Effect of allogeneic stem cell transplantation in patients with minimally differentiated acute myeloid leukemia

Satoshi Yamasaki1, Jun Aoki2, Jinichi Mori3, Kaito Harada4, Masashi Sawa5, Naoyuki Uchida6, Kazuki Ohashi7, Takahiro Fukuda8, Shiro Koh9, Heiwa Kanamori10, Hiroyasu Ogawa11, Hirokazu Okumura12, Tatsuo Ichinohe13, Yoshinobu Kanda14, Junji Tanaka15, Yoshiko Atsuta16, Masamitsu Yanada17, Shingo Yano18

1Department of Hematology and Clinical Research Institute, National Hospital Organization Kyushu Medical Center, Fukuoka, Japan
2Department of Hematology, Yokohama City University Medical Center, Yokohama, Japan
3Department of Hematology, Jyoban hospital, Iwaki, Japan
4Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, Japan
5Department of Hematology and Oncology, Anjo Kosei Hospital, Anjo, Japan
6Department of Hematology, Federation of National Public Service Personnel Mutual Aid Associations Toranomon Hospital, Tokyo, Japan
7Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan
8Division of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan
9Division of Hematology, Fuchu Hospital, Fuchu, Japan
10Division of Hematology, Kanagawa Cancer Center, Yokohama, Japan
11Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Kobe, Japan
12Department of Internal Medicine, Toyama Prefectural Central Hospital, Toyama, Japan
13Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
14Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan
15Department of Hematology, Tokyo Women’s Medical University, Tokyo, Japan
16Department of Healthcare Administration, Nagoya University Graduate School of Medicine and Japanese Data Center for Hematopoietic Cell Transplantation, Nagoya, Japan
17Department of Hematology and Oncology, Fujita Health University School of Medicine, Toyoake, Japan
18Division of Hematology and Oncology, Department of Internal Medicine, Jikei University School of Medicine, Tokyo, Japan

Objective: The objective of this study was to analyze factors associated with outcomes of allogeneic hematopoietic cell transplantation (HCT), and cytogenetic and disease risks at HCT among patients with minimally differentiated acute myeloid leukemia (AML) to identify the potential clinical efficacy of allogeneic HCT. Patients and Methods: We retrospectively analyzed 398 patients who received allogeneic HCT for minimally differentiated AML between 2000 and 2015. Results: According to cytogenetic and disease risks at HCT, we divided patients into four groups: 1) intermediate risk and complete remission (CR) (n=183) or 2) non-CR (n=90), and 3) poor risk and CR (n=66) or 4) non-CR (n=59). Median follow-up times for survivors were 8-42 months. Three-year overall survival (OS) in the four groups were 1) 59.7%, 2) 30.9%, 3) 58.7%, and 4) 9.7%, respectively. Multivariate Cox regression analysis showed that a poor risk, Eastern Cooperative Oncology Group performance status ≥1, and non-CR at HCT were independent predictors of poorer OS. Conclusion: Our data suggest that allogeneic HCT can be considered for patients with minimally differentiated AML. Prospective trials of allogeneic HCT are needed to improve the safety and efficacy of allografting in patients with minimally differentiated AML. 

(Original Article: Journal of Hematopoietic Cell Transplantation 8(2): 59-63, 2014.)
Introduction

Minimally differentiated acute myeloid leukemia (AML), "AML not otherwise specified with minimal differentiation", is classified in the 2016 WHO classification, which has no specific associated chromosomal abnormality and corresponds to AML-M0 in the French-American-British classification. This disease occurs in approximately 2%-5% of AML patients, including young infants and older adults, and their prognoses are usually very poor. The leukemic cells are medium sized, mimic lymphoblasts, and <3% exhibit cytochemical reactions of myeloperoxidase. The immunophenotypic characteristics of blasts are positivity for at least one myeloid antigen, such as CD13, CD33, and CD15, frequent expression of stem cell-associated antigens, including CD34 and CD117, nuclear terminal deoxynucleotidyl transferase positivity in approximately 50% of cases, CD7 positivity in approximately 40% of cases, and usually negativity for mature myeloid and monocytic markers. Frequent incidences of complex and unbalanced chromosomal changes have been reported, but most cases should be classified as AML with myelodysplasia-related changes by the 2016 WHO classification.

The objective of this study was to analyze factors associated with outcomes of allogeneic hematopoietic cell transplantation (HCT), and cytogenetic and disease risks at HCT among patients with minimally differentiated AML to identify the potential clinical efficacy of allogeneic HCT and/or novel therapies.

Patients and methods

Data Source

More than 200 Japanese transplant centers participate in the Transplant Registry Unified Management Program (TRUMP) database including physician-reviewed data with informed consent and yearly follow-ups. This study was approved by the Data Management Committee of the Japanese Society for Hematopoietic Cell Transplantation and Institutional Review Board of Kyushu Medical Center.

Patient Selection

A total of 851 patients aged >15 years, who received a first allogeneic HCT for de novo AML-M0 were eligible for the present study. Patients with myelodysplasia-related changes (n=140), a mixed phenotype (n=63), AML with maturation (n=3), AML without maturation (n=102), or no description (n=106) in the WHO classification were excluded. To evaluate the effect of the conditioning regimen, we excluded patients who received oral busulfan (BU, n=39), because intravenous BU is used for conditioning regimens in Japan. A total of 398 patients who received allogeneic HCT for minimally differentiated AML in the WHO classification from related or unrelated donors between January 2000 and December 2015, which had sufficient follow-up data, were included in this analysis and divided into four groups based on the cytogenetic risk status according to the National Comprehensive Cancer Network (NCCN) guidelines for AML and disease status at HCT. Because of the lack of data, FLT3ITD/NPM1 are unavailable in this study. Preparative regimens classified as myeloablative conditioning (MAC, n=281), including total body irradiation (TBI) >8 Gy+cyclophosphamide (CY, n=165), +cytarabine (CA, n=5), +melphalan (MEL, n=5) or fludarabine (FLU, n=4), and intravenous BU (ivBU)+CY (n=39), +FLU (n=55) or FLU+MEL (n=8) and reduced-intensity conditioning (RIC, n=117), including FLU+TBI 2-4 Gy+MEL (n=63) or TBI 2-4 Gy+ ivBU (n=42), FLU+ivBU+MEL (n=8) or CY (n=4) were defined by the Center for International Blood and Marrow Transplant Research functional criteria. Neutrophil engraftment was defined as the time to an absolute neutrophil count of ≥500/μL for 3 consecutive days. Primary graft failure was defined as failure of neutrophil engraftment by day 35. The incidence of grade II-IV acute graft-versus-host disease (GVHD) and the presence or absence of chronic GVHD were defined as reported previously. Patients were considered evaluable for GVHD if they had engraftment. The percentages of patients who relapsed or were refractory to allogeneic HCT were determined (Relapse). The survival rate and overall survival (OS) were calculated. All data were censored at the date of the last reported follow-up. Analyzed outcomes included non-relapse mortality (NRM, defined as any death while in continuous remission), disease-free survival (DFS), and OS.

Statistical Analysis

The frequencies and descriptive statistics of patient-, disease-, and transplantation-related variables of the four groups, intermediate risk and CR or non-CR, and poor risk and CR or non-CR, were calculated. The distributions of categorical and continuous variables of the four groups were compared using chi-squared and Kruskal-Wallis tests, respectively. Probabi-
ties of neutrophil engraftment, acute and chronic GVHD, NRM, and relapse were calculated using cumulative incidence curves to accommodate competing risks. Ninety-five percent confidence intervals (CIs) for all probabilities and \( P \)-values of pairwise comparisons were derived from pointwise estimates and calculated using standard techniques. The four groups were compared using Cox proportional hazards regression models for neutrophil engraftment, and acute and chronic GVHD. To accommodate competing risks, cumulative incidence curves for NRM and relapse of the four groups were estimated, and their differences were compared using Gray’s test. Competing risk events for NRM and relapse were relapse and death, respectively. For each group, the Fine and Gray model was employed to evaluate the effects of patient-, disease-, and transplantation-related variables on NRM.\(^{14}\)

Baseline patient variables included age at HCT and diagnosis, sex, Eastern Cooperative Oncology Group performance status (PS) at HCT and diagnosis, extramedullary region at diagnosis, the number of regimens before HCT, time from diagnosis to HCT, disease status at HCT, year at HCT, and HCT-specific comorbidity index (HCT-CI).\(^{15}\) Patient variables assessed before and after HCT included conditioning regimens (MAC vs RIC), stem cell source, human leukocyte antigen (HLA) matching, time of granulocyte-colony stimulating factor (G-CSF) treatment to promote engraftment and neutrophil engraftment, GVHD prophylaxis, incidence and onset of grades II-IV acute or chronic GVHD, secondary malignancies, relapse or refractory after HCT, and cause of death (AML vs transplantation-related mortality, TRM). All data were censored at the date of the last reported follow-up.

To evaluate OS, Kaplan-Meier curves of the four groups were compared based on the log-rank test, and multivariate Cox regression analysis was performed for each group. The Cox proportional hazard model included patient- and transplantation-related variables. The stepwise method included or excluded one independent variable at each step. A two-sided \( P \)-value of \(< 0.05 \) was considered to be statistically significant. All statistical analyses were performed using the Stata Version 14 statistical package (Stata Corporation, College Station, TX, USA) or EZR (Saitama Medical Center, \textit{http://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmedEN.html}), which is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0). More precisely, it is a modified version of R commander (version 1.6-3) designed to add statistical functions that are frequently used in biostatistics.\(^{16}\)

### Results

#### Patient Characteristics

Table 1 shows the patient-related variables in the four groups of minimally differentiated AML patients. Of the 398 patients with AML, 273 patients (69%) were intermediate risk and 125 (31%) were poor risk according to the cytogenetic risk status. The cytogenetic results are presented in the Table 2. According to the cytogenetic and disease risks at HCT, we divided the patients into four groups: intermediate risk and complete remission \( \text{(CR)} \) \( n = 183; \) first CR \( \text{(CR1)} \) \( n = 159, \) second CR \( \text{(CR2)} \) \( n = 24 \) or non-CR \( n = 90, \) and poor risk and CR \( n = 66; \) CR1 \( n = 61, \) CR2 \( n = 5 \) or non-CR \( n = 59 \). The percentages of young patients at both HCT and diagnosis in the poor risk and CR group, patients with lower PS at HCT, and number of regimens before HCT in both intermediate and poor risk and CR groups, as well as the time from diagnosis to HCT were shorter in the poor risk group compared with the intermediate risk group. Transplant-related characteristics and outcomes are shown in Table 3. The percentages of patients who underwent allogeneic HCT with RIC were higher in the poor risk and non-CR group compared with the others. Because the percentages of patients who received bone marrow and HLA-matched unrelated transplantations were higher in CR groups and those of patients who received cord blood stem cell \( \text{(CBSC)} \) and HLA-mismatched unrelated transplantations were higher in the non-CR group, neutrophil engraftment was later in the non-CR group compared with the CR group. There was no significant difference in the cumulative incidence of neutrophil engraftment among the four groups \( P = 0.131, \text{Figure 1A}. \)

Cumulative incidences of neutrophil engraftment in the four groups at 28 days were 93.7\% (95\% CI, 88.8\%-96.4\%) in the intermediate risk and CR group, 89.0\% (95\% CI, 79.6\%-94.0\%) in the intermediate risk and non-CR group, 95.3\% (95\% CI, 85.8\%-98.4\%) in the poor risk and CR group, and 90.3\% (95\% CI, 77.8\%-95.8\%) in the poor risk and non-CR group. Multivariate Cox regression analysis (Table 4) showed that receiving CBSCs and non-CR at HCT were independent predictors of later neutrophil engraftment. The percentages of patients who received cyclosporine and methotrexate for GVHD prophylaxis were higher for patients with intermediate risk. The percentages of patients who received tacrolimus for GVHD prophylaxis were higher for patients with poor risk. The incidence of \( \geq 2 \) acute GVHD \( (P = 0.940, \text{Figure 1B}) \) and chronic GVHD \( (P = 0.947, \text{Figure 1C}) \) were
### Table 1. Distribution of minimally differentiated AML according to cytogenetic risk and disease statuses at HCT

| Characteristics                      | Intermediate-risk | Poor-risk | \( P \) |
|--------------------------------------|-------------------|-----------|---------|
| **Disease status at HCT**            | CR (n=183)        | Non-CR (n=90) | CR (n=66) | Non-CR (n=59) |       |
| Median age (range) at HCT, years     | 50 (16-70)        | 50 (18-70) | 41 (16-63) | 51 (20-67) | 0.013\(^b\) |
| Median age (range) at diagnosis, years | 49 (15-70)        | 50 (17-69) | 40 (16-62) | 48 (20-67) | 0.013\(^b\) |
| Sex, n (%): male                     | 99 (54)           | 57 (63)   | 45 (68)   | 36 (61)   | 0.180\(^a\) |
|                                       | female            | 84 (46)   | 33 (37)   | 21 (32)   |            |
| ECOG PS at HCT, n (%): 0              | 112 (61)          | 25 (28)   | 48 (73)   | 15 (25)   | <0.001\(^a\) |
|                                       | 1                 | 64 (35)   | 54 (60)   | 16 (24)   | 26 (44)   |
|                                       | 2                 | 5 (3)     | 9 (10)    | 2 (3)     | 13 (22)   |
|                                       | ≥3                | 2 (1)     | 2 (2)     | 0         | 5 (8)     |
| ECOG PS at diagnosis, n (%): 0        | 67 (37)           | 28 (31)   | 30 (45)   | 18 (31)   | 0.098\(^a\) |
|                                       | 1                 | 92 (50)   | 49 (54)   | 32 (48)   | 25 (42)   |
|                                       | 2                 | 17 (9)    | 9 (10)    | 4 (6)     | 11 (19)   |
|                                       | ≥3                | 4 (2)     | 3 (3)     | 0         | 5 (8)     |
| Extranodular region at diagnosis, n (%) | 11 (6)            | 9 (10)    | 5 (8)     | 5 (8)     | 0.689\(^a\) |
| No. of regimens before HCT, n (%): 1  | 117 (64)          | 74 (82)   | 42 (64)   | 51 (86)   | <0.001\(^a\) |
|                                       | 2                 | 41 (22)   | 12 (13)   | 17 (26)   | 4 (7)     |
|                                       | ≥3                | 24 (13)   | 0         | 7 (11)    | 2 (3)     |
| Median time (range) from diagnosis to HCT, M | 7 (1-59) | 6 (1-74) | 6 (2-40) | 5 (1-37) | <0.001\(^b\) |
| Year at HCT, n (%): 2000-2005          | 18 (10)           | 4 (4)     | 10 (15)   | 4 (7)     | 0.335\(^b\) |
| 2006-2010                             | 69 (38)           | 34 (38)   | 21 (32)   | 19 (32)   |
| 2011-2015                             | 96 (52)           | 52 (58)   | 35 (53)   | 36 (61)   |
| HCl-CI at HCT n (%): 0                 | 127 (69)          | 50 (56)   | 45 (68)   | 34 (58)   | 0.277\(^a\) |
|                                       | 1                 | 44 (24)   | 29 (32)   | 14 (21)   | 15 (25)   |
|                                       | 2                 | 10 (5)    | 7 (8)     | 5 (8)     | 6 (10)    |
|                                       | ≥3                | 2 (1)     | 4 (4)     | 2 (3)     | 4 (7)     |

AML, acute myeloid leukemia; HCT, hematopoietic cell transplantation; CR, complete remission at HCT; ECOG, Eastern Cooperative Oncology Group; PS, performance status; M, month; HCl-CI, HCT-specific comorbidity index. Variables in the four groups were compared using \(^a\): Pearson's chi-squared test or the \(^b\): Kruskal-Wallis test.

### Table 2. Cytogenetic features of minimally differentiated AML according to cytogenetic risk and disease status at HCT

| Cytogenetic Feature                      | Intermediate-risk | Poor-risk | CR (n=183) | Non-CR (n=90) | CR (n=66) | Non-CR (n=59) | \( P \) |
|----------------------------------------|-------------------|-----------|------------|---------------|-----------|---------------|---------|
| Normal/constitutional                  | 147 (80%)         | 75 (83%)  | 0          | 0             |           |               |         |
| t (9;11)                               | 3 (2%)            | 0         | 0          | 0             |           |               |         |
| +8                                     | 2 (1%)            | 2 (2%)    | 0          | 0             |           |               |         |
| Other non-defined                      | 31 (17%)          | 13 (14%)  | 0          | 0             |           |               |         |
| Complex (≥3 clonal chromosomal abnormalities) | 0          | 0         | 50 (76%)   | 34 (58%)     |           |               |         |
| Monosomal karyotype                    | 0                 | 0         | 3 (4%)     | 0             |           |               |         |
| -5/5q−                                 | 0                 | 0         | 3 (4%)     | 4 (7%)        |           |               |         |
| -7/7q−                                 | 0                 | 0         | 4 (6%)     | 12 (20%)      |           |               |         |
| 11q23: non t (9;11)                    | 0                 | 0         | 1 (2%)     | 0             |           |               |         |
| inv (3)                                | 0                 | 0         | 2 (3%)     | 5 (8%)        |           |               |         |
| t (3;3)                                | 0                 | 0         | 1 (2%)     | 4 (7%)        |           |               |         |
| t (9;22)                               | 0                 | 0         | 2 (3%)     | 0             |           |               |         |

AML, acute myeloid leukemia; HCT, hematopoietic cell transplantation; CR, complete remission.
similar among the four groups. Cumulative incidences of acute GVHD in the four groups at 100 days were 37.6% (95% CI, 29.9%-44.5%) in the intermediate risk and CR group, 43.0% (95% CI, 31.1%-52.9%) in the intermediate risk and non-CR group, 42.8% (95% CI, 29.2%-53.8%) in the poor risk and CR group, and 43.6% (95% CI, 27.8%-55.9%) in the poor risk and non-CR group. Cumulative incidences of chronic GVHD in the four groups at 1 and 2 years were 41.1% and 47.1% (95% CI, 32.5%-48.6% and 37.8%-55.0%) in the intermediate risk and CR group, 42.2 and 48.0% (95% CI, 27.9%-53.7% and 32.2%-60.1%) in the intermediate risk and non-CR group, 43.4% and 52.1% (95% CI, 28.2%-55.4% and 35.0%-64.6%) in the poor risk and CR group, and 43.7% and unevaluable (95% CI, 22.3%-59.1% and unevaluable) in the poor risk and non-CR group, respectively. No independent predictor of acute or chronic GVHD was detected by multivariate Cox regression analysis. The onset of acute GVHD was later in non-CR groups compared with CR groups. The percentages of relapse or refractory after allogeneic HCT were higher in non-CR groups compared with CR groups. The median survival time of patients with poor risk and non-CR was 8 months, which was significantly shorter compared with that of the other groups. The percentages of death due to AML after allogeneic HCT were higher in non-CR groups compared with the CR groups. The percentage of TRM in the poor risk and non-CR group was significantly higher than those in the other groups. The cause of TRM in the four groups, 1) intermediate risk and CR (n=55) or 2) non-CR (n=34), and 3) poor risk and CR (n=18) or 4) non-CR (n=37) were as follows: infection (n=22, 12, 5, and 13, respectively)

### Table 3. Transplant-related characteristics and outcomes of minimally differentiated AML according to cytogenetic risk and disease statuses at HCT

| Characteristics                      | Intermediate-risk | Poor-risk | P       |
|--------------------------------------|-------------------|----------|---------|
|                                      | CR (n=183)        | Non-CR (n=90) | CR (n=66) | Non-CR (n=59) |       |
| Conditioning regimen, n (%) MAC      |                   |           |         |               |       |
| MAC                                  | 130 (71)          | 63 (70)  | 54 (82) | 34 (58)       | 0.032<sup>a</sup> |
| RIC                                  | 53 (29)           | 27 (30)  | 12 (18) | 25 (42)       |         |
| Stem-cell source, n (%) BM           |                   |           |         |               |       |
| BM                                   | 102 (56)          | 35 (39)  | 33 (50) | 18 (31)       | 0.014<sup>a</sup> |
| PBSC                                 | 33 (18)           | 24 (27)  | 16 (24) | 15 (25)       |         |
| CBSC                                 | 48 (26)           | 31 (34)  | 17 (26) | 26 (44)       |         |
| Donor, n (%) HLA-identical sibling   | 46 (25)           | 20 (22)  | 17 (26) | 14 (24)       | 0.003<sup>a</sup> |
| HLA-matched unrelated                 | 90 (49)           | 29 (32)  | 29 (44) | 16 (27)       |         |
| HLA-mismatched related               | 6 (3)             | 13 (14)  | 6 (9)   | 5 (8)         |         |
| HLA-mismatched unrelated             | 41 (22)           | 28 (31)  | 14 (21) | 24 (41)       |         |
| Time of G-CSF to promote engraftment, days | 13 (0–164)        | 16 (2–126) | 14 (2–71) | 15 (3–51) | 0.321<sup>1</sup> |
| Neutrophil engraftment, median (range), days | 17 (6–35)       | 19 (8–35) | 17 (11–34) | 19 (10–57) | 0.015<sup>a</sup> |
| GVHD prophylaxis, n (%)              |                   |           |         |               |       |
| CyA                                  | 77 (42)           | 25 (38)  | 20 (22) | 16 (27)       | 0.035<sup>a</sup> |
| TAC                                  | 104 (57)          | 44 (67)  | 66 (73) | 43 (73)       | 0.026<sup>a</sup> |
| MTX                                  | 159 (87)          | 64 (97)  | 60 (67) | 40 (68)       | <0.001<sup>a</sup> |
| MMF                                  | 11 (6)            | 12 (18)  | 4 (4)   | 6 (10)        | 0.191<sup>a</sup> |
| Missing                              | 2 (1)             | 0        | 2 (2)   | 0             |         |
| Cumulative incidences (95% CI) of grades II–IV acute GVHD at 100 days, % | 37.6 (29.9–44.5) | 43.0 (31.1–52.9) | 42.8 (29.2–53.8) | 43.6 (27.8–55.9) | 0.940<sup>c</sup> |
| Onset of acute GVHD, median (range), day | 18 (3–100)      | 27 (14–88) | 23 (11–61) | 25 (7–9)    | 0.022<sup>a</sup> |
| Cumulative incidences (95% CI) of chronic GVHD at 1 years, % | 41.1 (32.5–48.6) | 42.2 (27.9–53.7) | 43.4 (28.2–55.4) | 43.7 (22.3–59.1) | 0.947<sup>c</sup> |
| Onset of chronic GVHD, median (range), day | 112 (55–751)   | 117 (79–403) | 130 (41–698) | 90 (56–168) | 0.057<sup>a</sup> |
| Secondary malignancies, n (%)         | 5 (3)             | 1 (1)    | 2 (3)   | 0             | 0.491<sup>a</sup> |
| Relapse/refractory after HCT, n (%)   | 46 (25)           | 44 (49)  | 23 (35) | 33 (56)       | <0.001<sup>a</sup> |
| Median (range) follow-up of survivors, M | 41 (1–180)     | 37 (4–127) | 42 (3–141) | 8 (1–51)    | 0.019<sup>a</sup> |
| Cause of death, n (%) AML             | 24 (13)           | 23 (26)  | 13 (20) | 16 (27)       | 0.027<sup>a</sup> |
| TRM                                  | 55 (30)           | 34 (38)  | 18 (27) | 37 (63)       | <0.001<sup>a</sup> |

AML, acute myeloid leukemia; HCT, hematopoietic cell transplantation; MAC, myeloablative conditioning; RIC, reduced intensity conditioning; BM, bone marrow; PBSC, peripheral blood stem cell; CBSC, cord blood stem cell; HLA, human leukocyte antigen; G-CSF, granulocyte-colony stimulating factor; GVHD, graft-versus-host disease; CyA, cyclosporine A; TAC, tacrolimus; MTX, methotrexate; MMF, mycophenolate mofetil; n, total number of patient assessments; M, month; TRM, transplantation-related mortality. Variables in the four groups were compared using a: Pearson’s chi-squared test, the b: Kruskal-Wallis test, or c: Gray’s test.
tively), multiple organ failure (n = 9, 6, 5, and 11), hepatic sinusoidal obstruction syndrome (n = 4, 3, 0, and 1), acute GVHD (n = 4, 1, and 4), chronic GVHD (n = 2, 2, 1, and 4), pneumonitis (n = 1, 2, 2, and 1), idiopathic pneumonia syndrome (n = 1, 0, 1, and 2), transplantation-associated thrombotic microangiopathy (n = 1, 0, 0, and 3), rejection (n = 4, 1, 1, and 1), and hemorrhage (n = 6, 3, 1, and 1).

Regarding of secondary malignancies, five patients with secondary malignancies (tongue, esophageal, pancreatic, or renal cell cancers or teratoma) in the intermediate risk and CR group survived after cancer treatment, but one patient with secondary breast cancer in the intermediate risk and non-CR group and two patients with secondary malignancies (esophageal cancer or EBV-associated lymphoproliferative disorders) in the poor risk and CR group died of secondary malignancies. The higher percentages of patients who received CBSCs in non-CR groups may be associated with the later neutrophil engraftment and onset of acute GVHD.

**NRM and relapse**

The cumulative incidence of NRM with poor risk was significantly higher in the non-CR group compared with the CR group (P = 0.0060, Figure 2A). Cumulative incidences of NRM in the four groups at 1, 3, and 5 years were 19.2, 22.5, and 15.1% (95% CI, 13.2%--24.8%, 15.8%--28.8%, and 17.6%--31.9%) in the intermediate risk and CR group, 17.9%,
24.0%, and 29.8% (95% CI, 8.8%-26.2%, 12.8%-33.6%, and 13.6%-43.0%) in the intermediate risk and non-CR group, 14.5%, 16.4%, and 16.4% (95% CI, 5.2%-22.9%, 6.5%-25.2%, and 6.5%-25.2%) in the poor risk and CR group, and 35.1%, 54.4%, and 69.6% (95% CI, 19.1%-48.0%, 15.2%-75.4%, and 16.3%-88.9%) in the poor risk and non-CR group, respectively. The Fine and Gray model showed that 60 years of age at HCT, HCT-CI >2, and receiving CBSCs were significantly associated with NRM (Table 4).

The cumulative incidence of relapse with poor risk was significantly higher in the non-CR group compared with the CR group (P = 0.0094, Figure 2B). Cumulative incidences of relapse in the four groups at 1, 3, and 5 years were 14.1%, 27.7%, and 46.6% (95% CI, 8.4%-19.4%, 19.5%-35.1%, and 23.4%-40.5%) in the intermediate risk and CR group, 39.2%, 58.6%, and 66.5% (95% CI, 27.3%-49.1%, 44.1%-69.4%, and 47.9%-78.4%) in the intermediate risk and non-CR group, 17.2%, 40.7%, and 46.6% (95% CI, 6.8%-26.5%, 24.6%-53.4%, and 29.3%-59.6%) in the poor risk and CR group, and 63.3%, 82.2%, and unevaluable (95% CI, 44.0%-75.9%, 60.9%-91.9%, and unevaluable) in the poor risk and non-CR group, respectively. The Fine and Gray model showed that poor risk and non-CR at HCT were significantly associated with relapse (Table 4).

DFS and OS

Multivariate Cox regression analysis (Table 4) showed that poor risk, and PS ≥1 and non-CR at HCT were independent predictors of poorer DFS and OS. The 3-year DFS and OS were higher in patients with CR than in patients with non-CR at HCT (log-rank, P < 0.001: Figure 3A, 3B). In patients with non-CR, poor risk was associated with significant reductions in DFS and OS (log-rank, P < 0.001: Figure 3A, 3B). DFS in the four groups at 1, 3, and 5 years was 68.7%, 55.2%, and 49.7% (95% CI, 61.3%-79.8%, 35.2%-62.5%, and 12.4%-34.0%) in the intermediate risk and non-CR group, 70.1%, 48.9%, and 44.1% (95% CI, 57.1%-79.8%, 35.2%-61.2%, and 30.4%-56.9%) in the intermediate risk and non-CR group, 79.6%, 58.7%, and 56.0% (95% CI, 67.4%-87.6%, 44.6%-68.1%, and 41.1%-68.1%) in the poor risk and CR group, and 31.9, 9.7%, and unevaluable (95% CI, 20.1%-44.4%, 3.1%-20.9%, and un evaluable) in the poor risk and non-CR group, respectively.

Discussion

To our knowledge, this is the largest analysis of the inci-
dence, outcome, and risk factors of patients with minimally differentiated AML, who received allogeneic HCT. The major findings indicated that poor risk, and PS \( \geq 1 \) and non-CR at HCT were independent predictors of poorer DFS and OS.

Patients with minimally differentiated AML undergo allogeneic HCT because of poor outcomes.\(^3\)\(^-\)\(^6\) Although this analysis of the TRUMP database is one of the largest to assess patients with minimally differentiated AML, who underwent allogeneic HCT, it was not surprising that allogeneic HCT in poor risk, minimally differentiated AML patients with non-CR at HCT resulted in a higher NRM rate compared with CR at HCT. The advent of RIC has allowed increased use of allogeneic HCT in older patients without increasing NRM,\(^17\)-\(^20\), but it might be still controversial in older patients with minimally differentiated AML. The median age of patients who undergo HCT has increased, and we found that disease control at HCT and improvements in transplantations for older patients with higher HCT-CI and/or receive CBSCs are important for patients with minimally differentiated AML to improve outcomes after allogeneic HCT.

In this large series, DFS and OS were significantly worse in patients with non-CR. Higher PS was associated with poorer DFS and OS, and any finding associated with poorer NRM and relapse, except for poor risk and non-CR at HCT. Because poor outcomes have often been observed in minimally differentiated AML patients with poor risk cytogenetics, and there is no effective therapy for relapse or progression after allogeneic HCT, new therapies are needed to improve the safety and efficacy of allografting in patients with minimally differentiated AML.

Small patient numbers may confound recognition of small differences in outcomes. However, these encouraging results confirm the overall safety and tolerability of allogeneic HCT in patients deemed eligible for allogeneic HCT in transplantation centers. Current strategies for allogeneic HCT might not improve outcomes of patients with poor risk cytogenetics and non-CR at HCT, but analysis of the various conditioning regimens used for transplantation was beyond the scope of this study. Because the best preparative conditioning regimen for allogeneic HCT is still uncertain, carefully designed prospective trials are essential to determine the contribution of specific conditioning regimens to successful disease control. Additionally, the lack of mutation data, including FLT3-ITD/ NPM1, is a significant limitation in this study, and a more prolonged follow-up may be needed to better evaluate eligibility criteria for HCT.

In conclusion, our data suggest that allogeneic HCT can be considered for patients with minimally differentiated AML. A poor risk may be an indicator of allogeneic HCT for these patients. Prospective trials of allogeneic HCT and new therapies with targeted agents, such as FLT3-ITD,\(^18\)-\(^20\) are needed to improve the safety and efficacy of allografting in patients with minimally differentiated AML.

Table 4. Multivariate analysis of neutrophil engraftment, NRM, relapse, and DFS/OS of patients with minimally differentiated AML

| Variable | Neutrophil engraftment \(^1\) | NRM \(^2\) | Relapse \(^1\) | DFS \(^1\) | OS \(^1\) |
|----------|------------------|------------------|------------------|------------------|------------------|
|          | HR 95% CI P      | HR 95% CI P      | HR 95% CI P      | HR 95% CI P      | HR 95% CI P      |
| >60 y.o at HCT | 1.181 1.126 0.014 | -2.816          |                  |                  |                  |
| HCT-CI>2 at HCT | 1.290 1.023 0.013 | -1.625          |                  |                  |                  |
| Poor-risk\(^a\) | 1.687 1.208 0.0021 | -2.356          | 1.602 1.229 <0.001 | -2.089          | 1.600 1.207 0.0010 | -2.121 |
| CBSC\(^b\) | 2.764 2.172 <0.001 | 1.290 1.023 0.031 | -3.516          |                  |                  |
| PS≧1 at HCT | 1.502 1.134 0.0045 | 1.669 1.232 <0.001 | -1.989          |                  |                  |
| Non-CR at HCT | 1.298 1.044 0.018 | 3.240 2.393 <0.001 | 2.262 1.712 <0.001 | 2.080 1.546 <0.001 | -2.799 |

NRM, non-relapse mortality; DFS, disease-free survival; OS, overall survival; HR, hazard ratio; CI, confidence interval; y.o., years old; HCT, hematopoietic cell transplantation; HCT-CI, HCT-specific comorbidity index; CBSC, cord blood stem cell; PS, performance status; CR, complete response. \(^1\) Multivariate competing event statistics by Fine and Gray models for NRM and relapse or \(^2\) multivariate Cox proportional hazards regression analysis of neutrophil engraftment, DFS, and OS were performed. Variables were the \(^a\) cytogenetic risk status or the \(^b\) stem cell source.
Acknowledgments

We appreciate the patients and clinical staff for their participation in the study. We are very grateful to the Japanese Data Center for Hematopoietic Cell Transplantation for data management, and to the Clinical Research Institute of Kyushu Medical Hospital for their editorial support. This study was supported by The Practical Research Project for Allergic Diseases and Immunology (Research Technology of Medical Transplantation) of the Japan Agency for Medical Research and Development (AMED). We thank Edanz Group (www.edanzediting.com) for editing a draft of this manuscript.

Author’s contributions

SY contributed to the study design, data analysis, and manuscript preparation; JA, JM, KH, MY and SY contributed to manuscript preparation; MS, NU, KO, TF, SK, HK, HO, and HO provided clinical data; TI, YK, JT and YA managed clinical data; and all of them reviewed the manuscript.

Conflict of interest disclosure

The authors declare that there are no competing financial interests regarding this article.

References

1. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016; 127: 2391–2405.
2. Bennett JM, Catovsky D, Daniel MT, et al. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-M0). Br J Haematol. 1991; 7: 325–329.
3. Stasi R, Del Poeta G, Venditti A, et al. Analysis of treatment failure in patients with minimally differentiated acute myeloid leukemia (AML-M0). Blood. 1994; 83: 1619–1625.
4. Amadori S, Venditti A, Del Poeta G, et al. Minimally differentiated acute myeloid leukemia (AML-M0): a distinct clinicobiologic entity with poor prognosis. Ann Hematol. 1996; 72: 208–215.
5. Béné MC, Bernier M, Casasnovas RO, et al. Acute myeloid leukemia M0: haematological, immunophenotypic and cytogenetic characteristics and their prognostic significance: an analysis in 241 patients. Br J Haematol. 2001; 113: 737–745.
6. Cuneo A, Ferrant A, Michaux JL, et al. Cytogenetic profile of minimally differentiated (FAB M0) acute myeloid leukemia: correlation with clinicobiologic findings. Blood. 1995; 85: 3688–3694.
7. Atsuta Y, Suzuki R, Yoshimi A, et al. Unification of hematopoietic stem cell transplantation registries in Japan and establishment of the TRUMP System. Int J Hematol. 2007; 86: 269–274.
8. Atsuta Y. Introduction of Transplant Registry Unified Management Program 2 (TRUMP2): scripts for TRUMP data analyses, part I (variables other than HLA-related data). Int J Hematol. 2016; 103: 3–10.
9. Yanada M, Mori J, Aoki J, et al. Effect of cytogenetic risk status on outcomes for patients with acute myeloid leukemia under-
going various types of allogeneic hematopoietic cell transplantation: an analysis of 7812 patients. Leuk Lymphoma. 2017; 28: 1–9.

10. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology, Acute Myeloid Leukemia (Version 2.2016) (https://www.nccn.org/professionals/physician_gls/pdf/aml.pdf) Accessed 20 January 2017.

11. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: Defining the dose spectrum—Report of a workshop convened by the Center for International Blood and Marrow Transplant Research. Biol Blood Marrow Transplant. 2009; 15: 367–369.

12. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995; 15: 825–828.

13. Filipovich AH, Weisdorf D, Pavletic S, et al. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. Biol Blood Marrow Transplant. 2005; 11: 945–956.

14. Fine J, Gray R. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc. 1999; 94: 496–509.

15. Sorror M, Storer B, Sandmaier BM, et al. Hematopoietic cell transplantation-comorbidity index and Karnofsky performance status are independent predictors of morbidity and mortality after allogeneic nonmyeloablative hematopoietic cell transplantation. Cancer. 2008; 112: 1992–2001.

16. Kanda Y. Investigation of the freely available easy-to-use software ’EZR’ for medical statistics. Bone Marrow Transplant. 2013; 48: 452–458.

17. McClune BL, Weisdorf DJ, Pedersen TL, et al. Effect of age on outcome of reduced-intensity hematopoietic cell transplantation for older patients with acute myeloid leukemia in first complete remission or with myelodysplastic syndrome. J Clin Oncol. 2010; 28: 1878–1887.

18. Kao HW, Liang DC, Wu JH, et al. Gene mutation patterns in patients with minimally differentiated acute myeloid leukemia. Neoplasia. 2014; 16: 481–488.

19. Perl AE, Altman JK, Cortes J, et al. Selective inhibition of FLT3 by gilteritinib in relapsed or refractory acute myeloid leukaemia: a multicentre, first-in-human, open-label, phase 1–2 study. Lancet Oncol. 2017; 18: 1061–1075.

20. Cortes J, Perl AE, Döhner H, et al. Quizartinib, an FLT3 inhibitor, as monotherapy in patients with relapsed or refractory acute myeloid leukaemia: an open-label, multicentre, single-arm, phase 2 trial. Lancet Oncol. 2018; 19: 889–903.