no intervention was specified and the therapy was left to the decision of attending physicians. However, their OS data were reported.

Best supportive care, including administration of antibiotics and platelet transfusion from blood cell separators, was given if indicated. When patients had life-threatening infections during neutropenia, the use of granulocyte colony-stimulating factor (G-CSF) was permitted.

Response criteria and statistical analysis

CR was defined as the presence of all the following criteria: <5 % of blasts in bone marrow, no leukemic blasts in peripheral blood, recovery of peripheral neutrophil counts over 1,000/μL and platelet counts over 100,000/μL, and no evidence of extramedullary leukemia. CR had to continue for at least 4 weeks, but the date of CR was defined as the first day when these criteria were fulfilled. Relapse was defined as the presence of at least one of the following: recurrence of more than 10 % leukemic cells in bone marrow, any leukemic cells in peripheral blood, and appearance of extramedullary leukemia.

Overall survival (OS) was calculated from the diagnostic day to death by any cause, and censored at the last follow-up. Relapse-free survival (RFS) for patients who achieved CR was measured from the date of CR to relapse or death by any cause, and censored at the last follow-up.

This was a multi-institutional randomized phase 3 study with a 2 × 2 factorial design. The primary end point of the first randomization was CR rate, and the secondary endpoints were OS and RFS. For the second randomization, the primary end point was RFS and the secondary endpoint was OS, and Kaplan–Meier product limit estimation was used to determine OS and RFS. A sample size of 98 patients per group was estimated to have a power of 70 % at a 5 % level of significance (single-sided) to demonstrate 10 % non-inferiority in CR rate (60 vs. 55 %). Statistical testing for the non-inferior trial was performed according to the method of Blackwelder [20]. To test the factors to predict CR, χ^2 test and Wilcoxon rank-sum test were used for univariate analysis, and the multiple logistic regression model was used for multivariate analysis. For comparison of OS and RFS, the log-rank test and the generalized Wilcoxon test were used for univariate analysis and Cox's proportional hazard model was used for multivariate analysis. SAS ver. 8.2 (SAS Institute Inc., Cary, NC, USA) was used for the analysis. p values <0.05 (two-sided) were considered statistically significant. Analysis was done on an intent-to-treat basis. This study is registered at http://www.umin.ac.jp/ctrj/ as C000000220 for the randomization study on eligible patients and C000000224 for the observation study on non-eligible patients.

Results

Patient population and characteristics

Of 375 patients registered, 130 patients were either judged as non-eligible by the attending physicians because of various reasons listed in eligibility criteria, including 6 patients with FAB-M3, or eligible but gave no informed consent to enter the randomized study. Of 245 eligible and consented patients, 122 were assigned to the fixed-scheduled therapy and 123 to the individualized therapy. One in the former group and two in the latter were unevaluable due to insufficient data. Pretreatment characteristics of 242 evaluable patients are presented in Table 1. Overall, the median age was 71, and 47 patients (19 %) were of age 75 or older. Successful cytogenetic data were reported in 231 patients (95 %), including 113 patients (91 %) in 124 observation cohort excluding M3. There were no major imbalances between the two randomized groups, although there were fewer patients with favorable cytogenetics and more with adverse cytogenetics in the fixed-scheduled group ($p = 0.1338$) (Table 1).

In the individualized therapy group, during the first course of the induction therapy, 45 patients received additional doses of DNR and BHAC from day 9, and 13 patients received the additional doses from day 11, and, during the second course, 11 patients received additional doses from day 9 and 2 from day 11.

Overall treatment results

Of 242 evaluable patients, 150 (62.0 %) achieved CR. Of 121 patients in the fixed-scheduled group, 73 (60.3 %) obtained CR, and of 121 in the individualized group 77 (63.6 %) achieved CR ($p = 0.6913$). In the fixed-scheduled group, 56 patients (46.3 %) achieved CR after the first course, while in the individualized group 56 patients (46.3 %) achieved CR after the first course. Of 53 (43.8 %)

Table 1 Patient characteristics

	Fixed-scheduled	Individualized	Non-randomized	Total
No. of patients	121	121	124	366
Age (years)				
65–69	54	51	29	134
70–74	42	48	36	126
75–79	25	22	32	79
80–	0	0	27	27
Median (range)	70 (65–79)	71 (65–79)	74 (65–92)	
Chromosome				
Favorable	6	14	7	27
Intermediate	91	92	91	274
Adverse	18	10	15	43
Unknown	6	5	11	22
FAB classification				
M0	10	8	10	28
M1	24	23	32	79
M2	48	52	45	145
M4	18	18	17	53
M5	13	16	12	41
M6	5	3	5	13
M7	3	1	3	7
Sex				
Male	75	65	72	212
Female	46	56	52	154
PS				
0	110	113	103	326
1	6	8	9	23
2	5	0	4	9
3			6	6
4			2	2

patients who had received additional chemotherapy during the first course of the individualized therapy, 22 (41.5 %) achieved CR (Table 2). There was no statistically significant difference in CR rates between the two groups regarding cytogenetics, gender, age, PS or FAB classification (data not shown).

The individualized group received significantly larger dosages of BHAC ($p < 0.001$) and DNR ($p < 0.001$) during the first course of induction therapy (Table 3). Myelosuppression judged by the period of leukocyte count <1,000/μL after the first course of induction therapy was significantly severer in the individualized group ($p = 0.040$) (Table 4). Early death within 30 days occurred in 5 (4.1 %) patients in the fixed-scheduled group and 4 (3.3 %) in the individualized group. There was no statistically significant difference in the incidence of complications between the two groups (Table 4).

Significant prognostic factors for the achievement of CR in all patients were cytogenetic risk group and gender (Table 2). Eighteen (90 %) of 20 patients with favorable risk cytogenetics, 120 (65.6 %) of 183 patients with intermediate risk, and 7 (25 %) of 28 with adverse risk achieved CR, respectively ($p < 0.0001$). Seventy-four (72.5 %) of 102 female patients achieved CR, while 76 (54.5 %) of 140 male patients attained it ($p = 0.0048$). These 2 factors were statistically significant and independent prognostic factors by the multivariate analysis (Table 5). Since this randomized study only included elderly patients who had met the eligibility criteria and agreed to enter the study, PS was 0 in 223 patients (92 %), 1 in 14 (6 %) and 2 in 5 (2 %). Paradoxically, patients with PS 1 or 2 had higher CR rate (84.2 %) compared with those with PS 0 (60.1 %) by the univariate analysis ($p = 0.0478$), but the difference was not statistically significant by the multivariate analysis ($p = 0.0998$).

Table 2 Response to induction therapy

Response by induction	Fixed-scheduled	Individualized	Total	*p* value
CR	73 (60.3 %)	77 (63.6 %)	150 (61.9 %)	0.6913
Non CR	48 (39.7 %)	44 (36.4 %)	92 (38.0 %)	
CR after first course	56 (46.3 %.)	56 (46.3 %)	114 (47.1 %)	
CR after second course	17 (14.0 %)	21 (17.4 %)	39 (16.1 %)	
Response by age group	65–69 years	70–74 years	75–79 years	
CR	64/106 (61.0 %)	56/90 (62.2 %)	30/47 (63.8 %)	0.9429
Response by PS	PS 0	PS 1	PS 2	
CR	134/223 (60.1 %)	11/14 (78.6 %)	5/5 (100.0 %)	0.0804
Response by PS	PS 0	PS 1 + 2		
CR	134/223 (60.1 %)	16/19 (84.2 %)		0.0478
Response by gender	Male	Female		
CR	76/140 (54.3 %)	74/102 (72.5 %)		0.0048
Response by cytogenetic risk[a]	Favorable	Intermediate	Adverse	
CR	18/20 (90.0 %)	120/183 (65.6 %)	7/28 (25.0 %)	<0.0001

[a] Cytogenetic data of 11 patients were not available

Table 3 Total administered dosage of behenoyl cytarabine (BHAC) and daunorubicin (DNR)

	BHAC (mg/m^2)		DNR (mg/m^2)	
	Average	Mean (range)	Average	Mean (range)
First course				
Fixed-scheduled (n = 121)	1,605	1,600 (200–3,000)	109	120 (40–240)
Individualized (n = 121)	1,851	1,600 (160–3,840)	139	120 (12–440)
p value	<0.001		<0.001	
Second course				
Fixed-scheduled (n = 42)	1,633	1,600 (1,600–2,400)	106	105 (60–180)
Individualized (n = 44)	1,732	1,600 (160–4,200)	123	120 (12–315)
p value	0.234		0.026	

Table 4 Toxicity during induction therapy

	Fixed-scheduled (n = 121)	Individualized (n = 121)	
Leukopenia			
G3/4	119 pts. (98.3 %)	117 pts. (96.7 %)	p = 0.513
G4	107 pts. (88.4 %)	111 pts. (91.7 %)	
Median duration of leucocytes <1,000/μl in G4 pts.			
1st course	14 days (2–52)	17 days (2–78)	p = 0.04
2nd course	15.5 days (2–32)	17.5 days (2–35)	p = 0.24
Use of G-CSF			
1st course	40 pts. (33.1 %)	44 pts. (36.4 %)	p = 0.686
2nd course	15 pts. (34.9 %)	11 pts. (24.4 %)	p = 0.352
Hemorrhage (CNS, pulmonary, GI): G3/4,	3 pts. (2.5 %)	3 pts. (2.5 %)	p = 1
Infection: G3/4	13 pts. (10.7 %)	11 pts. (9.1 %)	p = 0.72
Febrile neutropenia: G3/4	45 pts. (37.2 %)	48 pts. (39.7 %)	p = 0.696

Table 5 Multivariate analysis for achievement of complete remission and overall survival

Variable	Classification	No. of patients	Odds ratio	95 % CI	p value
Multivariate analysis for complete remission					
Treatment group	Fixed/individualized	115/116	0.973	0.543–1.744	0.9263
Age		231	1.000	0.925–1.081	0.9979
Sex	Male/female	133/98	2.192	1.200–4.003	0.0106
PS	0/1 + 2	213/18	3.065	0.808–11.634	0.0998
Cytogenetic risk	Favorable/Adverse	20/28	28.435	5.108–158.288	0.0001
	Intermediate/Adverse	183/28	4.764	1.878–12.086	0.0010
Multivariate analysis for overall survival					
Treatment group	Fixed/Individualized	115/116	1.037	0.751–1.430	0.8264
Age		231	1.003	0.961–1.047	0.8924
Sex	Male/female	133/98	0.747	0.530–1.052	0.0952
PS	0/1 + 2	213/18	0.833	0.365–1.902	0.6650
Cytogenetic risk	Favorable/adverse	20/28	0.390	0.185–0.820	0.0130
	Intermediate/adverse	183/28	0.422	0.261–0.680	0.0004

Analyzed in 231 patients by excluding 11 patients whose cytogenetic data were not available

Of 150 patients who had achieved CR, 63 patients were randomly assigned to receive ubenimex during 3 courses of consolidation therapy, plus 3 more months thereafter, and 60 received no ubenimex. All courses of consolidation therapy were administered to 65 (84.4 %) of 77 patients in the individualized group and 58 (79.5 %) of 73 patients in the fixed-scheduled group (p = 0.5248). There was no significant difference between patients receiving ubenimex or none, regarding myelosuppression and non-hematological toxicity, as well as complications during the consolidation therapy.

At a median follow-up of 39 months (range 2–76 months), predicted 4-year OS was 18.3 % for the fixed-scheduled group

and 17.1 % for the individualized group ($p = 0.807$) (Fig. 2a), and predicted 4-year RFS for patients who had achieved CR was 8.8 % for the former group and 17.9 % for the latter ($p = 0.467$) (Fig. 2b). Significant prognostic factors for OS were cytogenetic risk group and gender (Table 5). Predicted 2-year OS for patients with favorable cytogenetic risk was 56.4 %, while that for patients with intermediate risk was 35.8 % and for patients with adverse risk was 12.6 % ($p < 0.0001$ both for favorable and intermediate risk groups vs. adverse risk group) (Fig. 3). Predicted 4-year OS for female patients was 24.4 %, while that for male patients was 13.5 % ($p = 0.0018$) (Fig. 4). By the multivariate analysis, cytogenetic risk group was a significant prognostic factor ($p < 0.0001$), but the significance regarding gender was marginal ($p = 0.0106$) (Table 5). It is of note that there was no significant difference in OS among patients of age 65–69, 70–74 and 75–79 (Fig. 5). For 124 patients in the non-randomized observation cohort, predicted 1-, 2-, 3- and 4-year OS was 46.5, 33.7, 26.5 and 21.6 %, respectively, which did not differ from the randomized cohorts (Fig. 2a).

Among patients who had obtained CR, predicted 4-year OS was 32.3 % for 63 patients in the ubenimex group, and 18.7 % for 60 patients in the control group ($p = 0.111$) (Fig. 6a). Predicted 4-year RFS was 16.4 % for the former group and 10.4 % for the latter, in favor of the ubenimex group ($p = 0.061$ by the log-rank test and $p = 0.014$ by the generalized Wilcoxon test) (Fig. 6b).

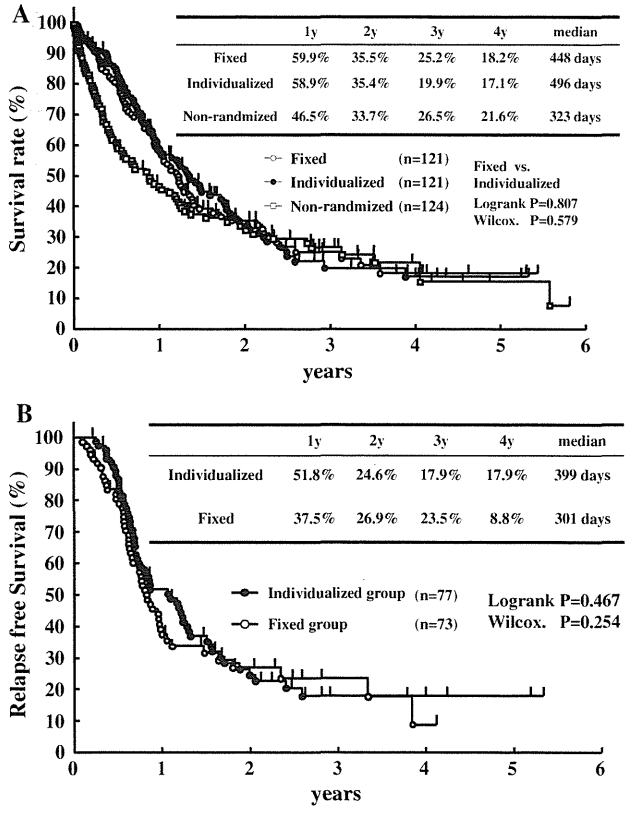

Fig. 2 Overall survival and relapse-free survival. Overall survival rate in three groups (**a**). There was no significant difference in each group. Relapse-free survival in fixed and individualized group (**b**). There was no significant difference in each group

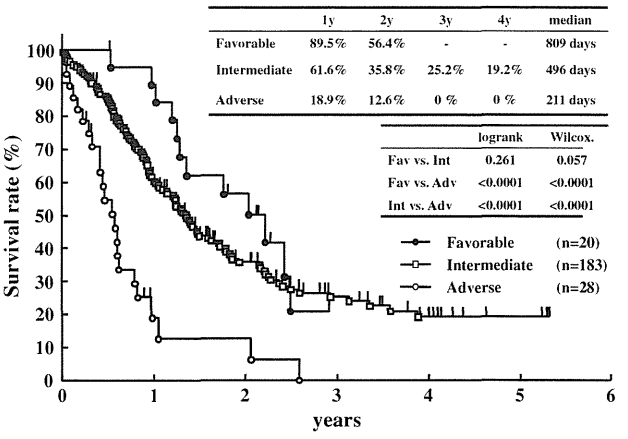

Fig. 3 Overall survival according to cytogenetics. Survival rate decreases down according to cytogenetics group

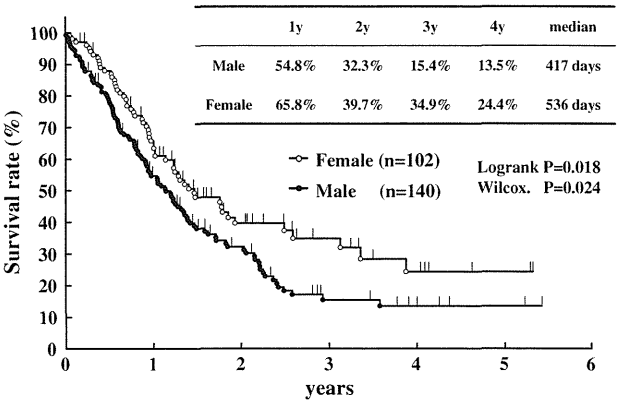

Fig. 4 Overall survival according to gender. There was significant difference between male and female

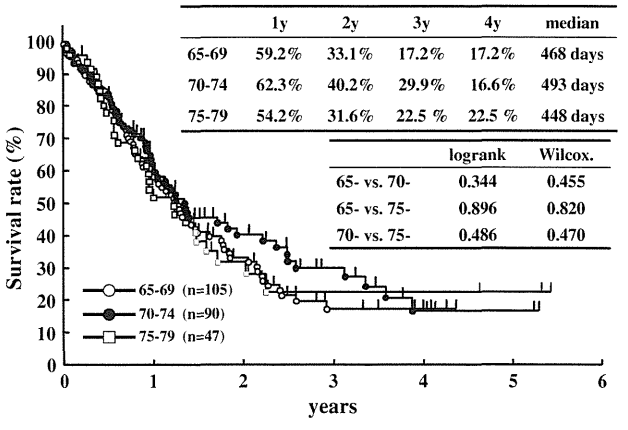

Fig. 5 Overall survival according to age. There was no significant difference in three age groups

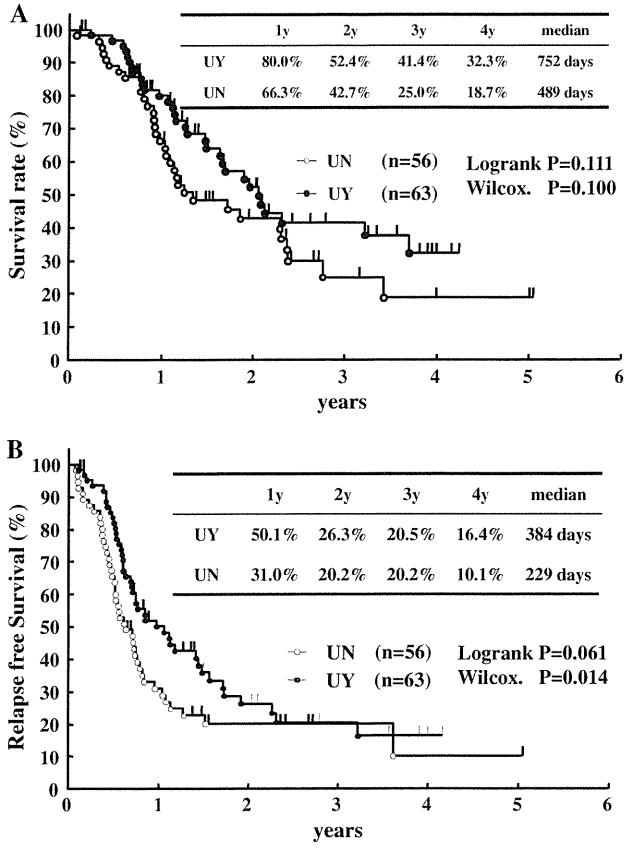

Fig. 6 Overall survival and relapse free survival for with ubenimex group and without ubenimex group. Overall survival (**a**). There was no significant difference between two groups. Relapse-free survival (**b**). There was significant difference by Wilcoxon analysis. UY is with ubenimex group, UN is without ubenimex group

Discussion

Aging generally causes comorbidity, poor performance status, decreased immune competency, deficient stem cell reservoir in bone marrow and so on, and inevitably puts patients at a great disadvantage for receiving intensive chemotherapy. Additionally, elderly AML is biologically associated with higher frequency of adverse karyotypes such as complex abnormalities and aberrations of chromosomes 5 or 7, MDR1 expression, antecedent MDS and secondary AML. Thus, the treatment outcome of elderly patients with AML is much poorer than that of younger patients, when treated with currently available intensive therapy using cytotoxic drugs [1–6].

Recently, HOVON/AMLSG/SAKK group reported that escalation of the dose of DNR to twice the conventional dose to elderly patients of age 60–83 (median 67) with AML or high-risk refractory anemia resulted in higher CR rate (64 vs. 54 %, $p = 0.0002$) without additional toxic effects, but that OS did not differ significantly between the two groups. Subset analysis, however, revealed that only patients of age 60–65 in the escalated-treatment group had significantly higher CR rate (73 vs. 51 %), event-free survival (29 vs. 14 %), and OS (38 vs. 23 %) than patients of the same age range in the conventional dose group, indicating that there was no advantage in the escalated treatment to patients older than 65 [21].

In Japan, where people enjoy the longest life expectancy in the world, JALSG has regarded patients as elderly when they were 65 years or older, since the AML95 study started in 1995, after the analysis of the treatment outcomes of preceding AML87, AML89 and AML92 studies in which patients of age 65 or older were included [9–11]. Thus, even in the HOVON/AMLSG/SAKK study, the question of recommendable treatment for elderly patients older than 65 remains unsettled.

Most drug therapies are generally carried out in a response-oriented and individualized manner, and physicians adjust dosage and treatment period depending on the response of patient's symptoms to administered drugs. However, cancer chemotherapy is generally carried out by fixed dosage and period, because the nadir of myelosuppression, the most important toxic effect of cytotoxic drugs, appears 7–10 days after the discontinuation of drugs. Myelosuppression is usually judged by leukocyte or platelet counts in the peripheral blood, but, if it is judged by bone marrow itself, it is possible to obtain information on myelosuppression directly and earlier.

We attributed the higher CR rates of our previous JALSG studies for adult AML: AML87 [9], AML89 [10] and AML92 [11], to response-oriented individualized therapy, which administered highly intensive but not too toxic doses of anti-leukemia drugs, especially DNR. Disappointingly, however, a prospective randomized study for AML of younger patients of age <65, the JALSG-AML95 failed to demonstrate that response-oriented individualized therapy was superior to the fixed-scheduled therapy, although IDR instead of DNR was used in combination with Ara-C in this study [15]. Both regimens resulted in very high CR rates: 79 and 82 %, respectively, but leukocytopenia was significantly severer and its duration significantly longer, and early death within 30 days tended to occur more frequently in the individualized group. We speculated that, if DNR instead of IDR had been used, the CR rate of the fixed-scheduled group might have been lower like around 70 % as reported from other large scale multicenter studies.

In the present study with elderly patients, we again prospectively compared a fixed-scheduled therapy with a response-oriented individualized therapy, utilizing DNR and BHAC. BHAC has been chosen because this analogue of Ara-C is administered by 3-h infusion, instead of 24-h continuous infusion required for Ara-C, and thus is more conveniently given especially to elderly patients, and also

because BHAC in combination with DNR, 6MP and PSL produced over 70 % CR rates in adult AML in the previous JALSG studies.

Again, however, we could not demonstrate that the response-oriented individualized therapy was not inferior to the fixed-scheduled therapy. CR rate and OS were almost the same in both groups. Patients in the individualized therapy group, being given additional drugs on day 8 and thereafter, showed severer myelosuppression, but the 30-day mortality rates were almost the same in both groups.

Ubenimex is a small molecule inhibitor of leucine aminopeptidase and has various immunomodulatory properties via macrophage or T cell activation. A myeloid lineage marker, CD 13, has been identified as aminopeptidase N36. Ubenimex inhibits aminopeptidase N, and increases the sensitivity of leukemia cells to apoptosis through the inhibition of cell-surface aminopeptidase N activities by hampering the degradation of endothelial cell-derived interleukin 8 [16, 22–24]. In the JALSG AML89 study for younger patients with AML, however, we could not demonstrate that ubenimex given after the end of maintenance chemotherapy improved DFS of AML patients [25]. In this study for elderly AML, ubenimex given orally during and after the consolidation therapy did not clearly improve OS, although RFS in the ubenimex group was longer than that in no-ubenimex control group ($p = 0.061$ by the log-rank test and $p = 0.014$ by the generalized Wilcoxon test).

Cytogenetic risk factor was the most important prognostic factor in this study. Although the number of cytogenetically favorable risk group was small (9 %), 90 % achieved CR and predicted 2-year OS was 56 %. Of patients with intermediate risk cytogenetics, 66 % achieved CR and predicted 4-year OS was 19 %. Of patients with adverse risk cytogenetics, only 25 % achieved CR and predicted 3-year OS was 0 %. Thus, elderly patients with favorable and intermediate risk karyotypes seemed to be benefitted from the present chemotherapy, but not those with adverse risk cytogenetics.

One interesting observation from this study was that female elderly patients had significantly higher CR rate and better OS compared with male patients. Although our female patients tended to have less adverse risk cytogenetics, gender was an independent significant factor for the achievement of CR ($p = 0.0106$) and marginal one for OS ($p = 0.0952$) by the multivariate analysis. In our past adult AML studies, there has been no such observation. ECOG reported that female gender was one of the independent prognostic factors to predict a long-term survival of more than 3 years among 1,414 adult AML patients, but karyotypes were not included in their analysis [26]. German Study Alliance Leukemia recently proposed a novel prognostic model for elderly patients with AML, based on the data of 909 patients entered into the prospective trial, but female gender was not a prognostic factor in achievement of CR, or in OS, either [27]. On the other hand, in childhood leukemia, female patients generally have better prognosis, although no clear explanation has been provided so far. The average remaining life expectancy of Japanese female of age 65 in 2002 was 22.4 years which is 5 years longer than Japanese male of the same age (17.4 years), and this may apply to leukemia patients. However, the higher CR rate is not explainable by this statistics of average life expectancy. Another notable observation was that age was not prognostic factor in the present setting. If patients are eligible for rather strict inclusion criteria as in this study, chronological age alone should not be regarded as a single bad prognostic factor.

In conclusion, we could not demonstrate that the response-oriented individualized therapy gave a better treatment outcome in elderly AML of age 65 or older. Ubenimex given concomitantly during consolidation therapy and thereafter showed a marginal benefit in RFS, but was not impressive. The treatment of elderly AML is still being explored, and new effective therapeutic drugs, especially pathogenic molecule-specific target drugs, are desperately awaited for the treatment of this leukemia, which is increasing in number all over the world.

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ORIGINAL ARTICLE

Phase 1 trial of gemtuzumab ozogamicin in combination with enocitabine and daunorubicin for elderly patients with relapsed or refractory acute myeloid leukemia: Japan Adult Leukemia Study Group (JALSG)-GML208 study

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Abstract We conducted a phase 1 study of a combination of gemtuzumab ozogamicin (GO) plus conventional chemotherapy in elderly patients (≥65 years old) with relapsed or refractory CD33-positive acute myeloid leukemia (AML). Patients received a standard dose of enocitabine (200 mg/m^2 × 8 days) and daunorubicin (30 mg/m^2 × days 1–3) plus an escalating dose of GO (1.5–5 mg/m^2 on day 4). The dose escalation of GO was done according to a standard 3 + 3 design following a modified Fibonacci sequence. No dose-limiting toxicities were observed in three patients (median age, 71) at level 1 (1.5 mg/m^2) or in three patients (median age, 73) at level 2 (3 mg/m^2). Neither veno-occlusive diseases nor sinusoidal obstructive syndromes were noted at either level. However, as GO was withdrawn from the US market in June 2010, based on a randomized study in newly diagnosed AML, we decided not to proceed to the level 3 (5 mg/m^2) in order to avoid possibly more severe adverse effects, and also because all six patients experienced grade 4 myelosuppression, with complete remission in three. This study showed that 3 mg/m^2 of GO in combination with enocitabine and daunorubicin may be a recommendable dose for a phase 2 study in Japanese elderly patients with CD33-positive AML. The study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (http://www.umin.ac.jp/ctr/) as UMIN000002603.

Keywords Acute myeloid leukemia · Elderly · Gemtuzumab ozogamicin · Chemotherapy

Introduction

Therapeutic strategy for acute myeloid leukemia (AML) generally consists of two phases: intensive combination chemotherapy to achieve complete remission (CR) followed by post-remission therapy to prevent relapse with 3–4 courses of intensive combination chemotherapy or with hematopoietic stem cell transplantation (HSCT). Recently, with these intensive treatments, nearly 80 % of

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younger AML patients less than 60 or 65 years obtain CR and nearly 50 % enjoy long-term survival [1].

However, it is not the case with elderly AML patients, as less than 60 % of them achieve CR and less than 20 % secure long-term survival, partly because AML of elderly patients carries biologically unfavorable characteristics compared with those of younger patients, and partly because elderly patients cannot tolerate intensive therapies including HSCT [1–3]. Therefore, collective efforts have been made in the past decades to improve the prognosis of elderly AML patients by employing new drugs and strategies, without any remarkable progress [1, 2].

Gemtuzumab ozogamicin (GO) is a recombinant humanized anti-CD33 monoclonal antibody, conjugated to calicheamicin which is around 1000 times more potent than doxorubicin [4, 5], and a promising novel agent against AML. Since CD33 antigen is expressed on around 90 % of AML cells, GO is attached to the antigen on cell surface and rapidly internalized. Then, the linker of calicheamicin and CD33 is hydrolyzed, and isolated calicheamicin causes subsequent cell death in CD33-positive cells. GO was shown to be effective in patients with relapsed AML and was approved for relapsed elderly AML in the United States [6, 7], and also for relapsed/refractory AML in Japan in 2005 [8].

Since monotherapy with GO alone has resulted in limited efficacy in relapsed AML, combination of GO with conventional anti-leukemia drugs has been tested by several investigators, and resulted in favorable but some conflicting outcomes [9–11]. We conducted a phase 1 study of combination therapy of GO with enocitabine [behenoyl-arabinofuranosyl-cytosine (BHAC)] and daunorubicin (DNR) in elderly patients with relapsed/refractory AML, because there had been no phase 1 study to find the optimal dosage of GO in combination with conventional chemotherapy for elderly AML including Japan where only monotherapy was approved by its national medical insurance. Instead of cytarabine (Ara-C), its analog, BHAC, was chosen for this study. BHAC is resistant to deamination by cytidine deaminase because of enhanced lipophilicity by behenoylation at the 4-amino position of the cytosine, and is converted to cytarabine within and without leukemic cells. BHAC had been widely used for elderly AML patients in Japan owing to its easier administration method by 3-h infusion, compared with 24-h continuous infusion of Ara-C and less adverse events compared with Ara-C [12, 13].

Patients and methods

Patients

Three JALSG member institutions participated in this prospective study. The inclusion criteria of patients were as follows: (a) diagnosed as CD33-positive AML (excluding acute promyelocytic leukemia). The positivity was defined when 20 % or more blasts were positive for CD33 by flow-cytometry; (b) relapsed after the first CR (CR1) or were refractory to initial standard induction therapy; (c) 65–74 years old; (d) 0–1 by Eastern Cooperative Oncology Group (ECOG) performance status; (e) no active double cancer; (f) adequate cardiac, renal and hepatic function; (g) no uncontrolled infection; (h) no HIV, HBV and HCV infection. Cytogenetic abnormalities were grouped by standard criteria and classified according to the UK Medical Research Council (MRC) classification [14]. All patients were hospitalized during the therapy and received the best supportive care if needed.

Study design and treatment protocol

The primary objective of this study was to determine the maximum tolerable dose (MTD) from the dose-limiting toxicity (DLT) of GO in combination with standard doses of BHAC and DNR in elderly Japanese AML patients. All unknown ≥grade 3 and already known ≥grade 4 non-hematological toxicities, and persistent ≥grade 4 neutropenia, lymphopenia or anemia by the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 that occurred, unrelated to leukemia progression until 30 days after the treatment, were considered DLTs. Secondary objective was to evaluate the remission rate and other toxicities of the combination therapy.

Remission induction consisted of BHAC 200 mg/m^2 by 3-h intravenous infusion for 8 days, DNR 30 mg/m^2 by 30-min intravenous infusion for 3 days from day 1, and escalating dose of GO (provided by the Nonprofit Supportive Organization for Cooperative Study on Adult Leukemia Treatment, which purchased it from Weiss Co. Ltd, Japan, and distributed to each participating institution): 1.5 mg/m^2 (level 1), 3 mg/m^2 (level 2) and 5 mg/m^2 (level 3) by 2-h intravenous infusion on day 4. The dose of GO was escalated according to the standard 3 + 3 design (Fig. 1). To prevent the infusion reaction of GO, antihistamines and corticosteroids were given 1 h before the infusion.

The study was approved by the Institutional Review Board at each participating hospitals. Written informed consent was obtained from each patient before registration in accordance with the Declaration of Helsinki. The study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (http://www.umin.ac.jp/ctr/) as UMIN000002603.

Response criteria

Responses were evaluated according to the recommendations of the International Working Group [15]. A CR was

defined as disappearance of all clinical and/or radiologic evidence of the disease with ≤5 % marrow blasts, neutrophil count ≥1 × 10^9/L, and platelet count ≥100 × 10^9/L.

Results

The study was initiated in December 2008, and 3 patients were enrolled to the level 1. Median age of patients was 73 and all were refractory to initial induction therapy (Table 1). None experienced DLT although all had grade 4 neutropenia and thrombocytopenia and grade 3 febrile neutropenia (Table 2). Grade 1/2 non-hematological toxicities included hypoalbuminemia and hyperglycemia in 3, hyponatremia in 2, hyperbilirubinemia and hypokalemia in one. Then another 3 patients were enrolled to the level 2, with median age of 71 with one relapsed case and 2 refractory. Again none experienced DLT, although all had grade 4 neutropenia and thrombocytopenia and 2 had grade 3 febrile neutropenia. Grade 1/2 non-hematological toxicities included hypoalbuminemia and diarrhea in 3, hyperglycemia, hyponatremia and elevated aminotransferases in 2, hyperbilirubinemia, elevated creatinine and pruritis in one. In June 2010, however, GO was withdrawn from the US market based on the result of a randomized study in newly diagnosed younger AML patients conducted by the South Western Oncology Group (SWOG), which resulted in the lack of improvement in CR rate or relapse-free survival (RFS) and in the higher fatal induction adverse-event rate in the GO arm [11]. Therefore, by the recommendation of the independent data and safety monitoring committee, we decided not to proceed to the level 3 (5 mg/m^2) in order to avoid plausible higher adverse events to patients and partly because all 6 patients in the levels 1 and 2 experienced grade 4 myelosuppression.

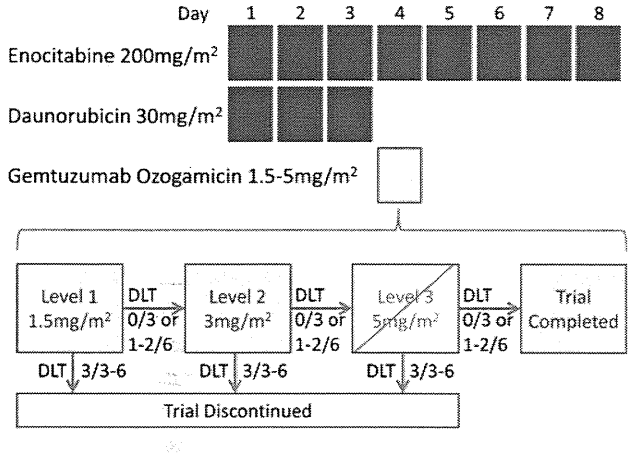

Fig. 1 Treatment schedule. Gemtuzumab ozogamicin (GO) was scheduled to be escalated from 1.5 to 3 and 5 mg/m^2

Table 1 Patient characteristics and outcome of treatment

Age	65, 73, 74	69, 71, 74
Male/female	1/2	2/1
WBC, ×10^9/L	1.1, 3.4, 3.5	2.0, 3.5, 6.1
Blasts (%)	13.9, 18.9, 61.0	29.2, 41.9, 55.8
CD33 positivity (%)	45.8, 84.6, 99.1	82.2, 94.0, 99.3
Relapsed/refractory	0/3	1/2
Months after onset	1, 1, 1	1, 3, 14
Cytogenetics		
Intermediate	3	1
Adverse	0	2
Performance status		
0	3	1
1	0	2
Outcome of treatment		
Hypoplastic marrow on day 15	3	3
CR	2	1
Alive on day 38	3	3
Alive after 6 months	3	2

The number of patients is given in each column

WBC white blood cells

In all 6 patients, sufficient hypoplasia of bone marrow was obtained on day 15. Two patients at the level 1 and one at the level 2 obtained CR, of whom both at the level 1 were refractory cases. Karyotypes of these two cases were 46,XY,i(14)(q10) (9/20 cells) and 47,XX, +8 (10/23 cells), respectively. Another CR case after relapse at the level 2 showed a normal female karyotype. One patient at the level 2 died at 5 months due to disease progression, but other 5 were alive at least for 6 months after the initiation of this treatment.

Discussion

Although GO, a CD33-targeting humanized antibody conjugated with calicheamicin, is a promising agent for AML by its unique mode of action, the efficacy of its monotherapy is limited, resulting in 25–30 % CR in relapsed AML patients in USA and Europe as well as in Japan [6–8]. Therefore, the role of GO in combination with conventional anti-leukemia drugs has been explored by several investigators [16]. In Japan, JALSG previously conducted a phase 1 study of GO in combination with standard induction chemotherapy in relapsed/refractory younger AML patients, and reported that 5 mg/m^2 of GO caused DLTs in 3 of 3 patients and the recommendable dose of GO for a phase 2 study was 3 mg/m^2 in combination with standard doses of DNR or idarubicin (IDR) and Ara-C in younger AML patients [17].

In the present phase 1 study for elderly AML patients, no DLT was observed up to 3 mg/m^2 GO in combination

with BHAC and DNR, but we did not enroll patients for the scheduled 5 mg/m^2 according to the recommendation of the independent data and safety monitoring committee, mainly because fatal toxicity profiles had occurred in the SWOG S0106 study by using 6 mg/m^2 of GO [11] and in the JALSG AML206 study 5 mg/m^2 of GO [17].

Although this study for elderly patients was different from our previous study in younger patients, we used the standard drug combination and dosage for both younger and elderly AML. Therefore, our decision to regard 3 mg/m^2 as the recommendable dose for a phase 2 study, without enrolling patients further to the level 3 or 5 mg/m^2 of GO, would be justified.

Combination therapy of GO with conventional chemotherapy for elderly AML patients has already been tested by other investigators as summarized in Table 3 [10, 18–25]. Although the stage of leukemia and the dosage of GO, as well as combined anti-leukemia drugs and their dosage, are different, the combination therapies have generated satisfactory CR rates (35–81 %) in elderly patients of age 50–83 years. Two Italian groups employed 3 mg/m^2 of GO in combination with chemotherapy, showing its feasibility in elderly patients [22, 23]. Therefore, we decided to initiate our phase 1 study with the half dose of GO (1.5 mg/m^2), considering the possible ethnic difference between Europeans and Japanese. Notably among these reports was the Korean group that conducted a phase 2 study in 37 newly diagnosed elderly AML patients of age $\geq$55 by 6 mg/m^2 of GO on day 1 combined with BHAC 300 mg/m^2 for 5 days and IDR 12 mg/m^2 for 3 days, and reported CR in 28 patients (78 %), CR with incomplete platelet recovery (CRp) in 2 (3 %), induction death in 2, grade 4 adverse events in 2, and 25 patients (68 %) living at the time of report [24]. Although this was not a randomized study, their high CR/CRp rate in elderly AML patients is quite satisfactory.

As mentioned above, but reported only as an abstract so far, SWOG conducted a randomized study comparing DNR 60 mg/m^2 for 3 days and Ara-C 100 mg/m^2 for 7 days versus DNR 45 mg/m^2 for 3 days and Ara-C 100 mg/m^2 for 7 days plus GO 6 mg/m^2 on day 4 in 627 newly diagnosed younger AML patients of age 18–60 [11]. There were no differences in CR rates (66 vs. 69 %) nor in RFS, but the rate of fatal adverse events possibly attributable to treatment was significantly higher in the GO arm (5.8 vs. 0.8 %, $P = 0.002$). It should be argued that the doses of DNR were not the same in the above two groups, and their CR rates are substantially lower even in the DNR + Ara-C arm as compared with those of other cooperative study groups including JALSG, which reported nearly 80 % CR in younger AML patients of age 15–64 years [26]. Nevertheless, GO has been withdrawn from the US market based on the SWOG study.

On the contrary, however, the United Kingdom Medical Research Council reported that a substantial proportion of younger patients with AML had improved survival with the addition of GO to induction chemotherapy with little additional toxicity [9]. They conducted a randomized study comparing GO 3 mg/m^2 on day 1 combined with DNR and Ara-C or with DNR, Ara-C and etoposide in 1113 patients, predominantly younger than age 60. The addition of GO was well tolerated with no significant increase in toxicity, with no overall difference in CR (82 vs. 83 %) or survival. However, a predefined analysis by cytogenetics showed highly significant interaction with GO in induction therapy ($P = 0.001$); significant survival benefit for patients with favorable cytogenetics, no benefit for patients with poor-risk disease, and a trend for benefit in intermediate-risk patients. An internally validated prognostic index identified that approximately 70 % of patients had a predicted benefit of 10 % by GO in 5-year survival.

Thus, the efficacy and role of GO in combination with conventional chemotherapy have not been determined even

Table 2 Hematological toxicities

	Neutrophils			Platelets		
Registration number in each cohort:	1	2	3	1	2	3
Level 1						
Days to nadir	17	18	15	14	14	15
Count at nadir, $\times 10^9$/L	0.016	0	0.009	18	5	15
CTCAE grade	4	4	4	4	4	4
Days to recover to grade 2	36	30	22	22	30	25
Level 2						
Days to nadir	15	15	18	15	13	9
Count at nadir, $\times 10^9$/L	0.067	0.038	0	16	13	15
CTCAE grade	4	4	4	4	4	4
Days to recover to grade 2	22	27	35	29	No	27

Table 3 GO combined chemotherapy for elderly patients with AML

References	Phase	Age	Status of AML	Combined agents	Dose (mg/m^2)	Days treated	Dose of GO (mg/m^2)	Schedule of GO	No. of pts	Grade 5 (*n*)	VOD (*n*)	CR or CRp (%)	Median OS (m)
De Angelo [18] (abstr)	2	62–78	New	Ara-C	100	7	6, 6	d1, d8	21	0	0	48	13. 4
Piccaluga [19]	Not mentioned	50–71	New/Rel/Ref	Ara-C	100	7	6, 4	d1, d8	9	1	0	56	6
Brunnberg [25]	2	60–83	New	Ara-C	100	7	6, 4	d1, d8	57	11	5	58	10
Stone [20]	1/2	52–69	Rel/Ref	Ara-C	3000	5	9	d7	37	7	0	35	8.9
Stone [20]	1/2	52–69	Rel/Ref	Ara-C	3000	5	9, 4. 5	d7, d14	7	4	0	Not reported	Not reported
Fianchi [21]	Not mentioned	65–78	New/Rel/Ref	Ara-C	100	5–7	6	d9	53	7	1	45	9
				G-CSF	5 μg/kg	8							
Clavio [22]	Not mentioned	60–80	New	Ara-C	1000	3	3	d4	46	1	0	52	8
				IDR	5	3							
				Fludarabine	30	3							
Pirrotta [23]	Not mentioned	65–77	New	Ara-C	1000 × 2	3	3	d4	10	0	0	60	10.5
				IDR	5	3							
				Fludarabine	25 × 2	3							
Eom [24]	Not mentioned	55–76	New	BHAC	300	5	6	d1	37	2	2	78.4	Not reported
				IDR	12	3							
Castaigne [10]	3	50–70	New	Ara-C	200	7	3, 3, 3	d1, d4, d7	139	9	3	81	34
				DNR	60	3							

New newly diagnosed, *Rel* relapsed, *Ref* refractory, *Ara-C* cytarabine, *BHAC* enocitabine, *IDR* idarubicin, *DNR* daunorubicin, *VOD* veno-occlusive disease, *CR* complete remission, *CRp* CR with incomplete platelet recovery

in AML of younger patients yet. The present study demonstrates that 3 mg/m^2 of GO with conventional BHAC + DNR can be administered safely in elderly patients with relapsed/refractory AML. Further study with this combination regimen will be warranted for elderly AML, for which the standard therapy has not been established so far, as either initial or salvage therapy.

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Conflict of interest The authors have no conflict of interest.

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