Sequential Intensified Conditioning Regimen Allogeneic Hematopoietic Stem Cell Transplantation in Adult Patients with Intermediate or High-Risk Acute Myeloid Leukemia in Complete Remission: a Study From the ALWP of the EBMT

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Sequential intensified conditioning regimen allogeneic hematopoietic stem cell transplantation in adult patients with intermediate or high-risk acute myeloid leukemia in complete remission: a study from the ALWP of the EBMT

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Highlights

- FLAMSA provides an efficient disease control in intermediate and high-risk AML.
- Leukemia-free survival at 2 years was 52.8% (95%CI, 46.4-59.2).
- Overall-free survival at 2 years was 56.1% (95%CI, 49.7-62.6).

Abstract

Post-transplant relapse is the leading cause of treatment failure in acute myeloid leukemia (AML) patients after reduced intensity conditioning allogeneic hematopoietic stem cell transplantation (allo-HSCT). In order to improve their outcome, we evaluated the outcome of a sequential intermediate intensity conditioning regimen combining fludarabine, cytosine arabinoside, amsacrine, cyclophosphamide and either total body irradiation or busulfan (FLAMSA) in patients with intermediate or high risk AML in first or second complete

remission (CR). A total of 265 patients (median age 55 years; range, 19-76 years) with AML who underwent allo-HSCT using a FLAMSA regimen were included. At the time of transplant, 216 (81.5%) were in CR1 and 49 (18.5%) in CR2. Cytogenetic was intermediate in 114 (43%) and poor in 42 (15.8%) patients, while 109 (41.1%) patients had a secondary AML. With a median follow-up of 46 months (range, 1-145), the Kaplan-Meier estimate of overall and leukemia-free survival at 2 years were 56.1% (95%CI, 49.7-62.6) and 52.8% (95%CI, 46.4-59.2), respectively. At 2 years, the cumulative incidences of relapse and non-relapse mortality were 22.8% (95%CI, 17.6-28.4) and 24.0% (95%CI, 18.8-29.5), respectively. In multivariate analysis, patients' age and cytogenetics were the only parameters with a significant impact on overall survival. These data suggest that the FLAMSA sequential intermediate conditioning regimen provides an efficient disease control in intermediate and high-risk AML patients, including those in CR2 and with secondary AML.

Keywords: Acute myeloid leukemia; complete remission; allogeneic hematopoietic-stem-cell transplantation; sequential conditioning regimen; intermediate intensity conditioning regimen

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective post-remission consolidation treatment, potentially curative, in acute myeloid leukemia (AML) patients[1, 2]. Reduced intensity conditioning (RIC) regimens have been developed to control or overcome toxicity and non-relapse mortality (NRM) associated with allo-HSCT[2]. RIC rely on the graft-versus-leukemia (GVL) effect mediated by the graft's immune cells[3]. RIC allo-HSCT is now widely used for AML patients with intermediate or high-risk cytogenetics, particularly in older or heavily pretreated patients and in those with medical comorbidities[2]. While a significant proportion of patients are cured after RIC allo-HSCT, relapse after transplant is still the leading cause of treatment failure in the RIC setting. In patients transplanted in complete remission (CR), AML cytogenetic status and prior myelodysplastic syndrome or cytotoxic therapy are strong predictors of relapse. Therefore, the effectiveness of different intermediate intensity conditioning regimens to enhance GVL, while safely minimizing NRM has been evaluated[2, 4, 5]. One such strategy is the so-called "sequential conditioning regimen", combining a short course of intensive chemotherapy followed by a RIC allograft. Thus, the Munich group developed the FLAMSA sequential strategy combining a short course of intensive chemotherapy to improve disease control using fludarabine (Flu) 30 mg/m^2/d, intermediate dose cytosine arabinoside (Ara-C) 2 g/m^2/d and amsacrine (Ams) 100 mg/m^2/d from day -12 to -9, followed, after a 3 days rest, by RIC using 4 Gy total body irradiation (TBI) on day -5, cyclophosphamide (Cy) 40-60 mg/Kg/d on days -4 and -3 and anti-thymocyte globulin (ATG) from days -4 to -2. This strategy has shown encouraging results in

relapsed or refractory AML patients'[6, 7]. In addition, Schmid et al. reported an effective disease control and a low NRM with this strategy in 23 patients with high-risk AML in CR[8]. Thereafter, 4 Gy TBI has been replaced by IV busulfan (Bu) 6.4 mg/kg total dose (or equivalent oral dose) in order to decrease the toxicity associated with TBI in elderly patients or in patients with severe comorbidities[9, 10].

Larger studies are needed to evaluate the role of TBI or Bu based FLAMSA sequential regimen in patients with AML in CR. We report here on 265 patients with AML in first or second CR subjected to a FLAMSA sequential allo-HSCT, TBI and Bu based FLAMSA are compared. In addition the contribution of prophylactic DLI is assessed in the subgroup of patients alive and free of disease at 6 months.

Patients and methods

Study design and data collection

This retrospective multicenter analysis was performed and approved by the Acute Leukemia Working Party (ALWP) of the EBMT group registry. The EBMT is a voluntary working group of more than 500 transplant centers; all centers are required to report annually all stem cell transplantations and follow-up. Use of patients' personal information for research purposes is authorized through the signature of an informed consent by the patients. This study included all adult patients (age >18 years) with AML in first or second morphological CR, who underwent a bone marrow (BM) or G-CSF-mobilized peripheral blood stem cells (PBSC) allo-HSCT, from an HLA matched-related (MRD) or unrelated (UD) donor between 2002 and 2014. In addition, to be eligible patients must have available

cytogenetics data, or secondary AML and receive a so-called "sequential" conditioning regimen. The latter was defined by the use of a short intensive course of chemotherapy combining Flu, intermediate dose Ara-C and Ams, followed after a 3 days rest by a RIC regimen combining Cy and either TBI 4 Gr or IV Bu 6.4 mg/kg total dose (or equivalent oral dose of Bu). Cytogenetic was classified according to the European Leukemia Net[11]. All allogeneic grafts were obtained from HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ-matched donors. A single HLA mismatch of 10 was allowed at the antigen or allele level. A list of the participating centers is available online (supplemental file).

Statistical analysis

Endpoints included overall survival (OS), leukemia-free survival (LFS), cumulative incidence of relapse (CIR), NRM, acute and chronic graft-versus-host disease (GVHD). All outcomes were measured from the time of allo-HSCT. OS was based on death, regardless of the cause. LFS was defined as survival with no evidence of relapse. NRM was defined as death in CR. Patients alive without relapse were censored at the time of last contact.

OS and LFS rates were calculated by the Kaplan-Meier estimator. Cumulative incidence functions were used to estimate the probabilities of acute and chronic GVHD, NRM, and relapse to accommodate competing risks. NRM and relapse were the competing risks. For acute and chronic GVHD, the competing risk was death without the event. For all prognostic analyses, patients' median age and median year of transplant were used as a cut-off point.

Univariate analyses were performed using the log-rank test for OS and LFS, and Gray's test for cumulative incidences. Chronic GVHD was analyzed as a time-dependent variable. For multivariate regression a Cox proportional hazards

model was build. Results were expressed as hazard ratio (HR) with 95% confidence interval (CI). All tests were two-sided and the type-1 error rate was fixed at 0.05. A landmark analysis was conducted 6 months after allo-HSCT on patients alive and free of disease to evaluate the impact of preemptive donor lymphocyte infusion (DLI) within the first 6 months on outcome. Patients developing grade II-IV aGVHD or cGVHD before DLI (group DLI) or within the first 6 months (group no DLI) were excluded from the landmark analysis. Statistical analyses were performed with SPSS 19 (SPSS Inc./IBM, Armonk, NY) and R 3.0.1 (R Development Core Team, Vienna, Austria) software packages.

Results

Patient and donor characteristics

A total of 265 patients were included in this study. Patients' and donors' characteristics are summarized in **Table 1**. The median age of recipients was 55 (range, 19-76) years. At transplantation, 216 patients (81.5%) were in CR1 and 49 (18.5%) in CR2. The median time between AML diagnosis and transplantation was 135 (43-225) days in patients with AML in CR1 and 627 (135-1701) in CR2. One hundred and nine patients (41.1%) had a secondary AML and 156 (58.9%) had a *de novo* AML including 114 (43.0%) with intermediate risk and 42 (15.8%) with high-risk cytogenetics. Of note, no patients had low risk cytogenetic among *de novo* AML. Seventy-four donors (27.9%) were MRD and 191 (72.1%) were UD. The stem cell source was BM in 14 cases (5.3%) and G-CSF-mobilized PBSCs in the remaining 251 (94.7%). All patients except for seven have received *in* vivo T cell depletion using ATG. ATG was Thymoglobulin in 102 patients (median total dose, 6 mg/kg; inter-quartile range [IQR], 5-7) and ATG Fresenius in 129 patients (median total dose 60 mg/kg; IQR, 30-60); ATG administered was unknown in 24 patients.

One hundred and fifty-nine patients (60%) were treated with a TBI based (TBI group) and 106 (40%) with a modified Bu based FLAMSA regimen (Bu group, 96 IV Bu and 10 oral Bu). The comparison between the TBI and Bu groups is shown on **table 1**. Compared to the TBI group, patients in the Bu group were significantly older [61 years (range, 25-74) *versus* 52 years (range, 19-76); $p<0.0001$], were transplanted more recently [2011 (2005-2014), versus 2009 (2002-2014); $p<0.0001$] and included more secondary AML [59.4% *versus* 28.9%; $p<0.0001$]. The median follow-up among surviving patients was 46

(range, 1-145) months, being significantly longer in the TBI group, 50 (range, 1-145) months, compared to that in the Bu group, 27 (range, 3-106) months (p=0.006).

Engraftment and GVHD

Engraftment was successful in 153 patients (96.2%) in the TBI and 101 (95.3%) in the Bu group, respectively (p=0.56). The median time to neutrophil recovery was significantly longer in the TBI group: 17 (range, 10-74) days compared with 14 (range, 8-112) in the Bu group (p<0.0001). The day-30 cumulative incidence of absolute neutrophil count > 0.5×10^9/L was 93.9% (95% confidence interval [CI], 90.1-96.2).

The day-100 cumulative incidence of grade II-IV aGVHD was 28.5% (95% CI, 23.1-34.1), being 30.3% (95% CI, 23,2-37.7) in the TBI group and 25.7% (95% CI, 17.6-34.6) in the Bu group (p=0.45). At 2 years, the cumulative incidence of cGVHD was 31.8% (95% CI, 25.9-37.9), being 33.5% (95%CI, 25.7-41.3) in the TBI group versus 29.1% (95% CI, 20-38.8) in the Bu group (p=0.79).

Outcome

Univariate and multivariate analyses of transplantation-related events are summarized in **Tables 2 and 3,** respectively. At 2 years, the cumulative incidence of NRM was 24.0% (95% CI, 18.8-29.5) (**Figure 1A**), being 19.4% (95% CI, 13.5-26.2) in the TBI and 31.1% (95% CI, 24.0-38.4) in the Bu groups (p=0.02). In multivariate analysis, there was no significant difference in NRM between the TBI and the Bu group (HR, 1.11; 95%CI, 0.62-2.01; p=0.72). NRM was related mainly to infection (n=31) and GVHD (n=19), others causes being hemorrhage (n=5), sinusoidal obstruction syndrome (SOS, n=2), cardiac toxicity (n=2), secondary malignancy (n=1), others (n=9), unknown (n=9). At 2 years, the

cumulative incidence of relapse was 22.8% (95% CI, 17.6-28.4) (**Figure 1B**), with 21.2% (95% CI, 50.7-66.8) in the TBI and 25.7% (95% CI, 32.8-53.7) in the Bu group (p=0.77). In multivariate analysis, there was no significant difference in relapse incidence between the two groups (HR, 1.27; 95%CI, 0.68-2.37; p=0.4). The only parameter with a significant impact on relapse incidence in multivariate analysis was cytogenetic status: relapse was significantly increased in patients with poor as compared to intermediate cytogenetics (HR, 1.96; 95%CI, 1.03-3.72; p=0.04).

The Kaplan-Meier estimate of OS at 2 years was 56.1% (95% CI, 49.7-62.6) (**Figure 1C**), being 62.0% (95% CI, 54.0-70.0) in the TBI and 46.7% (95%CI, 36.1-57.3) in the Bu group (p=0.14). In multivariate analysis, there was no significant difference in OS between the TBI group and the Bu group (HR, 1.09; 95%CI, 0.70-1.69; p=0.70). The only parameters with a significant impact on OS in multivariate analysis were cytogenetic status and patients' age. OS was significantly lower in patients with poor as compared to intermediate cytogenetics (HR, 1.26; 95%CI, 1.06-2.92; p=0.03) and in older patients (HR, 1.21; 95%CI, 1.01-1.45; p=0.04). The Kaplan-Meier estimate of LFS at 2 years was 52.8% (95% CI, 46.4-59.2) (**Figure 1D**): 58.8% (95% CI, 50.7-66.8) in the TBI and 43.2% (95%CI, 32.8-53.7) in the Bu group (p=0.14). In multivariate analysis, there was no significant difference in LFS between the TBI group and the Bu group (HR, 1.16; 95%CI, 0.76-1.78; p=0.48). No parameter had a significant impact on LFS in multivariate analysis.

Prophylactic DLI

Ninety-six patients alive and disease free at 6 months and without a history of grade II-IV aGVHD or cGVHD before DLI were eligible for the landmark analysis.

Of these, 21 received preemptive DLI within the first 6 months, based on physician decision, while 75 did not. The outcome was significantly improved in the former group of patients. The 2-year LFS was 95% (95% CI, 86-100) in the DLI group versus 76% (95% CI, 67-86) in the no DLI group (p=0.03), and the 2-year OS was 100% (95% CI, 100-100) in the DLI group versus 81% (95% CI, 72-91) in the no DLI group (p=0.10). The cumulative incidence of NRM and relapse were 0% and 5% (95% CI, 0-20), respectively, in the DLI group, versus 4% (95% CI, 1-11) and 19% (95% CI, 11-29), respectively, in the no DLI group, (p= 0.18 and 0.11 respectively). The 2-year cumulative incidence of cGVHD was significantly higher in the DLI group, 26% (95% CI, 9-46) versus 15% (95% CI, 8-24) in the no DLI group (p=0.48).

Discussion

Intermediate intensity conditioning regimens have been developed to decrease disease recurrence while minimizing NRM after RIC regimen. This retrospective study is the largest so far evaluating the so-called FLAMSA sequential intermediate intensity conditioning regimen in AML in CR.

The risk of relapse was notably high in our patients: 57% had an unfavorable karyotype or a secondary AML, and 18.5% were in CR2. Notably, the cumulative incidences of relapse and LFS were 22.8% and 52.8%, respectively. These results compare favorably with previous studies evaluating RIC regimens for AML, with relapse incidences up to 41% and LFS below 50%[12-17], but moreover with the results of myeloablative conditioning regimens, with relapse incidences ranging from 24% to 29%[12, 16]. Our results are also comparable to those of other intermediate conditioning regimens, combining Flu, ATG and 3 days of Bu[4],

associated with a cumulative incidence of relapse of 29.1% and a LFS of 57%[5]. Overall, this regimen is associated with a good disease control in intermediate and high risk AML, as compared to that achieved in previous studies.

In patients with a median age of 55 years, and aged up to 76 years, we reported a 2-year cumulative incidence of NRM of 24%. This may seem high for a RIC regimen, Russel et al. reported a reduced NRM of 6% after RIC compared to 22% after MAC in non-favorable AML in first CR[16], however, RIC regimen was non-myeloablative with low-dose TBI in most patients, leading to a cumulative incidence of relapse of 36% after RIC transplant, compare to only 22.8% in our study. Scott et al. recently reported the preliminary results of the BMT CTN 0901 randomized protocol comparing MAC versus RIC in AML and myelodysplastic syndrome in CR[18]. NRM was significantly lower after RIC allo-SCT: 4.4%, versus 15.8% after MAC, while corresponding relapse rate were 48.3% versus 13.5%. NRM rate are lower in this study, including in the MAC group, consisting of a majority of reduced toxicity regimen combining Flu and Bu. However, this study includes only selected patients up to 65 years, while in our cohort patients up to 76 years were treated. Therefore our NRM is comparable to the NRM reported in some of the largest studies on RIC regimen for AML[12, 13].

Regarding conventional MAC regimen, similar rate of NRM have been reported in prospective studies: 22% in the study by Russel et al. [16] and 25/7% in the study by Lee et al.[19]. However, in these prospective studies, patients were selected and younger with a median age of 42 and 41 years old respectively, compare to a median age of 55 years in our study. Therefore our NRM rate compare favorably with traditional myeloablative regimen. Previous report evaluating FLAMSA sequential regimen reported a 2-year NRM of 22% both in

relapse/refractory AML[7] and in AML in CR[8]. Of note, we reported only 2 deaths related to cardiac toxicity, a known side effect of amsacrine[20] and there was no increase in the mortality related to sinusoidal obstruction syndrome, with only 2 deaths reported.

Taken together, the low cumulative incidence of relapse and the NRM led to an OS of 56.1% at 2 years, which compare favorably with result of the previously cited studies evaluating both RIC (OS range from 36% to 55%)[12-16] and myeloablative regimens (OS range from 57% to 53%)[12, 16].

The patients' outcome was similar using either the TBI or the Bu based FLAMSA regimen. Although NRM was higher in patients receiving Bu in univariate analysis, after adjustment for patient age, among others, there was no difference in NRM between the two conditioning regimens in multivariate analysis. Given TBI is a the major risk factor of long-term complications [21] and the favorable safety profile associated with the use of IV Bu[22], IV Bu appears as an effective alternative to TBI in the FLAMSA sequential approach.

Attention must be paid to elderly patients when using FLAMSA sequential regimen. While McClure et al. reported no impact of patients' age on the outcome after RIC regimen allo-SCT[23], older age at transplant was associated with a significantly lower OS in our multivariate analysis. This difference seems to be related to an increased NRM in elderly patients; however, given the retrospective nature of this analysis, we were not able to identify the exact nature of those deaths. Ultimately, careful screening of co-morbidities must be performed in elderly patients before using intermediate intensity conditioning such as the FLAMSA sequential regimen.

In multivariate analysis, poor risk cytogenetic status was associated with a

significant increase in relapse and a decrease in OS. Therefore, despite the increased cytotoxicity, the FLAMSA sequential regimen does not overcome the bad prognosis of poor-risk cytogenetic. Development of a new strategy to decrease relapse risk in those patients remains indispensable. In our study, some patients received prophylactic DLI based on physician decision and, while we recognize that given the retrospective nature of our study, exact reason guiding decision to give prophylactic DLI is unknown, use of prophylactic DLI seems to be a valuable option to decrease relapse risk. Indeed, in a landmark analysis of patients alive and disease-free at 6 months, the LFS was significantly improved in patients who received prophylactic DLI, compared to those who did not. Furthermore, other strategies, such as early administration of the hypomethylating agent azacytidine or of FLT3-specific tyrosine kinase inhibitor seem promising[24, 25].

Given his retrospective nature, our study does have several obvious biases. Molecular marker and minimal residual disease before transplant were not available for all patients, precluding the evaluation of their prognostic value. As it was not a prospective study, the choice of the allocation to the FLAMSA regimen was based on physicians' preferences and we cannot exclude a bias in patients' selection. However, the homogeneity of the data in term of disease and transplant characteristics' strengthen our study and make the conclusion more robust.

Overall, our results suggest that the sequential FLAMSA conditioning regimen using either TBI or Bu may be a valid approach for intermediate or high risk AML transplanted in CR. This regimen is associated with a low incidence of relapse, although disease progression is still expected in patients with high-risk

cytogenetic. However, advanced age patients have an increased risk of NRM and particular attention should be paid to comorbidities, supportive care and dose of TBI or Bu used in this patients. Our result pave the way for future studies that should compare such sequential approaches with standard approached and include minimal residual monitoring to decipher the exact impact of the chemotherapy include in our sequential regimen before the RIC allo-SCT. Overall, our study provides a framework for further refinement of intermediate intensity conditioning designed to improve disease control without increasing toxicity in AML in CR.

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Authors' contributions

F.M., M.L., B.N.S., M.Mohty and A.N. designed the research and/or analyzed data; G.S., J.B, A.G., J.T., M.Michallet, N.K., C.S., A.H., M.H. and M.Mohty provided important clinical data; F.M. wrote the first draft of the manuscript; and all authors approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests in relation with the content of this analysis.

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References

[1] Dohner H, Weisdorf DJ, Bloomfield CD. Acute Myeloid Leukemia. N Engl J Med. 2015;373:1136-1152.

[2] Sengsayadeth S, Savani BN, Blaise D, Malard F, Nagler A, Mohty M. Reduced intensity conditioning allogeneic hematopoietic cell transplantation for adult acute myeloid leukemia in complete remission - a review from the Acute Leukemia Working Party of the EBMT. Haematologica. 2015;100:859-869.

[3] Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75:555-562.

[4] Mohty M, Malard F, Blaise D, et al. Reduced-toxicity conditioning with fludarabine, once-daily intravenous busulfan, and antithymocyte globulins prior to allogeneic stem cell transplantation: results of a multicenter prospective phase 2 trial. Cancer. 2015;121:562-569.

[5] Oudin C, Chevallier P, Furst S, et al. Reduced-toxicity conditioning prior to allogeneic stem cell transplantation improves outcome in patients with myeloid malignancies. Haematologica. 2014;99:1762-1768.

[6] Schmid C, Schleuning M, Ledderose G, Tischer J, Kolb HJ. Sequential regimen of chemotherapy, reduced-intensity conditioning for allogeneic stem-cell transplantation, and prophylactic donor lymphocyte transfusion in high-risk acute myeloid leukemia and myelodysplastic syndrome. J Clin Oncol. 2005;23:5675-5687.

[7] Schmid C, Schleuning M, Schwerdtfeger R, et al. Long-term survival in refractory acute myeloid leukemia after sequential treatment with chemotherapy and reduced-intensity conditioning for allogeneic stem cell transplantation. Blood. 2006;108:1092-1099.

[8] Schmid C, Schleuning M, Hentrich M, et al. High antileukemic efficacy of an intermediate intensity conditioning regimen for allogeneic stem cell transplantation in patients with high-risk acute myeloid leukemia in first complete remission. Bone Marrow Transplant. 2008;41:721-727.

[9] Kröger N, Zabelina T, Wolschke C, et al. Induction Chemotherapy Followed Immediately by Busulfan-Based Reduced Conditioning and Allografting in Elderly Patients with Advanced MDS or sAML. Blood. 2015;114:3387-3387.

[10] Christopeit M, Badbaran A, Alawi M, et al. Correlation of somatic mutations with outcome after FLAMSA-busulfan sequential conditioning and allogeneic stem cell transplantation in patients with MDS. Eur J Haematol. 2015.

[11] Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet. Blood. 2010;115:453-474.

[12] Aoudjhane M, Labopin M, Gorin NC, et al. Comparative outcome of reduced intensity and myeloablative conditioning regimen in HLA identical sibling allogeneic haematopoietic stem cell transplantation for patients older than 50 years of age with acute myeloblastic leukaemia: a retrospective survey from the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT). Leukemia. 2005;19:2304-2312.

[13] Valcarcel D, Martino R, Caballero D, et al. Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival. J Clin Oncol. 2008;26:577-584.

[14] Warlick ED, Paulson K, Brazauskas R, et al. Effect of postremission therapy before reduced-intensity conditioning allogeneic transplantation for acute myeloid leukemia in first complete remission. Biol Blood Marrow Transplant. 2014;20:202-208.
[15] Yeshurun M, Labopin M, Blaise D, et al. Impact of postremission consolidation chemotherapy on outcome after reduced-intensity conditioning allogeneic stem cell transplantation for patients with acute myeloid leukemia in first complete remission: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Cancer. 2014;120:855-863.
[16] Russell NH, Kjeldsen L, Craddock C, et al. A comparative assessment of the curative potential of reduced intensity allografts in acute myeloid leukaemia. Leukemia. 2015;29:1478-1484.
[17] Schmid C, Labopin M, Nagler A, et al. Treatment, risk factors, and outcome of adults with relapsed AML after reduced intensity conditioning for allogeneic stem cell transplantation. Blood. 2012;119:1599-1606.
[18] Scott BL, Pasquini MC, Logan B, et al. Results of a Phase III Randomized, Multi-Center Study of Allogeneic Stem Cell Transplantation after High Versus Reduced Intensity Conditioning in Patients with Myelodysplastic Syndrome (MDS) or Acute Myeloid Leukemia (AML): Blood and Marrow Transplant Cl…. Blood. 2015;126:LBA-8-LBA-8.
[19] Lee JH, Joo YD, Kim H, et al. Randomized trial of myeloablative conditioning regimens: busulfan plus cyclophosphamide versus busulfan plus fludarabine. J Clin Oncol. 2013;31:701-709.
[20] Pai VB, Nahata MC. Cardiotoxicity of chemotherapeutic agents: incidence, treatment and prevention. Drug safety. 2000;22:263-302.
[21] Mohty M, Malard F, Savani BN. High-Dose Total Body Irradiation and Myeloablative Conditioning before Allogeneic Hematopoietic Cell Transplantation: Time to Rethink? Biol Blood Marrow Transplant. 2015;21:620-624.
[22] Russell JA, Kangarloo SB. Therapeutic drug monitoring of busulfan in transplantation. Curr Pharm Des. 2008;14:1936-1949.
[23] 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.
[24] Goodyear OC, Dennis M, Jilani NY, et al. Azacitidine augments expansion of regulatory T cells after allogeneic stem cell transplantation in patients with acute myeloid leukemia (AML). Blood. 2012;119:3361-3369.
[25] Schiller GJ, Tuttle P, Desai P. Allogeneic Hematopoietic Stem Cell Transplantation in FLT3-ITD-Positive Acute Myelogenous Leukemia: The Role for FLT3 Tyrosine Kinase Inhibitors Post-Transplantation. Biol Blood Marrow Transplant. 2016;22:982-990.

448 **Figure legend:**

449 **Figure 1.** Outcome after allo-HSCT. Cumulative incidence of non-relapse

450 mortality (A); cumulative incidence of relapse (B); overall survival (C); and

451 leukemia-free survival (D). NRM indicates non-relapse mortality.

452

453 **Table 1.** Study population and transplant characteristics
454

Characteristic (%)	Total (n = 265)	FLAMSA TBI 4 Gy (n=159)	FLAMSA Bu (n=106)	P
Patient age, median (range)	55 (19-76)	52 (19-76)	61 (25-74)	<0.0001
Patients < 55 years	132 (49.6%)	104 (65.4%)	28 (26.4%)	
Patients ≥ 55 years	133 (50.4%)	55 (34.6%)	78 (73.6%)	
Year of transplant, median (range)	2010 (2002-2014)	2009 (2002-2014)	2011 (2005-2014)	<0.0001
Patient gender (female)	122 (46.2%)	82 (51.9%)	40 (37.7%)	0.02
Donor gender (female)	80 (30.5%)	52 (33.3%)	28 (26.4%)	0.23
Female donor to male patient	26 (10.0%)	16 (10.3%)	10 (9.4%)	0.81
Karnofsky performance status				
≥90%	179 (70%)	107 (71%)	72 (70%)	
80%	65 (26%)	39 (26%)	26 (25%)	0.82
≤70%	10 (4%)	5 (3%)	5 (5%)	
Unknown	11	8	3	
Donor CMV negative to patient CMV positive	51 (19.6%)	34 (21.8%)	17 (16.0%)	0.41
Disease status				
CR1	216 (81.5%)	127 (79.9%)	89 (84.0%)	0.40
CR2	49 (18.5%)	32 (20.1%)	17 (16.0%)	
Median time from diagnosis to transplant				
CR1 patients, days (range)	135 (43-225)	130 (43-948)	154 (43-827)	0.02
CR2 patients, days (range)	627 (135-1701)	631 (135-1508)	547 (166-1701)	0.36
Cytogenetic risk				
Intermediate	114 (43.0%)	82 (51.6%)	32 (30.2%)	
Poor	42 (15.8%)	31 (19.5%)	11 (10.4%)	<0.0001
Secondary AML	109 (41.1%)	46 (28.9%)	63 (59.4%)	
Donor type				
Matched related donor	74 (27.9%)	50 (31.4%)	24 (22.6%)	0.12
Unrelated donor*	191 (72.1%)	109 (68.6%)	82 (77.4%)	
Stem cell source				
BM	14 (5.3%)	10 (6.3%)	4 (3.8%)	0.37
PBSC	251 (94.7%)	149 (93.7%)	102 (96.2%)	
In vivo T-cell depletion				
No	7 (2.6%)	5 (3.1%)	2 (1.9%)	0.53
Yes	258 (97.4%)	154 (96.9%)	104 (98.1%)	
GVHD prophylaxis				
CsA + MMF	216 (81.8%)	129 (81.6%)	87 (82.1%)	0.93
Others	48 (18.2%)	29 (18.4%)	19 (17.9%)	

455 Abbreviations : CMV indicates cytomegalovirus; CR1. first complete remission; CR2. second
456 complete remission; AML. acute myeloid leukemia; BM. bone marrow; PBSC. peripheral blood stem
457 cells; TNC. total nucleated cells; Bu. busulfan; TBI. total body irradiation; GVHD. graft-versus-host
458 disease; CsA. Ciclosporine A; MMF. mycophenolate mofetil.
459 * 49 patients (%) received a mismatched unrelated donor, being 27 in the TBI group and 22 in the
460 Bu group.

461 **Table 2.** Transplant-related events: univariate analysis
462

	NRM	RI	OS	LFS
Conditioning				
TBI group	19.4% (13.5-26.2)	21.2 % (15-28.2)	62 % (54-70)	58.8 % (50.7-66.8)
Bu group	31.1% (24-38.4)	25.7 % (17-35.2)	46.7 % (36.1-57.3)	43.2 % (32.8-53.7)
P value	**0.02**	0.77	0.14	0.14
Patient age				
< 55 years	17.8% (11.5-25.3)	23.9 % (16.4-32.1)	62.2 % (53.2-71.2)	57.9 % (48.8-67.1)
≥ 55 years	29.7% (21.8-38)	21.8 % (14.9-29.5)	50.6 % (41.6-59.7)	48.1 % (39.2-57)
P value	**0.008**	0.90	**0.005**	**0.02**
Status at transplant				
CR1	23.2% (17.6-29.4)	23 % (17.3-29.3)	57.5 % (50.4-64.6)	53.2 % (46.1-60.4)
CR2	27.1% (21.1-33.5)	22 % (11.2-35.2)	50.6 % (36.1-65.2)	50.8 % (36.3-65.3)
P value	0.68	0.70	0.98	0.99
Patient gender				
Male	28.2 % (20.8-36.1)	23.8 % (16.8-31.4)	49.4 % (40.7-58.1)	47.6 % (38.9-56.2)
Female	18 % (12-25.1)	21.7 % (14.4-30.1)	65.4 % (56.2-74.5)	59.8 % (50.3-69.3)
P value	0.052	0.54	**0.049**	0.10
Donor gender				
Male	22.9 % (16.9-29.5)	23.7 % (17.5-30.5)	55.1 % (47.4-62.8)	53.4 % (45.7-61.1)
Female	27.5 % (21-34.3)	21.6 % (12.7-32)	56.6 % (44.7-68.4)	49.4 % (37.5-61.4)
P value	0.22	0.58	0.39	0.48
Female to male				
Yes	23.6% (18.1-29.5)	23 % (17.5-29)	56 % (49.1-62.8)	53.2 % (46.3-60)
No	27.7% (21.9-33.7)	24 % (9.4-42.2)	54.5 % (34.6-74.4)	46.2 % (26.3-66.1)
P value	0.32	0.95	0.28	0.34
Donor				
MRD	13.8% (7-22.8)	30.6 % (20.3-41.5)	62.5 % (51-73.9)	54.4 % (42.7-66.1)
UD	28.4% (18.5-39.1)	19.5 % (13.8-26)	53.5 % (45.8-61.2)	52.1 % (44.4-59.8)
P value	**0.04**	**0.047**	0.54	0.94
Year				
< 2010	13.9% (8.3-20.9)	23.5 % (16.2-31.6)	66.3 % (57.6-75)	61.9 % (53-70.9)
≥ 2010	33.3% (25-41.8)	23.3 % (16-31.6)	46.3 % (37.1-55.5)	43.4 % (34.2-52.5)
P value	**0.0008**	0.61	**0.003**	**0.02**
Cytogenetics				
Intermediate	20.6 % (12.6-30)	21.7 % (14.5-29.8)	61.2 % (52-70.4)	56.9 % (47.5-66.2)
Poor	19.6 % (11.8-28.9)	35.2 % (20.6-50.2)	47.3 % (31.6-62.9)	45.2 % (29.6-60.7)
Secondary AML	30.3 % (20.9-40.3)	19.1 % (11.4-28.3)	53.7 % (42.9-64.5)	50.6 % (39.8-61.4)
P value	0.10	0.08	0.46	0.69

463 Abbreviations : NRM indicates non-relapse mortality; RI, relapse incidence; OS, overall survival;
464 LFS, leukemia-free survival; TBI, total body irradiation; Bu, busulfan; CR1, first complete
465 remission; CR2, second complete remission; MRD, matched related donor; UD, unrelated donor;
466 CMV, cytomegalovirus; AML acute myeloid leukemia.
467 Bold denotes statistically significant.

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469

Table 3. Transplant-related events: multivariate analysis

Outcome	Hazard ratio (95% confidence interval)	P value
Non-relapse mortality		
Busulfan versus TBI based conditioning	1.11 (0.62-2.01)	0.72
Age at transplant (per 10 years)	1.18 (0.93-1.50)	0.18
Status at transplant (CR2 versus CR1)	1.31 (0.69-2.50)	0.41
Unrelated donor versus MRD	1.77 (0.92-3.39)	0.09
Cytogenetic		
Poor versus intermediate	1.07 (0.47-2.41)	0.87
Secondary AML versus intermediate	1.32 (0.73-2.39)	0.35
Patient gender (female versus male)	0.68 (0.39-1.19)	0.18
Female donor to male patient versus others	1.45 (0.65-3.26)	0.37
Year of transplant	1.11 (0.99-1.24)	0.07
Relapse incidence		
Busulfan versus TBI based conditioning	1.27 (0.68-2.37)	0.44
Age at transplant (per 10 years)	1.23 (0.96-1.58)	0.11
Status at transplant (CR2 versus CR1)	1.24 (0.62-2.48)	0.54
Unrelated donor versus MRD	0.62 (0.36-1.07)	0.08
Cytogenetic		
Poor versus intermediate	**1.96 (1.03-3.72)**	**0.04**
Secondary AML versus intermediate	0.89 (0.48-1.68)	0.73
Patient gender (female versus male)	0.82 (0.48-1.41)	0.48
Female donor to male patient	0.81 (0.34-1.95)	0.64
Year of transplant	0.98 (0.87-1.09)	0.69
Overall survival		
Busulfan versus TBI based conditioning	1.09 (0.70-1.69)	0.70
Age at transplant (per 10 years)	**1.21(1.01-1.45)**	**0.04**
Status at transplant (CR2 versus CR1)	1.35 (0.83-2.19)	0.23
Unrelated donor versus MRD	1.11 (0.73-1.69)	0.63
Cytogenetic		
Poor versus intermediate	**1.76 (1.06-2.92)**	**0.03**
Secondary AML versus intermediate	1.17 (0.75-1.84)	0.49
Patient gender (female versus male)	0.71 (0.48-1.07)	0.10
Female donor to male patient versus others	1.15 (0.63-2.10)	0.65
Year of transplant	1.08 (0.99-1.18)	0.07
Leukemia-free survival		
Busulfan versus TBI based conditioning	1.16 (0.76-1.78)	0.48
Age at transplant (per 10 years)	1.19 (1.00-1.41)	0.05
Status at transplant (CR2 versus CR1)	1.23 (0.77-1.98)	0.39
Unrelated donor versus MRD	0.98 (0.66-1.47)	0.94
Cytogenetic		
Poor versus intermediate	1.50 (0.92-2.47)	0.11
Secondary AML versus intermediate	1.09 (0.71-1.68)	0.68
Patient gender (female versus male)	0.76 (0.52-1.11)	0.16
Female donor to male patient versus others	1.10 (0.61-1.99)	0.76
Year of transplant	1.04 (0.97-1.13)	0.28

Abbreviations: Bu indicates busulfan; CR2, second complete remission; CR1, first complete remission; MRD, matched related donor; AML, acute myeloid leukemia.
Bold denotes statistically significant.