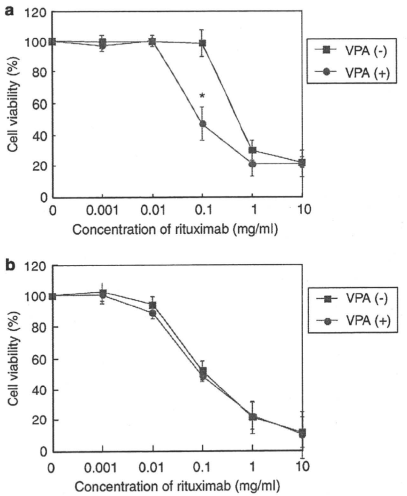

Figure 3 HDAC inhibitors augment the cytotoxic activity of rituximab *in vitro*. BJA-B (**a**) and HBL-2 (**b**) cells were pretreated with the vehicle (−) or VPA (+) for 48 h, washed, and further incubated for 24 h in the presence of human complement (1:4 dilution) and rituximab at the indicated concentrations. Cell viability was determined by modified MTT assays and shown as % untreated control. Each point represents the means ± s.d. (bars) of three independent experiments. *P*-values were calculated by paired Student's *t*-test (*$P<0.01$).

HDAC inhibitors enhance the cytotoxic effect of rituximab in vitro *and* in vivo

Finally, we examined whether the increased expression of CD20 was linked to the augmentation of the cytotoxic activity of rituximab. To this end, we first carried out *in vitro* complement-dependent cytotoxicity assays and found that HDAC inhibitors significantly enhanced the effect of rituximab at suboptimal concentrations (representative results of VPA-pretreated BJA-B cells are depicted in Figure 3a). The enhancement was not observed in HBL-2 and TK cells, in which HDAC inhibitors did not further increase CD20 expression (representative results of VPA-pretreated HBL-2 cells are depicted in Figure 3b). Next, we sought to determine whether this effect could be reproduced *in vivo* using mouse lymphoma models. First, we established lymphoma models by injecting BJA-B cells into non-obese diabetic/severe combined immunodeficiency mice intravenously. The mice were pretreated with either VPA or vehicle, followed by the administration of either rituximab or control IgG. Injected cells showed the enhancement of CD20 expression (Figure 4a) along with histone hyperacetylation (Figure 4b) *in vivo* after treatment with HDAC inhibitors. As expected, the increase in CD20 expression led to the enhancement of anti-lymphoma effects of rituximab; the numbers of CD45-positive cells were significantly lower in the bone marrow of mice pretreated with VPA (Figure 4c). However, VPA failed to prolong the survival of recipient mice compared with vehicle-treated control (data not shown). This might be due to neuronal toxicity of the drug, because VPA-treated mice showed a decrease in food intake and mobility. In addition, intravenously injected BJA-B cells seemed to grow too rapidly to be treated with rituximab alone. We therefore performed the *in vivo* experiment with the combination of another HDAC inhibitor romidepsin and subcutaneous inoculation of lymphoma cells. As shown in Figure 4d, the size of the subcutaneous tumor constantly increased in vehicle/IgG-treated control mice, and importantly, neither HDAC inhibitor alone nor rituximab alone inhibited tumor growth, consistent with the general consensus that Burkitt lymphoma is untreatable by these drugs as monotherapy.[3,4,7] In contrast, tumor growth was significantly retarded when two agents were combined (Figure 4d and Supplementary Figure S6). These results strongly suggest that HDAC inhibitors can potentiate the effects of rituximab *in vivo* by enhancing the expression of CD20.

Discussion

It is now widely accepted that rituximab is essential for the treatment of diffuse large B-cell lymphoma, follicular lymphoma and mantle cell lymphoma.[3,4] There are two emerging issues to be resolved for better usage of this drug: weak effects on some tumors[6–8] and drug resistance.[5,9–11] In this study, we clearly demonstrated that HDAC inhibitors, VPA and romidepsin, potentiated the cytotoxic effect of rituximab against Burkitt lymphoma, which has innate resistance to rituximab,[7] by enhancing the expression of surface CD20 antigen both *in vitro* and *in vivo*. We also analyzed the mechanisms of CD20 upregulation and found that HDAC inhibitors acetylated core histones of *CD20* promoter to enhance the binding of Sp1, leading to transactivation of the *CD20* gene in BJA-B and Namalwa cells. These results suggest that the innate resistance of these cells to rituximab is attributable to epigenetic silencing of CD20, which could be overcome by HDAC inhibitors. It is possible that the same mechanism also underlies acquired resistance in lymphomas heavily treated with rituximab. Indeed, Tomita *et al*.[46] have reported that an HDAC inhibitor, trichostatin A, could restore CD20 expression in a lymphoma cell line established from a patient with follicular lymphoma at the time of CD20-negative transformation after repeated treatment with rituximab. Taken together, HDAC inhibitors are promising agents to overcome both innate and acquired resistance to rituximab in patients with various B-cell malignancies.

HDAC inhibitors appear to upregulate the expression of CD20 specifically, because other B-cell markers such as CD10, CD21, CD43 and CD44 were unaffected. The mechanism underlying this specificity is not fully understood, but previous studies with regard to lineage-restricted expression of these molecules provide a plausible explanation. The expression of CD21 and CD44 was shown to be independent of Sp1,[47,48] a principal mediator of *CD20* transactivation by HDAC inhibitors. Transcription of *CD10* and *CD43* is governed by Sp1, but is also under the control of DNA methylation in a lineage-specific manner.[49,50] It is possible that Sp1 is not accessible to methylated *CD10* and *CD43* promoters of Burkitt lymphoma cells even after the treatment with HDAC inhibitors, although the involvement of other factors cannot be ruled out.

Owing to their unique mechanisms of action, HDAC inhibitors are expected to be effective for tumors resistant to conventional anticancer drugs, and indeed, exerted beneficial effects on peripheral T-cell lymphomas and malignant melanoma in experimental and pilot clinical studies.[16,40] However,

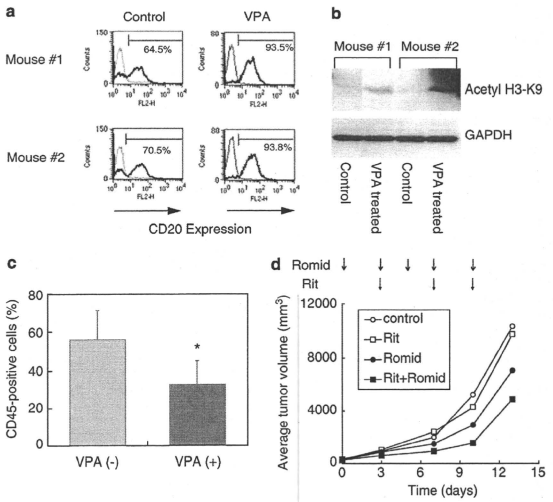

Figure 4 HDAC inhibitors augment the cytotoxic activity of rituximab *in vivo*. (**a**) We injected BJA-B cells into non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice intravenously, and treated them with the vehicle (control) or valproic acid at 150 mg/kg twice a day (VPA) from 4 days after transplantation. CD45-positive cells were isolated from the bone marrow of recipient mice after 48 h, and subjected to flow cytometric analysis for CD20 expression (**a**) and immunoblot analysis for histone H3 acetylation and GAPDH (**b**). (**c**) We determined the percentages of bone marrow CD45-positive cells using flow cytometry after 14 days of transplantation. The graph shows the means ± s.d. (bars) of samples from five mice. *P*-values were calculated by Student's *t*-test (*$P < 0.05$). (**d**) We inoculated Namalwa cells into the right flank of NOD/SCID mice, and started the following treatments when tumors were measurable (day 0): control (open circle), 0.9% NaCl on days 0, 3, 5, 7 and 10, followed by mouse IgG (25 mg/kg) on days 3, 7 and 10; rituximab alone (Rit, open square), 0.9% NaCl on days 0, 3, 5, 7 and 10, followed by rituximab (25 mg/kg) on days 3, 7 and 10; romidepsin alone (Romid, closed circle), romidepsin (0.1 mg/kg) on days 0, 3, 5, 7 and 10, followed by mouse IgG (25 mg/kg) on days 3, 7 and 10; romidepsin plus rituximab (Rit + Romid, closed square), romidepsin (0.1 mg/kg) on days 0, 3, 5, 7 and 10, followed by rituximab (25 mg/kg) on days 3, 7 and 10. Five mice were enrolled in each group. Caliper measurements of the longest perpendicular tumor diameters were performed every alternate day to estimate the tumor volume using the following formula: $4/3\pi \times (\text{width}/2)^2 \times (\text{length}/2)$, which represents the three-dimensional volume of an ellipse.

recent clinical trials concluded that HDAC inhibitors had only limited clinical activity when used as monotherapy.[33,34,36–38] Our present study suggests a more effective way of their application; the ability of HDAC inhibitors to modify the expression of surface molecules may provide a new avenue for the clinical application of HDAC inhibitors, for example, as immunopotentiators or chemosensitizers. Hence, the combination with HDAC inhibitors not only enhances anti-lymphoma activity, but also expands the clinical utility of rituximab for other disorders, such as autoimmune diseases.

Conflict of interest

The authors declare no conflict of interest.

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References

1 LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood* 2008; **112**: 1570–1580.
2 Bonavida B. Rituximab-induced inhibition of antiapoptotic cell survival pathways: implications in chemo/immunoresistance, rituximab unresponsiveness, prognostic and novel therapeutic interventions. *Oncogene* 2007; **26**: 3629–3636.
3 Coiffier B. Rituximab therapy in malignant lymphoma. *Oncogene* 2007; **26**: 3603–3613.
4 Cheson B, Leonard JP. Monoclonal antibody therapy for B-cell non-Hodgkin's lymphoma. *N Engl J Med* 2008; **359**: 613–626.
5 Smith MR. Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene* 2003; **22**: 7359–7368.
6 Hainsworth JD, Litchy S, Barton JH, Houston GA, Hermann RC, Bradof JE *et al*. Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: a phase II trial of the Minnie Pearl Cancer Research Network. *J Clin Oncol* 2003; **21**: 1746–1751.
7 Thomas DA, Faderl S, O'Brien S, Bueso-Ramos C, Cortes J, Garcia-Manero G *et al*. Chemoimmunotherapy with hyper-CVAD plus rituximab for the treatment of adult Burkitt and Burkitt-type lymphoma or acute lymphoblastic leukemia. *Cancer* 2006; **106**: 1569–1580.
8 Johnson NA, Boyle M, Bashashati A, Leach S, Brooks-Wilson A, Sehn LH *et al*. Diffuse large B-cell lymphoma: reduced CD20

expression is associated with an inferior survival. *Blood* 2009; **113**: 3773–3780.
9 Kennedy GA, Tey S-K, Cobcroft R, Marlton P, Cull G, Grimmett K *et al.* Incidence and nature of CD20-negative relapses following rituximab therapy in aggressive B-cell non-Hodgkin's lymphoma: a retrospective review. *Br J Haematol* 2002; **119**: 412–416.
10 Ferreri AJ, Dognini GP, Verona C, Patriarca C, Doglioni C, Ponzoni M. Re-occurrence of the CD20 molecule expression subsequent to CD20-negative relapse in diffuse large B-cell lymphoma. *Haematologica* 2007; **92**: e1–e2.
11 Hiraga J, Tomita A, Sugimoto T, Shimada K, Ito M, Nakamura S *et al.* Down-regulation of CD20 expression in B-cell lymphoma cells after treatment with rituximab-containing combination chemotherapies: its prevalence and clinical significance. *Blood* 2009; **113**: 4885–4893.
12 Esteller ME. Epigenetics in cancer. *N Engl J Med* 2008; **358**: 1148–1159.
13 Ropero S, Esteller M. The role of histone deacetylases (HDACs) in human cancer. *Mol Oncol* 2007; **1**: 19–25.
14 Yang X-J, Seto E. The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nat Rev Mol Cell Biol* 2008; **9**: 206–218.
15 Zhu P, Martin E, Mengwasser J, Schlag P, Janssen K-P, Gottlicher M. Induction of HDAC2 expression upon loss of APC in colorectal tumorigenesis. *Cancer Cell* 2004; **5**: 455–463.
16 Kobayashi Y, Ohtsuki M, Murakami T, Kobayashi T, Sutheesophon K, Kitayama H *et al.* Histone deacetylase inhibitor FK228 suppresses the Ras-MAP kinase signaling pathway by upregulating Rap1 and induces apoptosis in malignant melanoma. *Oncogene* 2006; **25**: 512–524.
17 Wada T, Kikuchi J, Nishimura N, Shimizu R, Kitamura T, Furukawa Y. Expression levels of histone deacetylases determine the cell fate of hematopoietic progenitors. *J Biol Chem* 2009; **284**: 30673–30683.
18 Maiso P, Carvajal-Vergara X, Ocio EM, Lopez-Perez R, Mateo G, Gutlerrez N *et al.* The histone deacetylase inhibitor LBH589 is a potent antimyeloma agent that overcomes drug resistance. *Cancer Res* 2006; **66**: 5781–5789.
19 Kikuchi J, Wada T, Shimizu R, Izumi T, Akutsu M, Mitsunaga K *et al.* Histone deacetylases are critical targets of bortezomib-induced cytotoxicity in multiple myeloma. *Blood*, e-pub ahead of print 29 March 2010; doi:10.1182/blood-2009-07-235663.
20 Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007; **26**: 5541–5552.
21 Murakami T, Sato A, Chun NAL, Hara M, Naito Y, Kobayashi Y *et al.* Transcriptional modulation using HDACi depsipeptide promotes immune cell-mediated tumor destruction of murine B16 melanoma. *J Invest Dermatol* 2008; **128**: 1506–1516.
22 Diermayr S, Himmelreich H, Durovic B, Mathys-Schneeberger A, Siegler U, Langenkamp U *et al.* NKG2D ligand expression in AML increases in response to HDAC inhibitor valproic acid and contributes to allorecognition by NK-cell lines with single KIR-HLA class I specificities. *Blood* 2008; **111**: 1428–1436.
23 Sutheesophon K, Nishimura N, Kobayashi Y, Furukawa Y, Kawano M, Itoh K *et al.* Involvement of the tumor necrosis factor (TNF)/TNF receptor system in leukemic cell apoptosis induced by histone deacetylase inhibitor depsipeptide (FK228). *J Cell Physiol* 2005; **203**: 387–397.
24 Lundqvist A, Abrams SI, Schrump DS, Alvarez G, Suffredin D, Berg M *et al.* Bortezomib and depsipeptide sensitize tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. *Cancer Res* 2006; **66**: 7317–7325.
25 Maeda T, Towatari M, Kosugi H, Saito H. Up-regulation of costimulatory/adhesion molecules by histone deacetylase inhibitors in acute myeloid leukemia cells. *Blood* 2000; **96**: 3847–3856.
26 Gialitakis M, Kretsovali A, Spilianakis C, Kravariti L, Mages J, Hoffmann R *et al.* Coordinated changes of histone modifications and HDAC mobilization regulate the induction of MHC class II genes by Trichostatin A. *Nucleic Acids Res* 2006; **34**: 765–772.
27 Suzuki O, Abe M. Cell surface *N*-glycosylation and sialylation regulate galectin-3-induced apoptosis in human diffuse large lymphoma. *Oncol Rep* 2008; **19**: 743–748.
28 Stein R, Qu Z, Chen S, Solis D, Hansen HJ, Goldenberg DM. Characterization of a humanized IgG4 anti-HLA-DR monoclonal antibody that lacks effector cell functions but retains direct antilymphoma activity and increases the potency of rituximab. *Blood* 2006; **108**: 2736–2744.
29 Kikuchi J, Shimizu R, Wada T, Ando H, Nakamura M, Ozawa K *et al.* E2F-6 suppresses growth-associated apoptosis of human hematopoietic progenitor cells by counteracting proapoptotic activity of E2F-1. *Stem Cells* 2007; **25**: 2439–2447.
30 Gillies SD, Lan Y, Williams S, Carr F, Forman S, Raubitschek A *et al.* An anti-CD20-IL-2 immunocytokine is highly efficacious in a SCID mouse model of established human B lymphoma. *Blood* 2005; **105**: 3972–3978.
31 Bertolini F, Fusetti L, Mancuso P, Gobbi A, Corsini C, Ferrucci PF *et al.* Endostatin, an antiangiogenic drug, induces tumor stabilization after chemotherapy or anti-CD20 therapy in a NOD/SCID mouse model of human high-grade non-Hodgkin lymphoma. *Blood* 2000; **96**: 282–287.
32 Haddad PM, Das A, Ashfaq M, Wieck A. A review of valproate in psychiatric practice. *Expert Opin Drug Metab Toxicol* 2009; **5**: 539–551.
33 Kuendgen A, Strupp C, Aivado M, Bernhardt A, Hildebrandt B, Haas R *et al.* Treatment of myelodysplastic syndromes with valproic acid alone or in combination with all-*trans* retinoic acid. *Blood* 2004; **104**: 1266–1269.
34 Kuendgen A, Schmid M, Schlenk R, Knipp S, Hildebrandt B, Steidl C *et al.* The histone deacetylase (HDAC) inhibitor valproic acid as monotherapy or in combination with all-trans retinoic acid in patients with acute myeloid leukemia. *Cancer* 2006; **106**: 112–119.
35 Tisdale JE, Tsuyuki RT, Oles KS, Penry JK. Relationship between serum concentration and dose of valproic acid during monotherapy in adult outpatients. *Ther Drug Monitor* 1992; **14**: 416–423.
36 Bryd JC, Marcucci G, Parthum MR, Xiao JJ, Klisovic RB, Moran M *et al.* A phase 1 and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia. *Blood* 2005; **105**: 959–967.
37 Fouladi M, Furman WL, Chin T, Freeman III BB, Dudkin L, Stewart CF *et al.* Phase I study of depsipeptide in pediatric patients with refractory solid tumors: a children's oncology group report. *J Clin Oncol* 2006; **24**: 3678–3685.
38 Klimek VN, Fircanis S, Maslak P, Guernah I, Baum M, Wu N *et al.* Tolerability, pharmacodynamics, and pharmacokinetics studies of depsipeptide (romidepsin) in patients with acute myelogenous leukemia or advanced myelodysplastic syndromes. *Clin Cancer Res* 2008; **14**: 826–832.
39 Sutheesophon K, Kobayashi Y, Takatoku M, Ozawa K, Kano Y, Furukawa Y. Histone deacetylase inhibitor depsipeptide (FK228) induces apoptosis in leukemic cells by facilitating mitochondrial translocation of Bax, which is enhanced by the proteasome inhibitor bortezomib. *Acta Haematol* 2006; **115**: 78–90.
40 Piekarz RL, Robey R, Sandor V, Bakke S, Wilson WH, Dahmoush L *et al.* Inhibitor of histone deacetylation, depsipeptide (FR901228), in the treatment of peripheral and cutaneous T-cell lymphoma: a case report. *Blood* 2001; **98**: 2865–2868.
41 Jilani I, O'Brien S, Manshuri T, Thomas DA, Thomazy VA, Imam M *et al.* Transient down-modulation of CD20 by rituximab in patients with chronic lymphocytic leukemia. *Blood* 2003; **102**: 3514–3520.
42 Lapalombella R, Yu B, Triantafillou G, Liu Q, Butchar JP, Lozanski G *et al.* Lenalidomide down-regulates the CD20 antigen and antagonizes direct and antibody-dependent cellular cytotoxicity of rituximab on primary chronic lymphocytic leukemia cells. *Blood* 2008; **112**: 5180–5189.
43 Thevenin C, Lucas BP, Kozlow EJ, Kehrl JH. Cell type- and stage-specific expression of the CD20/B1 antigen correlates with the activity of a diverged octamer DNA motif present in its promoter. *J Biol Chem* 1993; **268**: 5949–5956.
44 Himmelmann A, Riva A, Wilson GL, Lucas BP, Thevenin C, Kehrl JH. PU.1/Pip and basic helix loop helix zipper transcription factors interact with binding sites in the CD20 promoter to help confer lineage- and stage-specific expression of CD20 in B lymphocytes. *Blood* 1997; **90**: 3984–3995.

45 Corcoran LM, Karvelas M, Nossal GJV, Ye Z-S, Jacks T, Baltimore D. Oct-2, although not required for early B-cell development, is critical for later B-cell maturation and for postnatal survival. *Genes Dev* 1993; **7**: 570–582.

46 Tomita A, Hiraga J, Kiyoi H, Ninomiya M, Sugimoto T, Ito M *et al*. Epigenetic regulation of CD20 protein expression in a novel B-cell lymphoma cell line, RRBL1, established from a patient treated repeatedly with rituximab-containing chemotherapy. *Int J Hematol* 2007; **85**: 49–57.

47 Cruickshank MN, Fenwick E, Karimi M, Abraham LJ, Ulgiati D. Cell- and stage-specific chromatin structure across the complement receptor 2 (*CR2/CD21*) promoter coincide with CBF1 and C/EBP-β binding in B cells. *Mol Immunol* 2009; **46**: 2613–2622.

48 Maltzman JS, Carman JA, Monroe JG. Transcriptional regulation of the *Icam-1* gene in antigen receptor- and phorbol ester-stimulated B lymphocytes: Role for transcription factor EGR1. *J Exp Med* 1996; **183**: 1747–1759.

49 Ikawa Y, Sugimoto N, Koizumi S, Yachie A, Saikawa Y. Dense methylation of types 1 and 2 regulatory regions of the CD10 gene promoter in infant acute lymphoblastic leukemia with MLL/AF4 fusion gene. *J Pediatr Hematol Oncol* 2010; **32**: 4–10.

50 Kudo S. Methyl-CpG-binding protein MeCP2 represses Sp1-activated transcription of the human leukosialin gene when the promoter is methylated. *Mol Cell Biol* 1998; **18**: 5492–5499.

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ORIGINAL ARTICLE

Allogeneic stem cell transplantation versus chemotherapy as post-remission therapy for intermediate or poor risk adult acute myeloid leukemia: results of the JALSG AML97 study

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Abstract We prospectively compared allogeneic hematopoietic stem cell transplantation (allo-HSCT) with chemotherapy as a post-remission therapy in a multicenter trial (JALSG AML97) of adult patients with intermediate or poor risk acute myeloid leukemia (AML). Of 503 patients aged 15–50 years old registered between December 1997 and July 2001, 392 achieved complete remission (CR). CR patients classified in the intermediate or poor risk group using a new scoring system were tissue typed. Seventy-three with and 92 without an HLA-identical sibling were assigned to the donor and no-donor groups. Of 73 patients in the donor group, 38 (52%) received allo-HSCT during CR1 and 17 (23%) after relapse. Intention-to-treat analysis revealed that the relapse incidence was reduced in the donor group (52 vs. 77%; $p = 0.008$), and the disease-free survival (DFS) improved (39 vs. 19%; $p = 0.016$), but overall survival (OS)

For the Japan Adult Leukemia Study Group (JALSG).

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was not significantly different (46 vs. 29%; $p = 0.088$). The OS benefit was seen in the patients aged 36–50 years old (49 vs. 24%; $p = 0.031$), suggesting an advantage of allo-HSCT among older patients with leukemia that is more resistant to chemotherapy than that among younger patients.

Keywords AML · Allogeneic hematopoietic stem cell transplantation · Post-remission chemotherapy

1 Introduction

Around 70–80% of newly diagnosed patients with adult acute myeloid leukemia (AML) achieve complete remission (CR) when treated with cytarabine (AraC) and anthracycline, usually daunorubicin (DNR) or idarubicin (IDR). However, only about one-third of these patients remain disease free for more than 5 years [1–5]. Intensified post-remission chemotherapy has improved the survival rates of patients with AML, especially of younger patients [6]. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered to be the most intensive post-remission treatment consisting of high-dose chemoradiotherapy and allo-immune mechanisms. However, the powerful anti-leukemic effects of this treatment are counterbalanced by a high incidence of treatment-related mortality (TRM). Thus, allo-HSCT has not always been considered superior to chemotherapy [7, 8]. Intensified chemotherapy with high-dose Ara-C confers promising results on good risk patients [9] for whom allo-HSCT is currently abstained in the first CR (CR1). The Japan Adult Leukemia Study Group (JALSG) AML97 protocol committee circulated a questionnaire among the institutions participating in JALSG regarding their policy about indications for allo-HSCT among AML patients in CR1. The findings revealed that good risk patients in CR1 did not undergo an allo-HSCT at most of these institutions. Cytogenetic profile has been widely used to classify the patients with AML [7–13]; however, cytogenetic studies are not always foolproof. The JALSG established a scoring system that adopted significant factors including cytogenetic results from previous JALSG AML trials [14]. We applied this scoring system to stratify patients and conducted a prospective, multicenter cooperative study (AML97) to compare allo-HSCT with chemotherapy among intermediate and poor risk patients with AML in CR1.

2 Patients and methods

2.1 Patients and study design

The JALSG AML97 study was implemented between December 1997 and July 2001 at 103 institutions where the ethical committees approved the protocol. Adult patients aged from 15 to 64 years newly diagnosed with de novo AML according to the French–American–British (FAB) classification at each institution were eligible, but those with acute promyelocytic leukemia (APL) were excluded. Peripheral blood and bone marrow smears of the registered patients were stained with May-Giemsa, peroxidase, and esterase at Nagasaki University and subsequently reviewed by a central review committee. All patients provided written informed consent to participate before registration in this study.

The chemotherapeutic design of AML97 has been described elsewhere in detail [15]. In short, all the patients were treated with the same induction therapy consisted of AraC (100 mg/m^2, continuous infusion, days 1–7) and IDR (12 mg/m^2 days 1–3). If the patients did not achieve remission after the first induction therapy, then the same therapy was given again. For patients who did not achieve a CR even after second induction therapy, no further treatment was defined in this study. In the comparison between allo-HSCT and chemotherapy as post-remission therapy, these patients were not included in the analysis. All patients who achieved CR were randomized to receive either 4 courses of consolidation therapy without maintenance therapy (group A) or the conventional JALSG post-remission regimen with maintenance therapy (group B) [3]. The results of the two post-remission chemotherapeutic strategies (group A vs. group B) were comparable [15]. The CR patients were classified into good, intermediate or poor risk groups according to the scoring system described below. Intermediate or poor risk patients younger than 50 years old with living siblings were tissue typed. Patients with an HLA-identical sibling were assigned to undergo allo-HSCT soon after three courses of consolidation therapy (donor group), and those without living or HLA-identical siblings were assigned to the no-donor group that continued receiving chemotherapy.

Patients in the donor group with AST or ALT values fourfold higher than the normal range, serum bilirubin and creatinine more than 2 mg/dl, ejection fraction based on an echocardiogram of less than 50% or oxygen saturation according to pulse oximetry of less than 90% were ineligible for allo-HSCT, but were analyzed as a donor group one in an intention-to-treat fashion. Conditioning before transplantation and prophylaxis for graft-versus-host disease was performed according to each institutional standard. Either allogeneic peripheral blood or bone marrow was allowed to be the stem cell source.

2.2 Scoring system

We collected clinical and laboratory data (except for APL) from previous JALSG AML trials (AML87, $n = 234$

Table 1 JALSG scoring system

Scoring system		
System 1		
MPO positive blasts	>50%	+2
Age	≤50 years	+2
WBC	$\leq 2 \times 10^9$/l	+2
FAB subtypes	non-M0, M6, M7	+1
Performance status	0, 1, 2	+1
No. of induction	1	+1
t(8;21) or inv(16)	+	+1
Total score		
Good risk group		8–10
Intermediate risk group		5–7
Poor risk group		0–4
System 2		
MPO positive blasts	>50%	+2
Age	≤50 years	+2
WBC	$\leq 2 \times 10^9$/l	+2
FAB subtypes	non-M0, M6, M7	+1
Performance status	0, 1, 2	+1
Total score		
Good risk group		7–8
Intermediate risk group		4–6
Poor risk group		0–3

MPO myeloperoxidase, *WBC* white blood cell

patients; AML89, $n = 311$; AML92, $n = 986$), and then selected significant factors for achieving CR, disease-free survival (DFS) and overall survival (OS) using multivariate analysis [14]. According to the weight of significance, myeloperoxidase positivity of blasts, patient age, and WBC count at diagnosis were valued at 2 points, and FAB subtypes, performance status, numbers of inductions required to achieve CR, and favorable karyotypes of t(8;21) or inv(16) were valued at 1 point (Table 1, system 1). When we originally planned to use this system, cytogenetic data were not always available at diagnosis. Thus, we designed the system 2 that could be applied even without a cytogenetic data.

2.3 Statistical analysis

The aim of this study was to compare the efficacy of allo-HSCT and chemotherapy as a post-remission treatment, by evaluating DFS and OS rate. Forty-two patients were estimated for an evaluation of the primary endpoint of this study. The JALSG data management committee collected the clinical data from all participating institutions, then fixed them and analyzed the OS of each risk group in July 2004 and the relapse rate (RR), DFS, OS and TRM of the donor and no-donor groups in January 2009. The OS, DFS, RR and TRM were measured from the date of CR. The event for OS was death due to all causes, and patients were censored at the last observation date if alive. The events of DFS were death during CR or relapse. The RR was defined as the cumulative probability of relapse, censoring at death in CR. The events of TRM comprised death before relapse. We estimated OS, DFS, RR and TRM with their respective standard errors using the Kaplan–Meier method [16]. We compared the OS, DFS, RR and TRM between the patients with and without a donor using the log-rank test. Furthermore, the hazard ratio and the 95% confidence interval (CI) of the OS, DFS, RR and TRM were calculated using Cox regression analysis. The Wilcoxon rank-sum test was used for the continuous data, such as age and WBC count, while the Chi-square test was used for the ordinal data, such as the risk group and the frequency of allo-HSCT. All analyses were performed on the intention-to-treat principal with all patients in their allocated arms. Adding to the prospective comparison of the efficacy between allo-HSCT and chemotherapy, we also retrospectively performed subgroup analysis by age. Statistical analyses were conducted using the SAS software package (SAS Institute, Inc, Cary, NC).

3 Results

3.1 Study patients and genetical allocation

Five hundred and three de novo AML patients aged from 15 to 50 years participated in the AML97 comparison of allo-HSCT with chemotherapy as a post-remission therapy. Of 392 patients achieved CR, 62 patients were excluded from the analysis because of insufficient data mainly deficient clinical data at diagnosis which were essential to verify their classification. Three hundred and thirty evaluable patients were classified into the good ($n = 149$), intermediate ($n = 162$) or poor risk ($n = 19$) groups using the scoring system described above (Fig. 1). The 5-year OS

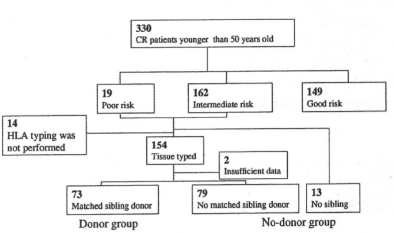

Fig. 1 Overview of patients included in analysis by risk classification, HLA typing, and donor availability

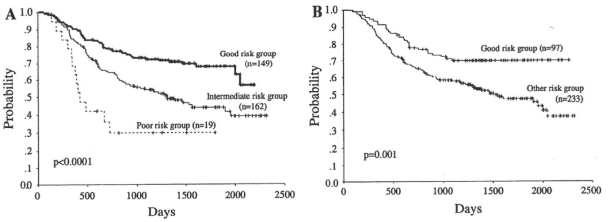

Fig. 2 Overall survival of patients in CR according to JALSG scoring system (**a**) and by cytogenetic studies (**b**)

rates of the CR patients with good, intermediate and poor risk were 68, 44 and 30%, respectively [hazard ratio (HR), 0.51 (good vs. intermediate) and 0.25 (good vs. poor), respectively; 95% confidential interval (CI), 0.35–0.73 (good vs. intermediate) and 0.14–0.48 (good vs. poor); $p < 0.0001$; Fig. 2a]. Among the intermediate and poor risk patients with living siblings, 154 patients and their siblings were examined for their HLA types. Seventy-three of these patients had an HLA-identical sibling and were assigned to the donor group. Thirteen patients with no siblings and 79 patients without an HLA-identical sibling were assigned to the no-donor group (92 patients). Finally, one patient in donor group and one patient in no-donor group were excluded from the analysis because of their insufficient data of survival (Fig. 1). The follow-up durations of the donor and no-donor groups were 1854 days (range 163–3176 days) and 1010 days (range 93–3008 days), respectively.

3.2 Patient characteristics of donor versus no-donor groups

Table 2 shows the characteristics of patients in the donor and no-donor groups. The distributions of these features were comparable in both groups with respect to age, gender, initial WBC count, MPO positivity of blasts, FAB subtype, performance status, prognostic risk according to JALSG score, presence of favorable cytogenetic abnormalities, and the groups of post-remission chemotherapy.

3.3 Donor group

Fifty-six patients (76%) in the donor group actually underwent allo-HSCT (Table 2). Thirty-eight patients (52%) received an allo-HSCT during CR1 at a median of 159 days (range 43–314 days) from CR1. Eighteen patients underwent allo-HSCT after relapse. The median times between CR1 and relapse and between CR1 and a transplantation were 183 days (range 39–757 days) and 248 days (range 157–973 days), respectively. Thirty and 24 patients were transplanted after undergoing a conditioning regimen with or without total body irradiation (TBI), respectively, and conditioning information was not available for 2 patients. The sources of transplanted stem cells were bone marrow cells ($n = 26$), peripheral blood cells ($n = 27$) and bone marrow cells together with peripheral blood cells ($n = 2$). Twenty-nine of the 56 patients in the donor group who underwent allo-HSCT remain alive. Twenty patients died of recurrent leukemia and 7 of transplant-related causes. Seventeen patients allocated to the donor group did not receive a transplantation for the following reasons; patients' refusal ($n = 6$), donors' refusal to donate ($n = 2$), physician's decision ($n = 1$), disease progression before transplantation ($n = 2$), donor health problems ($n = 2$) and unknown reasons ($n = 4$).

3.4 No-donor group

Of the 92 patients in the no-donor group, 42 eventually underwent HSCT (Table 2): autotransplantation ($n = 3$), allo-HSCT from HLA mismatched-related donors ($n = 4$), allo-HSCT from an HLA matched-unrelated donor ($n = 28$), and allo-HSCT from an HLA-mismatched unrelated donor ($n = 7$). Eleven patients underwent a transplantation during CR1 from an unrelated donor or mismatched-related donor at a median of 281 days (range 170–1700 days) from CR1, significantly later than those transplanted during CR1 in the donor group ($p < 0.001$). Thirty-one patients received a transplantation after relapse. The median times between CR1 and relapse and between CR1 and a transplantation were 329 days (range 92–876 days) and 519 days (range 167–1373 days), respectively.

3.5 Comparison of donor versus no-donor groups

The actual risk of relapse at 8 years was significantly lower in the donor group than in the no-donor group (52 vs. 77%, respectively, HR, 0.58; 95% CI, 0.39–0.88; $p = 0.008$; Table 3). The TRM did not significantly differ between the donor and the no-donor groups (16 vs. 17%, respectively, HR, 0.97; 95% CI, 0.34–2.80; $P = 0.959$; Table 3). Seven

Table 2 Patients' characteristics

	Donor	No-donor	*p*
Total number	73	92	
Age			
Median (range)	37 (16–50)	36 (15–50)	0.60[a]
15–35 years	33	46	
36–50 years	40	46	0.54[b]
Sex			
M/F	44/29	45/47	0.15[b]
WBC at diagnosis (10^9/l) (range)	3.8 (0.05–36.8)	5.1 (0.14–45.0)	0.16[a]
MPO positivity of blasts (range)	30 (0–100)	50 (0–100)	0.18[a]
FAB classification			
M0	4	6	
M1	18	25	
M2	22	24	
M4	20	23	
M5	7	14	
M6	1	0	
M7	1	0	0.67[b]
Performance status			
0–1	66	84	
2–3	7	8	0.70[b]
Risk classification by JALSG scoring system			
Intermediate	64	84	
Poor	9	8	0.45[b]
Cytogenetics			
t(8;21) or inv(16)	4	4	0.74[b]
Chemotherapy group			
Group A	38	42	
Group B	30	47	0.28[c]
Not randomized	5	3	
Allogeneic transplant			
During CR1	38	11	
		9 from UD	
		1 from MUD	
		1 from MRD	
After relapse	18	31	
No transplant	17	50	

UD HLA-matched unrelated donor, *MUD* HLA-mismatched unrelated donor, *MRD* HLA-mismatched related donor, *WBC* white blood count, *MPO* myeloperoxidase

[a] Mann–Whitney test

[b] Chi-square test

[c] Chi-square test excluding non-randomized

patients in the donor group and four in the no-donor group died of transplant-related causes during CR1. The lower RR in the donor group resulted in a significantly better DFS compared with the no-donor group (39 vs. 19%, respectively, HR, 0.63; 95% CI, 0.44–0.92; $P = 0.016$; Table 3; Fig. 3). The significant superiority of DFS in the donor group translated into a higher OS rate, but the difference in OS between the two groups did not reach statistical significance (46 vs. 29%, HR, 0.70; 95% CI, 0.47–1.06; $p = 0.088$; Table 3; Fig. 4).

The donor/no-donor analysis was performed on the intention-to-treat principal, which may underestimate the beneficial effect of allo-HSCT probably because of low compliance of transplantation. The 8-year DFS and OS of the recipients actually transplanted during CR1 ($n = 38$) in the donor group were significantly better than those of the patients not transplanted in the no-donor group ($n = 50$); 58 versus 27%, HR, 0.36; 95% CI, 0.20–0.66; $p < 0.001$, and 61 versus 24%, HR, 0.36; 95% CI, 0.19–0.68; $p = 0.001$, respectively.

Table 3 Effects of donor availability on outcome in donor and no-donor groups

Outcome	Donor			No-donor			*p*	HR (95% CI)
	n	No. of events	Probability of outcome at 8 years ±SE (%)	*n*	No. of events	Probability of outcome at 8 years ±SE (%)		
All patients	73			92				
RR		36	52 ± 6		67	77 ± 5	0.008	0.58 (0.39–0.88)
TRM		7	16 ± 6		7	17 ± 7	0.959	0.97 (0.34–2.80)
DFS		44	39 ± 6		74	19 ± 4	0.016	0.63 (0.44–0.92)
OS		37	46 ± 7		61	29 ± 6	0.088	0.70 (0.47–1.06)
Age ≤35	33			46				
RR		17	52 ± 9		31	70 ± 7	0.309	0.74 (0.41–1.33)
TRM		2	12 ± 8		3	15 ± 8	0.785	0.78 (0.13–4.71)
DFS		20	39 ± 9		34	26 ± 7	0.366	0.78 (0.45–1.35)
OS		18	42 ± 10		27	35 ± 9	0.860	0.95 (0.52–1.72)
Age >35	40			46				
RR		19	52 ± 9		36	85 ± 6	0.006	0.46 (0.26–0.81)
TRM		5	19 ± 8		4	19 ± 11	0.962	1.03 (0.27–3.92)
DFS		24	39 ± 8		40	12 ± 5	0.012	0.52 (0.31–0.87)
OS		19	49 ± 9		34	24 ± 7	0.031	0.54 (0.31–0.95)

RR relapse rate, *DFS* disease-free survival, *TRM* treatment-related mortality, *OS* overall survival

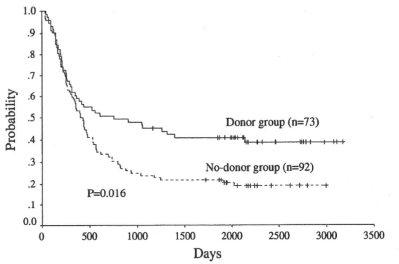

Fig. 3 Disease-free survival in donor and no-donor groups

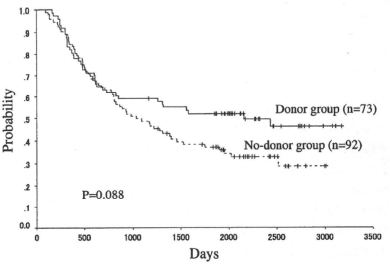

Fig. 4 Overall survival in donor and no-donor groups

3.6 Subset analysis according to patient age

The OS of the patients younger than 35 years of age were comparable between the donor and the no-donor groups (Fig. 5a). However, the OS of the patients aged >35 in the donor group was significantly better compared with the no-donor group (49 vs. 24%, respectively, HR, 0.54; 95% CI, 0.31–0.95; $p = 0.031$; Table 3; Fig. 5b). The RR, TRM, DFS and OS in the donor group were comparable between the two age categories (Table 3; Fig. 5c). In contrast, OS and DFS were marginally worse in the no-donor group of patients aged >35 than ≤35 years (Table 3; Fig. 5d). The distribution of the cytogenetic profile, risk by the JALSG scoring system, myeloperoxidase positivity of blasts, WBC count, FAB classification and performance status at diagnosis did not significantly differ between the two age categories in the no-donor group (data not shown).

4 Discussion

Many clinical trials have compared allo-HSCT with chemotherapy as a post-remission therapy for the patients with AML during CR1. Most of these targeted all patients in CR1 as a single population without prospective stratification by the prognostic factors. Thus, patients were simply assigned into the allo-HSCT or the chemotherapy groups according to donor availability [7, 10, 17, 18]. Here, we

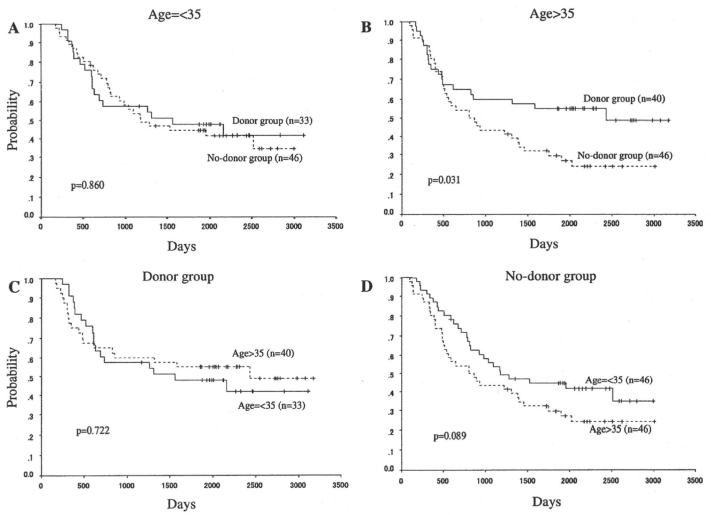

Fig. 5 Overall survival of patients according to age (**a** and **b** ≤35 and >35 years, respectively) and donor availability (**c** and **d**, donor and no-donor groups, respectively)

prospectively compared the effectiveness of allo-HSCT with chemotherapy among patients who were stratified into intermediate or poor risk groups according to JALSG scoring, which constitutes a new means of predicting the prognosis of AML. When this study was planned, as the availability of the cytogenetic study was expected to be variable, and the JALSG scoring system was revealed to be useful to stratify the patients, we adopted a scoring system to select the intermediate and poor risk patients. In contrary to our expectation, cytogenetic studies were performed in 99.2% of the registered patients and the results were available in 97% of the patients. Of 330 CR patients younger than 50 years old, cytogenetic studies disclosed that 97 had good prognostic chromosomal abnormalities, i.e., t(8;21) or inv(16). The OS was significantly better among patients with than without good prognostic cytogenetic profiles (70 vs. 47% at 5 years, with HR, 0.51; 95% CI, 0.34–0.77; $p = 0.001$; Fig. 2b). According to JALSG scoring, 87, 10 and 0 patients with good prognostic cytogenetic abnormalities corresponded to the good, intermediate and poor risk groups, respectively. More good risk patients were selected using this scoring system than by that using karyotype of AML cells alone and about 10% of patients who might be classified into the good risk group by cytogenetic profiles entered the comparison groups by the JALSG scoring system. The JALSG scoring system, which resembles the index used in the Bordeaux Grenoble Marseille Toulouse (BGMT) intergroup study [18], obviously separated patients with a good prognosis who should be excluded from the transplantation trials.

Allo-HSCT prevents AML relapse through intensive cytoreduction using high-dose chemoradiotherapy and graft-versus-leukemia effects. However, previous trials have not always shown advantages of this strategy on the survival of AML patients in CR1. Some studies have not found a benefit of allo-HSCT either on DFS or OS [7, 8], and some showed an advantage only on DFS [10, 17] compared with chemotherapy/auto-transplantation. Retrospective subgroup analysis and meta-analysis have shown a better OS in the donor group [10, 13, 19, 20], demonstrating the importance of limiting the indication of allo-HSCT for only the patients with an intermediate or poor risk.

The following issues should be considered regarding the prospective comparison of allo-HSCT with chemotherapy: assignment of patients according to sibling donor availability [21], low compliance of allo-HSCT for patients in the donor group, and allo-HSCT performed in the no-donor

group from unrelated donors. We could compare the effectiveness of treatment strategies using the intention-to-treat analysis. However, the intrinsic issues of this type of trial and recent advances in alternative stem cell sources will cause difficulties with future prospective comparison of allo-HSCT and chemotherapy using a similar study design.

Although the comparison was performed among patients in the intermediate and poor risk groups, the benefit of allo-HSCT was not significant in OS. Low compliance of allo-HSCT during CR1 in the donor group (52% in the current trial) and allo-HSCT in the no-donor group (total 45%; 11% during CR1) appeared to make the efficacy of allo-HSCT underestimated, especially with regard to OS. However, survival was significantly better among older patients in the donor group (Table 3; Fig. 5b), which seemed to contradict previous findings [19]. Age usually adversely affects allo-HSCT outcome, but it was not associated with the decrease of OS in the donor group in the present study (Table 3; Fig. 5c). Low incidence of TRM probably allowed the powerful anti-leukemic effect of allo-HSCT to function properly, indicating the advantage of allo-HSCT especially among older patients with leukemia that was more resistant to chemotherapy than that among younger patients [1] shown in the no-donor group (Fig. 5d), and caused a contrary result from HOVON/SAKK study. The recent reduction in TRM seemed to contribute much to these results as suggested by others [22, 23]. Different population of the cohorts selected by JALSG scoring and by cytogenetic profiles might also have influenced the present findings.

Molecular markers can be very useful for selecting patients who will most likely benefit from allo-HSCT during CR1 among those with a normal karyotype, which comprises the largest group of patients with AML [24]. The overall safety of allo-HSCT obviously needs improvement, and also patients with chemotherapy-resistant AML who could benefit from allo-HSCT should be identified. Thus, stratification of patients with AML should be improved using a combination of leukemic cell karyotype and, genetic markers and also other clinical findings.

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References

1. Ohno R, Kobayashi T, Tanimoto M, et al. Randomized study of individualized induction therapy with or without vincristine, and of maintenance-intensification therapy between 4 or 12 courses in adult acute myeloid leukemia. AML-87 Study of the Japan Adult Leukemia Study Group. Cancer. 1993;71(12):3888–95.
2. Kobayashi T, Miyawaki S, Tanimoto M, et al. Randomized trials between behenoyl cytarabine and cytarabine in combination induction and consolidation therapy, and with or without ubenimex after maintenance/intensification therapy in adult acute myeloid leukemia. The Japan Leukemia Study Group. J Clin Oncol. 1996;14(1):204–13.
3. Miyawaki S, Tanimoto M, Kobayashi T, et al. No beneficial effect from addition of etoposide to daunorubicin, cytarabine, and 6-mercaptopurine in individualized induction therapy of adult acute myeloid leukemia: the JALSG-AML92 study. Japan Adult Leukemia Study Group. Int J Hematol. 1999;70(2):97–104.
4. Buchner T, Hiddemann W, Berdel WE, et al. 6-Thioguanine, cytarabine, and daunorubicin (TAD) and high-dose cytarabine and mitoxantrone (HAM) for induction, TAD for consolidation, and either prolonged maintenance by reduced monthly TAD or TAD-HAM-TAD and one course of intensive consolidation by sequential HAM in adult patients at all ages with de novo acute myeloid leukemia (AML): a randomized trial of the German AML Cooperative Group. J Clin Oncol. 2003;21(24):4496–504.
5. Moore JO, George SL, Dodge RK, et al. Sequential multiagent chemotherapy is not superior to high-dose cytarabine alone as postremission intensification therapy for acute myeloid leukemia in adults under 60 years of age: Cancer and Leukemia Group B Study 9222. Blood. 2005;105(9):3420–7.
6. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med. 1994;331(14):896–903.
7. Cassileth PA, Harrington DP, Appelbaum FR, et al. Chemotherapy compared with autologous or allogeneic bone marrow transplantation in the management of acute myeloid leukemia in first remission. N Engl J Med. 1998;339(23):1649–56.
8. Harousseau JL, Cahn JY, Pignon B, et al. Comparison of autologous bone marrow transplantation and intensive chemotherapy as postremission therapy in adult acute myeloid leukemia. The Groupe Ouest Est Leucemies Aigues Myeloblastiques (GOELAM). Blood. 1997;90(8):2978–86.
9. Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res. 1998;58(18):4173–9.
10. Burnett AK, Wheatley K, Goldstone AH, et al. The value of allogeneic bone marrow transplant in patients with acute myeloid leukaemia at differing risk of relapse: results of the UK MRC AML 10 trial. Br J Haematol. 2002;118(2):385–400.
11. Byrd JC, Dodge RK, Carroll A, et al. Patients with t(8;21)(q22;q22) and acute myeloid leukemia have superior failure-free and overall survival when repetitive cycles of high-dose cytarabine are administered. J Clin Oncol. 1999;17(12):3767–75.
12. Schlenk RF, Benner A, Hartmann F, et al. Risk-adapted postremission therapy in acute myeloid leukemia: results of the German multicenter AML HD93 treatment trial. Leukemia. 2003;17(8):1521–8.
13. Suciu S, Mandelli F, de Witte T, et al. Allogeneic compared with autologous stem cell transplantation in the treatment of patients younger than 46 years with acute myeloid leukemia (AML) in first complete remission (CR1): an intention-to-treat analysis of the EORTC/GIMEMAAML-10 trial. Blood. 2003;102(4):1232–40.
14. Kuriyama K, Tomonaga M, Kobayashi T, et al. Trial to extract prognostic factors prior to the start of induction chemotherapy for adult AML. Berlin: Springer; 1998. p. 901–5.
15. Miyawaki S, Sakamaki H, Ohtake S, et al. A randomized, postremission comparison of four courses of standard-dose

consolidation therapy without maintenance therapy versus three courses of standard-dose consolidation with maintenance therapy in adults with acute myeloid leukemia: the Japan Adult Leukemia Study Group AML 97 Study. Cancer. 2005;104(12):2726–34.
16. Kaplan E, Meier P. Non parametric estimation from imcomplete observations. J Am Stat Assoc. 1958;53:457–81.
17. Keating S, de Witte T, Suciu S, et al. The influence of HLA-matched sibling donor availability on treatment outcome for patients with AML: an analysis of the AML 8A study of the EORTC Leukaemia Cooperative Group and GIMEMA. European Organization for Research and Treatment of Cancer. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. Br J Haematol. 1998;102(5):1344–53.
18. Jourdan E, Boiron JM, Dastugue N, et al. Early allogeneic stem-cell transplantation for young adults with acute myeloblastic leukemia in first complete remission: an intent-to-treat long-term analysis of the BGMT experience. J Clin Oncol. 2005;23(30):7676–84.
19. Cornelissen JJ, van Putten WL, Verdonck LF, et al. Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom? Blood. 2007;109(9):3658–66.
20. Yanada M, Matsuo K, Emi N, Naoe T. Efficacy of allogeneic hematopoietic stem cell transplantation depends on cytogenetic risk for acute myeloid leukemia in first disease remission: a metaanalysis. Cancer. 2005;103(8):1652–8.
21. Gray R, Wheatley K. How to avoid bias when comparing bone marrow transplantation with chemotherapy. Bone Marrow Transplant. 1991;7(Suppl 3):9–12.
22. Frassoni F, Labopin M, Gluckman E, et al. Results of allogeneic bone marrow transplantation for acute leukemia have improved in Europe with time–a report of the acute leukemia working party of the European group for blood and marrow transplantation (EBMT). Bone Marrow Transplant. 1996;17(1):13–8.
23. Bacigalupo A, Sormani MP, Lamparelli T, et al. Reducing transplant-related mortality after allogeneic hematopoietic stem cell transplantation. Haematologica. 2004;89(10):1238–47.
24. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1909–18.

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ORIGINAL ARTICLE

Randomized trial of response-oriented individualized versus fixed-schedule induction chemotherapy with idarubicin and cytarabine in adult acute myeloid leukemia: the JALSG AML95 study

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Abstract A multicenter, prospective, randomized study was conducted to compare a response-oriented individualized remission induction therapy with a standard fixed-schedule induction therapy, using idarubicin (IDR) and cytarabine (Ara-C), in adult patients with acute myeloid leukemia (AML). Newly diagnosed patients with AML of age less than 65 were randomly assigned to receive either of the two schedules. Both groups received IDR (12 mg/m^2) for 3 days and Ara-C (100 mg/m^2) for 7 days. In the individualized group, if the bone marrow on day 8 did not become hypocellular with less than 15% blasts, patients received additional IDR for one more day and Ara-C for 2 or 3 more days. Patients achieving complete remission (CR) received the same post-remission therapy. The CR rate was 79.4% for the individualized group ($n = 209$) and 81.9% for the fixed group ($n = 221$) ($p = 0.598$). At a median follow-up of 81 months, 7-year predicted overall survival was 37% for the individualized group and 39% for

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the fixed group ($p = 0.496$), and 7-year predicted event-free survival was 22% for the individualized group and 23% for the fixed group ($p = 0.546$). Thus, the present study could not demonstrate any advantage of a response-oriented individualized induction therapy over a fixed-schedule induction therapy in this protocol setting.

Keywords Acute myeloid leukemia · Response-oriented individualized induction therapy · Idarubicin · Cytarabine

1 Introduction

In Japan, a response-oriented individualized induction therapy has been used for adult acute myeloid leukemia (AML) since the reporting of the success of DCMP two-step therapy using daunorubicin (DNR), cytarabine (Ara-C), 6-mercaptopurine (6MP) and prednisolone (PSL), by Uzuka et al. in the mid 1970s [1]. They reported a complete remission (CR) rate of more than 80% in adult AML, which is currently not surprisingly high but was remarkable in the mid 1970s even for a single institutional study. A subsequent multi-institutional study conducted at the Koseisho Leukemia Study Group using this DCMP two-step protocol could not replicate the high CR rate, but a subset analysis revealed the first-step alone could induce almost the same CR rate as the two-step therapy [2]. Accordingly, a response-oriented individualized induction therapy, the BHAC-DMP therapy, using enocitabine (BHAC), Ara-C, 6MP, and PSL, was developed, and Ohno et al. [3] reported more than 80% CR in adult AML by a single institutional study.

The multi-institutional AML87 study conducted by the Japan Adult Leukemia Study Group (JALSG) confirmed the high CR rate of response-oriented individualized BHAC-DMP therapy in adult AML, reporting an 80% CR rate [4]. Subsequent JALSG studies, AML89 [5] and AML92 [6], also employed the response-oriented individualized induction therapy, and reported 81 and 77% CR rates, respectively, in adult patients of age less than 65 years 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-schedule induction therapies are used [7]. Therefore, even though the necessity for a randomized study was seriously discussed among JALSG members, it was not possible to find any fixed-schedule regimen worth comparing with the present individualized therapy.

In the above 3 JALSG studies, DNR was used as one of the key drugs. However, in the late 1980s, a new DNR analogue, idarubicin (IDR), was introduced clinically, and in the early 1990s, one single [8] and 2 multi-institutional studies [9, 10] reported that IDR plus Ara-C regimens could produce 70–80% CR rates in adult AML by fixed-schedule therapy, which were significantly higher than the 58–59% CR rates of DNR plus Ara-C regimens.

Consequently, after IDR had been approved in Japan in 1995, a randomized study using IDR and Ara-C was conducted, comparing a response-oriented individualized induction therapy with a fixed-schedule therapy in previously untreated adult patients with AML.

2 Patients and methods

2.1 Patients

From August 1995 to December 1997, 437 newly diagnosed adult patients with AML, aged 15–64 years, were consecutively registered from 79 institutions, which participated in JALSG. The enrolled number of patients per hospital varied from 1 to 23 with median number of 4, and about 60% of patients were registered from major hospitals listed in the institutions of the authors.

AML was 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 by May-Giemsa, peroxidase, and esterase staining. Then, diagnosis was reevaluated by the central review committee. FAB-M3 was not registered in this study. Eligibility criteria included adequate functioning of the liver (serum bilirubin level < 2.0 mg/dL), kidney (serum creatinine < 2.0 mg/dL), heart, and lungs, and an Eastern Cooperative Oncology Group performance status between 0 and 3. Patients were not eligible if they had prediagnosed myelodysplastic syndrome (MDS), but were eligible if they had no definite diagnosis of MDS, as confirmed by bone marrow histological analysis even when they had a previous history of

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hematological abnormality. Cytogenetic analyses were performed at either laboratories in participating hospitals or authorized commercial laboratories according to standard methods of G-banding. Cytogenetic abnormalities were grouped by standard criteria and classified according to the MRC classification [11]. The protocol was approved by institutional review board of each hospital. Informed consent was obtained from all patients before registration.

2.2 Treatment regimens

Patients were assigned randomly to receive either a response-oriented individualized induction therapy or a fixed-schedule induction therapy, using a centralized telephone procedure. All patients received IDR (12 mg/m^2/day, intravenously) from days 1 to 3 and Ara-C (100 mg/m^2/day, by 24-h continuous infusion) from days 1 to 7. Examination of bone marrow on the day 8 was evaluated at each participating hospital and the decision was made by the attending physician in charge of the hospital. In the individualized group, bone marrow aspiration was performed on day 8, and if the marrow was not severely hypoplastic and had more than 15% blasts, additional IDR was given on day 8 and Ara-C on days 8 to 10, or if the marrow was severely hypoplastic and had more than 15% blasts, additional IDR was given on day 8 and Ara-C on days 8 and 9. If patients suffered from documented infection on day 8, cancellation of additional chemotherapy was permitted according to the judgment of the attending physician (Fig. 1). The main aim of the individualized therapy was to give highly intensive but not too toxic doses of anti-leukemia drugs, especially IDR, to make the bone marrow severely hypoplastic, reduce the percentage of blasts to less than 5% within 10 days and obtain CR by the first course of induction therapy. In the fixed-schedule group (fixed 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 intervals. If patients did not achieve CR with two courses, they were judged as failure cases.

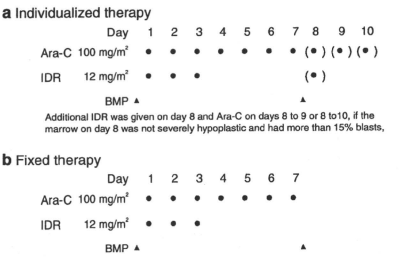

Fig. 1 Treatment scheme of induction therapy

All patients in both groups who had achieved CR planned to receive the same 3 courses of consolidation therapy. The first course consisted of mitoxantrone (MIT; 7 mg/m^2 by 30-min infusion on days 1–3) and Ara-C (200 mg/m^2 by 24-h continuous infusion on days 1–5). The second consisted of BHAC (200 mg/m^2 by 3-h infusion on days 1–7), DNR (50 mg/m^2 intravenously on days 1–3), 6MP (70 mg/m^2 orally on days 1–7), and etoposide (ETP; 100 mg/m^2 by 1-h infusion on days 1–5). The third consisted of BHAC (200 mg/m^2 on days 1–7) and aclarubicin (ACR; 14 mg/m^2 intravenously on days 1–7). Each consolidation course was given as soon as possible after WBC and platelet counts had recovered to more than 3,000/μL and 100,000/μL, respectively. Intrathecal methotrexate (15 mg), Ara-C (40 mg), and PSL (10 mg) were given after the second consolidation therapy for the prophylaxis of central nervous system leukemia.

After the completion of consolidation therapy, all patients planned to receive 6 courses of maintenance/intensification therapy every 2 months. The first course consisted of BHAC (170 mg/m^2 on days 1–5), DNR (40 mg/m^2 on days 1 and 4), and 6MP (70 mg/m^2 on days 1–7). The second consisted of BHAC (170 mg/m^2 on days 1–5) and MIT (5 mg/m^2 on days 1 and 2). The third consisted of BHAC (170 mg/m^2 on days 1–5), ETP (80 mg/m^2 on days 1, 3, and 5), and vindesine (2 mg/m^2 intravenously on days 1 and 8). The fourth consisted of BHAC (170 mg/m^2 on days 1–5), ACR (14 mg/m^2 on days 1–4) and 6MP (70 mg/m^2 on days 1–7), the fifth was the same as the first, and the sixth was the same as the third. Each course was given at 2-month intervals.

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 was permitted.

2.3 Response criteria and statistical analysis

CR was defined as the presence of all of the following: less than 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 first day of induction therapy to death by any cause and censored at the last follow-up. Event-free survival (EFS) was computed from the first day of induction therapy to relapse or death by any cause and censored at the last follow-up, and the survival time of patients who did not achieve CR was defined as 0 days. 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. Patients who underwent allogeneic bone marrow transplantation (BMT) were censored at the date of BMT or not censored according to the object of the analysis. Kaplan–Meier product-limit estimates were used to determine OS, EFS, and RFS. To test factors to predict CR, χ^2 test and Wilcoxon rank-sum test were used for univariate analysis and the multiple logistic regression model for multivariate analysis. For comparison of OS, EFS, and RFS, the log-rank test was used for univariate analysis and Cox's proportional hazard model for multivariate analysis. JMP software (SAS Institute Inc., Cary, NC, USA) was used for the analysis; p values less than 0.05 (two-sided) were considered statistically significant. Analysis was done on an intent-to-treat basis.

3 Results

3.1 Patient population and characteristics

Of 437 patients registered, 7 patients were judged as ineligible by the central review committee because of other diseases: one refractory anemia with excess of blast, 5 mixed-lineage leukemia, and one acute lymphoblastic leukemia (ALL), with 430 patients considered evaluable. Two hundred nine patients received the individualized therapy and 221 the fixed-schedule therapy. Pretreatment characteristics are presented in Table 1. There were no major imbalances between the two randomized groups. Overall, the median age was 44 years, and 154 patients (36%) were of age 50 years or older. Cytogenetic analysis was reported in 414 patients (96%), and the cytogenetic prognostic groups were equally distributed in both arms.

3.2 Overall treatment results

Of 430 evaluable patients, 347 (80.7%) achieved CR. Of 209 patients in the individualized group, 166 (79.4%) achieved CR, and of 221 in the fixed group, 181 (81.9%) obtained CR ($p = 0.516$) (Table 2). CR rates related to FAB classification, age, and cytogenetics are shown in Table 2, and there were no statistically significant differences between the two groups. In the individualized group, of 41 patients with favorable chromosomes, 39 (95%) achieved CR, of 133 with intermediate chromosomes, 109 (79%) achieved CR, and of 28 with adverse chromosomes, 17 (61%) achieved CR. In the fixed group, of 49 patients with favorable chromosomes,

Table 1 Pretreatment characteristics

	Individualized group ($n = 209$)	Fixed group ($n = 221$)
Median age (range)	44 years (15–64)	44 years (15–64)
PS 0	34.9%	38.5%
PS 1	42.6%	45.2%
PS 2	14.4%	9.5%
PS 3	8.1%	6.8%
Leukocyte counts > 50,000/μL	17.7%*	29.9%*
Peroxidase positivity ≥ 50%	62.8%	64.2%
Presence of Auer body (%)	37.5%	46.1%
Presence of trilineage dysplasia	25.4%	21.2%
LDH ≥ 500 IU/L	65.9%	69.1%
Cytogenetics		
Favorable	19.6%	22.2%
Intermediate	63.6%	59.7%
Adverse	13.4%	14.0%
Unknown	3.3%	4.1%

* $p < 0.05$

Table 2 CR rates related to FAB classification, age, and cytogenetics

	All cases		Individualized group		Fixed group	
	No.	CR (%)	No.	CR (%)	No.	CR (%)
FAB						
M 0	16	62.5	8	62.5	8	62.5
M 1	80	85.0	41	85.4	39	84.6
M 2	192	82.3	95	77.9	97	86.6
M 4	108	78.7	55	80.0	53	77.4
M 5	20	90.0	5	100.0	15	86.7
M 6	8	50.0	2	50.0	6	50.0
M 7	6	66.7	3	66.7	3	66.7
Age						
15–19	40	90.0	19	100.0	21	81.0
20–29	65	78.5	29	75.9	36	80.6
30–39	71	81.7	41	75.6	30	90.0
40–49	100	83.0	45	77.8	55	87.3
50–59	105	77.1	53	79.2	52	75.0
60–64	49	77.6	22	77.3	27	77.8
Cytogenetics						
Favorable	90	93.3	41	95.1	49	91.8
Intermediate	265	80.8	133	78.9	132	82.6
Adverse	59	62.7	28	60.7	31	64.5
Unknown	16	75.0	7	71.4	9	77.8
Total	430	80.7	209	79.4	221	81.9

45 (92%) achieved CR, of 132 with intermediate chromosomes, 109 (83%) achieved CR, and of 31 with adverse chromosomes, 20 (65%) achieved CR.

In the individualized group, 149 patients (71%) achieved CR after the first course, and 79 (38%) patients who had received additional chemotherapy during the first course, 56 (71%) achieved CR. In the fixed group, 159 (72%) achieved CR after the first course (Table 3; Fig. 2). CR rates between patients who had equal to or more than 15% of blasts in bone marrow on day 8 and those had less than 15% were not significantly different in the individualized group (75 and 63%, respectively; $p = 0.09$), but were significantly different in the fixed group (81 and 56%, respectively; $p < 0.001$).

Myelosuppression judged by the nadir of leukocyte counts and the period of leukocyte count less than 1,000/μL after the first course of induction therapy was significantly more severe in the individualized group, as shown in Table 4. Early death within 30 days occurred in 10 (4.8%) in the individualized group and 4 (1.8%) in the fixed group ($p = 0.105$). There were no statistically significant differences in the distribution or frequency of complications between the two groups.

Significant favorable prognostic features for the achievement of CR were cytogenetic risk group (favorable or intermediate), blast peroxidase positivity of 50% or more, and pretreatment LDH value of less than 500 IU/L. These features were independent by the logistic regression analysis and not different between the two groups.

All courses of consolidation therapy were administered to 72% of patients in the individualized group and 80% in the fixed group ($p = 0.087$), and all courses of maintenance therapy were administered to 36 and 41% ($p = 0.365$), respectively. The most common reason for these cancellations was relapse in both groups (34 and 42 patients, respectively). The second common reason was BMT in the first remission (22 and 12 patients, respectively).

At a median follow-up of 81 months, 23 patients underwent BMT in the first remission, 29 after relapse and 4 without remission in the individualized group, and 15, 32 and 7 patients, respectively, in the fixed group. If patients who underwent BMT were censored at the date of transplantation to decrease the influence of BMT, 7-year predicted OS was 37% for the individualized group and 39% for the fixed group ($p = 0.496$) (Fig. 3a), and 7-year predicted EFS was 22 and 23%, respectively ($p = 0.546$) (Fig. 3b). If patients who underwent BMT were not censored, 7-year predicted OS was 35 and 35%, respectively

Table 3 Effect of individualized induction therapy

	Patients (%)	CR after first course	
		n	%
Individualized group	209	149	71
Additional chemotherapy −	130 (62)	93	72
Additional chemotherapy +	79 (38)	56	71
Fixed group	221	159	72

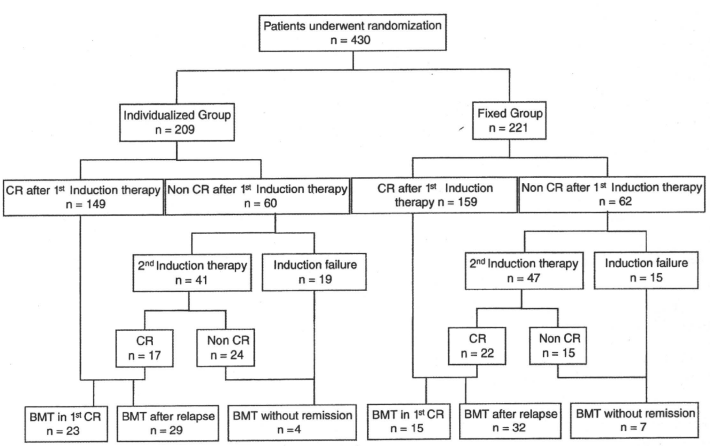

Fig. 2 Flow diagram: study design and outcome

Table 4 Comparison of treatment outcome

	Individualized group ($n = 209$)	Fixed group ($n = 221$)	p
CR rate (%)	79.4	81.9	0.516
After the first course	71.3	71.9	
After the second course	8.1	10.0	
Marrow blasts at day 8	12.9 ± 17.8%	11.1 ± 18.4%	0.021
Nadir of WBC[a]	328 ± 205/μL	394 ± 215/μL	0.0002
Period of WBC < 1,000/μL[a]	19.6 ± 9.8 days	17.8 ± 8.5 days	0.024
Days to CR[a]	38.9 ± 17.5	38.5 ± 16.2	0.802
Days till the consolidation therapy	49 ± 22	46 ± 18	0.157
Early death rate			
Within 30 days	4.8%	1.8%	0.105
Between 30 and 60 days	0.9%	1.4%	
Overall survival at 7 years	37%	39%	0.496
Event-free survival at 7 years	22%	23%	0.546

Data with ± denotes mean ± standard deviation

[a] After the initial course of induction therapy

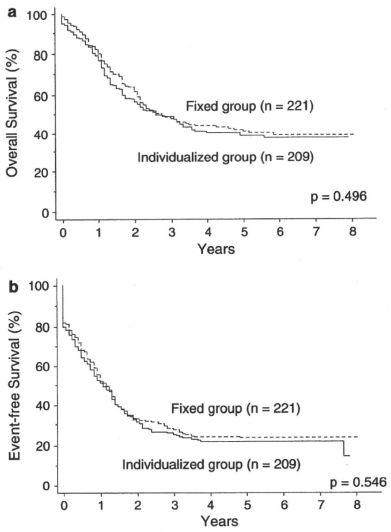

Fig. 3 Overall survival (**a**) and event-free survival (**b**). Predicted 7-year OS was 37% for the individualized group ($n = 209$) (*solid line*) and 39% for the fixed group ($n = 221$) (*dotted line*) ($p = 0.496$), and EFS was 22% for the individualized group (*solid line*) and 23% for the fixed group (*dotted line*) ($p = 0.546$)

($p = 0.840$), and 7-year predicted EFS was 23 and 24%, respectively ($p = 0.717$). Significant adverse prognostic features for OS were absence of Auer body, cytogenetic risk group (adverse), and age more than 30 years, and those for EFS were blast peroxidase positivity less than 50%, cytogenetic risk group (adverse), pretreatment LDH value equal or more than 500 IU/L, and FAB classification (M0, M6, or M7). When patients who underwent BMT were censored, RFS of CR patients was 27% for the individualized group and 29% for the fixed group ($p = 0.712$). Significant adverse prognostic features for RFS of CR patients were cytogenetic risk group (adverse) and FAB classification (M0, M6, or M7). There were no significant differences in these prognostic features between the two groups. However, among patients of age 50 years or older, the individualized group had significantly lower RFS (17%) than the fixed group (34%, $p = 0.026$), but there was no such difference of RFS (34 and 25%, respectively, $p = 0.194$) among patients of age less than 50 years.

4 Discussion

Most drug therapies are generally carried out in a response-oriented and individualized manner. Physicians adjust the dosage and treatment period depending on the response of patient's disease to the administered drugs. The reason why cancer chemotherapy is generally carried out by fixed dosage and period is because 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. However, if it is judged by bone marrow itself it is possible to obtain information on myelosuppression directly and earlier. Although the present individualized therapy requires frequent bone marrow aspirations and a prompt decision by attending physicians, well-trained hematology oncologists have little difficulty in

making such a decision. In addition, the present protocol states that, if the decision is difficult due to equivocal findings, additional drugs should be given.

It was considered that the higher CR rates of previous JALSG studies for adult AML: AML87 [4], AML89 [5] and AML92 [6], were due to response-oriented individualized therapy, giving highly intensive but not too toxic doses of anti-leukemia drugs, especially IDR, to make the bone marrow severely hypoplastic, reduce the percentage of blasts to less than 5% within 10 days, and aim to obtain CR by the first course of induction therapy. For example, in the AML89 study, the primary objective of which was to compare Ara-C with BHAC in remission induction therapy, 130 (82%) of 159 patients in the DNR + Ara-C + 6MP + PSL group achieved CR by this individualized induction therapy [5]. It is clear that without a prospective randomized study, one cannot argue whether the individual therapy is superior to a standard fixed-schedule remission induction therapy. However, it is noteworthy, that in the 3 randomized studies in the USA mentioned in Sect. 1, which compared IDR plus Ara-C with DNR plus Ara-C, the fixed-schedule therapy with DNR plus Ara-C resulted in merely 57–58% CR rates, while IDA plus Ara-C regimens produced 70–80% CR rates [8–10].

Disappointingly, the present study could not demonstrate that response-oriented individualized therapy was superior to the fixed-schedule therapy. Both regimens resulted in almost the same CR rates: 79 and 82%, respectively. Actually, both therapies produced very good CR rates. The results were interpreted as follows: IDR is a good but very powerful drug, therefore, additional IDR and Ara-C on day 8 or later may not be necessary and gave too much myelosuppression. In fact, in the individualized group, leukocytopenia was significantly more severe and its duration was significantly longer, and early death within 30 days tended to occur more frequently. From the present study it is suggested that response-oriented individualized therapy could be successful in cases where DNR is used as a key drug. Usui et al. [12] reported that the optimal dose of DNR in the induction therapy for newly diagnosed adult AML was approximately 280 mg/m^2 (40 mg/m^2 for 7 days), which was more than its conventional dose of 40–60 mg/m^2 for 3 days.

It is very interesting that among patients of age 50 years or older, the individualized group had significantly lower RFS than the fixed group, but there was no such difference in younger patients. However, we cannot clearly explain the real reason of this observation. There may be potential sources of bias in our subset analysis of clinical data that have many confounding factors. Therefore, we must be cautious in drawing a conclusion from this observation.

So far, CR rates around 80% for newly diagnosed adults of age less than 65 years with non-M3 AML seems to be the upper limit by currently available anti-leukemia drugs in multi-institutional studies [7]. To increase the CR rates and improve treatment outcomes, novel drugs other than cytotoxic ones such as all-*trans* retinoic acid (ATRA) for acute promyelocytic leukemia (APL) are needed. With ATRA in combination with conventional cytotoxic drugs such as IDR and Ara-C, CR rates around 95% and more than 80% overall survival for APL with PML/RARα can be obtained [13, 14]. The remarkable success of molecule targeting therapy with ATRA against APL as well as imatinib mesylate against chronic myeloid leukemia [15] and Philadelphia chromosome-positive ALL [16] with BCR/ABL is a good example. Specific molecule targeting therapy should be developed against pathogenic molecules responsible for leukemogenesis. Meanwhile, it is necessary to explore separate treatment regimens for prognostically different subtypes of AML with conventionally available modalities in order to increase the cure rate of adult leukemia.

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References

1. Uzuka Y, Liong SK, Yamagata S. Treatment of adult acute non-lymphoblastic leukemia using intermittent combination chemotherapy with daunomycin, cytosine arabinoside, 6-mercaptopurine and prednisolone-DCMP two step therapy. Tohoku J Exp Med. 1976;118(Suppl):217–25.
2. Cooperative Study Group on Leukemia and Allied Diseases. DCMP two-step therapy for acute myelogenous leukemia in adults. Jpn J Clin Oncol. 1978;8:133–40.
3. Ohno R, Kato Y, Nagura E, Murase T, Okumura M, Yamada H, et al. Behenoyl cytosine arabinoside, daunorubicin, 6-mercaptopurine, and prednisolone combination therapy for acute myelogenous leukemia in adults and prognostic factors related to remission duration and survival length. J Clin Oncol. 1986; 4:1740–7.
4. Ohno R, Kobayashi T, Tanimoto M, Hiraoka A, Imai K, Asou N, et al. Randomized study of individualized induction therapy with or without vincristine, and of maintenance-intensification therapy between 4 or 12 courses in adult acute myeloid leukemia. AML-87 Study of the Japan Adult Leukemia Study Group. Cancer. 1993;71:3888–95.
5. Kobayashi T, Miyawaki S, Tanimoto M, Kuriyama K, Murakami H, Yoshida M, et al. Randomized trials between behenoyl cytarabine and cytarabine in combination induction and cytarabine in combination induction and consolidation therapy, and with or without ubenimex after maintenance/intensification therapy in adult acute myeloid leukemia. J Clin Oncol. 1996;14:204–13.
6. Miyawaki S, Tanimoto M, Kobayashi T, Minami S, Tamura J, Omoto E, et al. No beneficial effect from addition of etoposide to daunorubicin, cytarabine, and 6-mercaptopurine in individualized induction therapy of adult acute myeloid leukemia: the JALSG-AML92 study. Int J Hematol. 1999;70:97–104.
7. Ohno Ryuzo. How high can we increase complete remission rate in adult acute myeloid leukemia? Int J Hematol. 2000;72:272–9.