RIC versus MAC UCBT in adults with AML: A report from Eurocord, the ALWP and the CTIWP of the EBMT

Frédéric Baron1,*, Annalisa Ruggeri2,3,*, Eric Beohou4, Myriam Labopin4, Guillermo Sanz5, Noel Milpied6,7 Mauricette Michallet8, Andrea Bacigalupo9, Didier Blaise10, Jorge Sierra11, Gérard Socié12, Jan J. Cornelissen13, Christoph Schmid14, Sebastian Giebel15, Norbert-Claude Gorin3,4, Jordi Esteve16, Fabio Ciceri17, Bipin N. Savani18, Mohamad Mohty1,3,19,20, Eliane Gluckman21 and Arnon Nagler4,22

1 University of Liège, Liege, Belgium
2 Eurocord, Hospital Saint Louis, AP-HP, and IUH University Paris VII, Paris, France
3 AP-HP, Hématologie Clinique et Thérapie Cellulaire, Hôpital Saint-Antoine, Paris, France
4 EBMT Paris Office, Hospital Saint Antoine, Paris, France
5 Hospital Universitario La Fe, Servicio de Hematología, Valencia, Spain
6 CHU Bordeaux, Hématologie Clinique et Thérapie Cellulaire-Hôpital Haut-leveque, Bordeaux, France
7 University of Bordeaux, Bordeaux, France
8 Service d’Hematologie du Centre Hospitalier de Lyon Sud, Pierre-Bénite, France
9 Ospedale San Martino, Department of Haematology II, Genova, Italy
10 Institut Paoli Calmettes (IPC), Aix Marseille University (AMU), UM105, Centre de Recherche en Cancerologie (CRCM), Inserm U1068, CNRS UMR7258 Marseille, Marseille, France
11 Hospital Santa Creu i Sant Pau, Hematology Department, Barcelona, Spain
12 AP-HP, Hematology Transplantation, Hospital Saint-Louis, Paris, France
13 Erasmus Medical Center-Daniel den Hoed Cancer Center, Rotterdam, The Netherlands
14 Klinikum Augsburg, Department of Hematology and Oncology, University of Munich, Augsburg, Germany
15 Maria Sklodowska-Curie Cancer Center and Institute of Oncology, Gliwice Branch, Gliwice, Poland
16 Department of Hematology, Hospital Clinic, Barcelona, Spain
17 Department of Hematology, Ospedale San Raffaele, Università degli Studi, Milano, Italy
18 Long Term Transplant Clinic, Vanderbilt University Medical Center, Nashville, TN, USA
19 Université Pierre & Marie Curie, Paris, France
20 INSERM, UMR 938, Paris, France
21 Eurocord, Hospital Saint Louis, AP-HP, and IUH University Paris VII, France Monacord, Centre Scientifique de Monaco, Monaco
22 Division of Hematology and Bone Marrow Transplantation, The Chaim Sheba Medical Center, Tel-Hashomer, Ramat-Gan, Israel

* These authors have contributed equally to the paper

Correspondence to: Frédéric Baron, email: f.baron@ulg.ac.be

Keywords: unrelated cord blood, AML, reduced-intensity, myeloablative, transplantation

Received: May 02, 2016  Accepted: May 14, 2016  Published: May 26, 2016

ABSTRACT

Nonrelapse mortality (NRM) is the first cause of treatment failure after unrelated cord blood transplantation (UCBT) following myeloablative conditioning (MAC). In the last decade, reduced-intensity conditioning (RIC) regimens have been developed with the aim of reducing NRM and allowing older patients and those with medical comorbidities to benefit from UCBT. The aim of the current retrospective study was to compare transplantation outcomes of acute myeloid leukemia (AML) patients given UCBT after either RIC or MAC. Data from 894 adults with AML receiving a single or double UCBT as first allograft from 2004 to 2013 at EBMT centers were included in
INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HCT) from HLA-identical sibling is the treatment of choice for selected patients with acute myeloid leukemia (AML) [1-3]. For AML patients who lack a suitable human leukocyte antigen (HLA)-identical sibling, unrelated cord blood transplantation (UCBT) is an adequate alternative to HLA-matched unrelated bone marrow/peripheral blood stem cell (PBSC) transplantation, particularly for patients at high risk of rapid disease relapse who urgently need a transplantation [4, 5].

Despite major improvements in the field, nonrelapse mortality (NRM) has remained the main cause of failure of UCBT for AML [5]. In the last decade, reduced-intensity conditionings (RIC) for UCBT have been developed with the aim of reducing NRM and allowing older/unfit patients to benefit from UCBT [6-9]. Although a recent study demonstrated low NRM after RIC UCBT for AML [10], a high incidence of disease relapse has also been observed with this approach [10-12]. This prompted us to perform the current retrospective registry study aimed at assessing the impact of the conditioning intensity on transplantation outcomes in patients receiving UCBT as treatment for AML.

RESULTS

Patient, disease and transplant characteristics

Patients and disease characteristics are described in Table 1. Briefly, 415 patients were given UCB after this study. 415 patients were given UCBT after RIC while 479 patients following a MAC. In comparison to MAC recipients, RIC recipients had a similar incidence of neutrophil engraftment and of acute and chronic graft-versus-host disease (GVHD). However, RIC recipients had a higher incidence of disease relapse and a lower NRM, translating to comparable leukemia-free (LFS), GVHD-free, relapse-free survival (GRFS) and overall survival (OS). These observations remained qualitatively similar after adjusting for differences between groups in multivariate analyses. In conclusion, these data suggest that LFS and OS are similar with RIC or with MAC in adults AML patients transplanted with UCBT. These observations could serve as basis for a future prospective randomized study.

Figure 1: UCBT outcomes in AML patients transplanted following RIC (n = 415) versus MAC (n = 479). The figures show the unadjusted curves for MAC patients and the adjusted curves for RIC recipients. Curves were adjusted for age at transplantation, recipient gender, year of transplantation, disease status, TBF conditioning or not, TCF conditioning, or not, and the use of ATG. LFS, leukemia-free survival; OS, overall survival; RI, relapse incidence and NRM, nonrelapse mortality.
Table 1: Patient and transplant characteristics

|                          | MAC \((n = 479)\) | RIC \((n = 415)\) | \(P\) value 1 |
|--------------------------|------------------|------------------|----------------|
| Median patient age, y (range) | 37 (18–68) | 54 (19-72) | <0.0001 |
| Median year of UCBT, y (range) | 2010 (2004-2013) | 2010 (2004-2013) | 0.16 |
| Recipient gender M, # (%) | 251 (52) | 185 (45) | 0.02 |
| Status at transplantation, # (%) |                |                  |                |
| CR1                      | 248 (52) | 200 (48) | 0.06 |
| CR2                      | 123 (26) | 131 (32) |                |
| CR3                      | 19 (4) | 7 (2) |                |
| Advanced                 | 89 (19) | 77 (19) |                |
| Cytogenetics, # (%) all patients |                |                | 0.0004 |
| Good risk\(^2\)         | 42 (9) | 20 (5) |                |
| Intermediate risk\(^1\) | 221 (46) | 229 (55) |                |
| High risk\(^4\)         | 50 (10) | 61 (15) |                |
| Not reported/failed      | 166 (35) | 105 (25) |                |
| Cytogenetics, # (%) patients in CR1 |        |        |                |
| Good risk\(^2\)         | 10 (4) | 0 (0) |                |
| Intermediate risk\(^1\) | 122 (49) | 111 (56) |                |
| High risk\(^4\)         | 29 (12) | 41 (20) |                |
| Not reported/failed      | 87 (35) | 48 (24) |                |
| Normal cytogenetics and FLT3-ITD+ | 25 (11) | 28 (12) |                |
| Missing                  | 71 (30) | 60 (26) |                |
| Conditioning regimen, # (%) |        |        | <0.0001 |
| TCF\(^3\)               | 85 (18) | 308 (74) |                |
| TBF\(^6\)               | 176 (37) | 21 (5) |                |
| BuCy                    | 41 (9) | 5 (1) |                |
| BuFlu                   | 40 (8) | 8 (2) |                |
| FluMel                  | 4 (1) | 15 (4) |                |
| TreoFlu                 | 7 (1) | 4 (1) |                |
| TBI-based but not TCF   | 82 (17) | 29 (7) |                |
| Others                  | 44 (9) | 25 (6) |                |
| Recipient CMV-seronegative, # (%) |        |        | 0.06 |
| Single                  | 341 (71) | 159 (38) | <0.0001 |
| Double                  | 138 (29) | 256 (62) |                |
| ATG, # (%)              | 267 (60) | 89 (23) | <0.0001 |
| TNC at infusion x 10\(^7\)/kg |        |        | <0.0001 |
| Median (range)          | 2.8 (0.2-40.3) | 3.5 (0.3-11.8) |                |
| Missing data (# of patients) | 127    | 82    |                |
| Number of HLA disparities, # (%) |        |        | 0.003 |
| 0-1 mismatch            | 166 (44) | 109 (33) |                |
| 2-4 mismatches          | 208 (56) | 217 (67) |                |
| Missing data            | 105 | 89 |                |
| Postgrafting immunosuppression, # (%) |        |        | <0.0001 |
| CSP (or tacrolimus) alone | 46 (10) | 22 (6) |                |
| CSP (or tacrolimus) + MMF | 218 (49) | 335 (86) |                |
| CSP + MTX               | 24 (5) | 11 (3) |                |
| CSP + MMF + MTX         | 6 (1) | 3 (1) |                |
| Post-transplant cy       | 8 (2) | 4 (1) |                |
| Other                   | 142 (32) | 14 (4) |                |
| Missing                  | 35 | 26 |                |
MAC, myeloablative conditioning; RIC, reduced-intensity conditioning; Y, year; M, male; UCBT, umbilical cord blood transplantation; CR, complete remission; #, number of patients; ATG, anti-thymocyte globulins; TNC, total nucleated cells; tac, tacrolimus; CSP, cyclosporine A; MMF, mycophenolate mofetil. TCF, total body irradiation (TBI), cyclophosphamide and fludarabine; TBF, Thiopepa, busulfan and fludarabine.

1, calculated with $c^2$ statistics for categorical variables and Mann-Whitney test for continuous variables; 2, defined as t(8;21), t(15;17), inv or del (16), or acute promyelocytic leukemia, these abnormalities only or combined with others; 3, defined as all cytogenetics not belonging to the good or high risk (including trisomias); 4, defined as 11q23 abnormalities, complex karyotype, abnormalities of chromosomes 5 and 7; 5, classified as RIC if the dose of TBI was < 6 Gy; 6 classified as RIC when the busulfan total dose was ≤ 8 mg/kg.

RIC while 479 patients were administered MAC. The most frequently used conditioning regimens were either TCF regimen, given in 18% of MAC recipients and 74% of RIC recipients, respectively, or TBF given in 39% of MAC recipients and 5% of RIC recipients. In comparison to MAC recipients, RIC recipients were almost 2 decades older [median age 54 (range, 19 - 72) years versus 37 (range, 18 - 68) years, $P < 0.001$], received more frequently a double UCBT (62% versus 29%, $P < 0.001$), received more frequently units with > or = 2 HLA-mismatches (67% vs 56%, $P = 0.003$), received higher TNC [median 3.5 (range, 0.3 - 11.8) 10E7 cells/kg versus 2.8 (range, 0.2 - 40.3) 10E7 cells/kg, $P < 0.0001$], and received less frequently ATG (23% versus 60%, $P < 0.0001$) in the conditioning. Disease status at UCBT was comparable in both groups with approximately half of the patients in first CR and 19% of patients not in CR in both groups. Median follow-up for survivors was 26 (range, 1.02 -118.2) months.

Engraftment and GVHD

Overall, CI of neutrophil engraftment at day 100 was not different in RIC (89%) and MAC (88%) recipients ($P = 0.8$) with the limitation that we did not systematically collect chimerism data and that autologous reconstitution is possible in RIC recipients. Median times for reaching 0.5 x 10^9/L neutrophils were 21 (range, 3-66) days in RIC patients versus 23 (range, 1-106) days in MAC patients, respectively.

In univariate analysis, there was a higher incidence of grade II-IV acute GVHD in RIC recipients (35% versus 26%, $P = 0.009$) while the incidence of grade III-IV acute GVHD was similar in both groups of patients (11% and 11%, $P = 0.9$). However, in multivariable analyses adjusting for single versus double UCBT, gender combination, use of ATG and HLA-compatibility the incidence of grade II-IV acute GVHD was comparable in RIC and MAC recipients (HR = 1.09, $P = 0.65$).

The 2-year cumulative incidence of chronic GVHD was similar in RIC and MAC recipients (23% and 23%, $P = 0.9$). In multivariable analysis, there was a trend for a higher incidence of chronic GVHD in patients receiving the TBF regimen [HR 1.7 (95% CI, 1.0-2.7), $P = 0.05$], while, interestingly, ATG failed to decrease the incidence of chronic GVHD [HR 1.4 (95% CI, 0.9-2.2), $P = 0.15$].

Relapse, NRM, LFS, OS

At 2-year, RIC recipients had a higher incidence of disease relapse (41% versus 23%, $P < 0.001$) but a lower NRM (19% versus 36%, $P < 0.001$), translating to similar LFS (40% versus 41%, $P = 0.8$) and OS (46% versus 43%, $P = 0.3$) than when compared to MAC recipients (Figure 1). In multivariate analyses, the use of RIC (versus MAC) regimen was associated with a higher incidence of relapse (HR = 1.6, 95% CI: 1.2-2.2; $P = 0.005$). LFS and OS were comparable (LFS: HR = 1.1, 95% CI:0.9-1.4; $P = 0.3$); (OS: HR = 1.0, 95% CI:0.8-1.3; $P = 0.9$) (Table 2). Factors associated with worse OS included older patient age (HR = 1.0, 95% CI: 1.0-1.0; $P = 0.02$), advanced disease (HR = 2.3, 95% CI: 1.8-2.9; $P < 0.0001$), while female recipients had better OS than male recipients (HR = 0.8, 95% CI:0.7-1.0; $P = 0.02$).

GRFS

GVHD-free, relapse-free survival (GRFS) has recently emerged as an important endpoint in allo-HCT. We thus compared GRFS in patients receiving UCBT after RIC or MAC. At 2-year, GRFS was similar in RIC and MAC recipients (30.9% versus 31.1%, $P = 0.86$). In multivariate analysis, GRFS was similar in RIC and MAC patients (HR = 1.0, 95% CI: 0.8-1.3; $P = 0.7$). Factors associated with worse GRFS included advanced disease (HR 1.8, 95% CI: 1.5-2.2, $P < 0.001$) while female recipients had better GRFS than male recipients (HR = 0.8, 95% CI:0.6-0.9; $P = 0.001$).

Additional Cox analyses for OS and LFS

To further dissect the impact of conditioning intensity on UCBT, we performed additional Cox analyses comparing UCBT outcomes among patients conditioned with RIC or MAC regimen separately for pre-transplant variables. The results of these analyses are presented graphically using Forest plots in Figures 2-3. RIC regimens were associated with lower NRM in each subgroup categories with median HR ranging from 0.3 to 0.9 (Figure 2A). This was particularly the case in the subgroup of younger patients (HR = 0.3, 95% CI: 0.2-0.6) and in those not given ATG (HR = 0.6, 95% CI: 0.4-0.9). However, RIC regimen was also associated with a
Figure 2: Forest plot analysis of cumulative incidence of nonrelapse mortality (A) and relapse (B) HR and 95% confidence intervals were computed using univariate Cox analyses.
|                                | P value | HR  | Lower | Upper |
|--------------------------------|---------|-----|-------|-------|
| **Relapse or death**           |         |     |       |       |
| RIC vs MAC                     | .348    | 1.1 | 0.9   | 1.4   |
| Age at tx (in years)           | .090    | 1.0 | 1.0   | 1.0   |
| Female vs Male                 | .019    | 0.8 | 0.7   | 1.0   |
| Year of tx                     | .816    | 1.0 | 1.0   | 1.0   |
| CR2 vs CR1                     | .257    | 1.1 | 0.9   | 1.4   |
| Advanced vs CR1                | .000    | 2.2 | 1.7   | 2.7   |
| Double vs Single               | .436    | 0.9 | 0.7   | 1.1   |
| TCF used                       | .166    | 0.8 | 0.6   | 1.1   |
| TBF used                       | .426    | 0.9 | 0.7   | 1.2   |
| ATG used                       | .061    | 1.3 | 1.0   | 1.6   |
| **Death**                      |         |     |       |       |
| RIC vs MAC                     | .859    | 1.0 | 0.8   | 1.3   |
| Age at tx (in years)           | .017    | 1.0 | 1.0   | 1.0   |
| Female vs Male                 | .023    | 0.8 | 0.7   | 1.0   |
| Year of tx                     | .731    | 1.0 | 1.0   | 1.0   |
| CR2 vs CR1                     | .117    | 1.2 | 1.0   | 1.5   |
| Advanced vs CR1                | .000    | 2.3 | 1.8   | 2.9   |
| Double vs Single               | .273    | 0.9 | 0.7   | 1.1   |
| TCF used                       | .221    | 0.8 | 0.6   | 1.1   |
| TBF used                       | .812    | 1.0 | 0.7   | 1.3   |
| ATG used                       | .085    | 1.2 | 1.0   | 1.6   |
| **RI**                         |         |     |       |       |
| RIC vs MAC                     | .005    | 1.6 | 1.2   | 2.2   |
| Age at tx (in years)           | .794    | 1.0 | 1.0   | 1.0   |
| Female vs Male                 | .354    | 0.9 | 0.7   | 1.1   |
| Year of tx                     | .552    | 1.0 | 0.9   | 1.0   |
| CR2 vs CR1                     | .632    | 0.9 | 0.7   | 1.3   |
| Advanced vs CR1                | .000    | 3.2 | 2.4   | 4.4   |
| Double vs Single               | .646    | 1.1 | 0.8   | 1.4   |
| TCF used                       | .417    | 0.9 | 0.6   | 1.2   |
| TBF used                       | .100    | 0.7 | 0.4   | 1.1   |
| ATG used                       | .841    | 1.0 | 0.7   | 1.3   |
| **NRM**                        |         |     |       |       |
| RIC vs MAC                     | .101    | 0.7 | 0.5   | 1.1   |
| Age at tx (in years)           | .018    | 1.0 | 1.0   | 1.0   |
| Female vs Male                 | .026    | 0.7 | 0.6   | 1.0   |
| Year of tx                     | .925    | 1.0 | 0.9   | 1.1   |
| CR2 vs CR1                     | .037    | 1.4 | 1.0   | 1.8   |
| Advanced vs CR1                | .090    | 1.4 | 1.0   | 2.0   |
| Double vs Single               | .110    | 0.8 | 0.5   | 1.1   |
| TCF used                       | .236    | 0.8 | 0.5   | 1.2   |
| TBF used                       | .867    | 1.0 | 0.7   | 1.5   |
| ATG used                       | .012    | 1.6 | 1.1   | 2.3   |

HR, hazard ratio; CI, confidence interval; P, p value; cGVHD, cumulative incidence of chronic graft-versus-host disease; NRM, cumulative incidence of non relapse mortality; RI, cumulative incidence of relapse; LFS, leukemia-free survival; OS, overall survival; Tx, transplantation; CR, complete remission; advanced, not in complete remission; ATG, anti-thymocyte globulin; RIC, reduced-intensity conditioning; MAC, myeloablative conditioning.
higher risk of relapse in each subgroup categories with HR ranging from 1.2 to 2.5 (Figure 2B). Interestingly, the association between RIC regimen and higher risk of relapse was not less pronounced in patients in CR1 at transplantation (HR = 2.5, 95% CI: 1.7-3.7) than in those in CR3+ or advanced disease (HR = 1.4, 95% CI HR 0.9-2.1), and was also observed in the subgroup of patients transplanted following TCF regimen (HR = 2.0, 95% CI 1.2-3.2).

As shown in Figure 3A, RIC and MAC patients had comparable LFS in most transplantation variable subgroup. Interestingly, the assessment of heterogeneity according to age group evidenced a I² = 4.3%, demonstrating a very low impact of age on the association between conditioning intensity and LFS. However, there was a suggestion for worse LFS with RIC in the subgroup of patients transplanted in first CR (HR = 1.3, 95% CI: 1.0-1.6), in the subgroup of patients given ATG (HR = 1.3, 95% CI: 1.0-1.7), and in patients infused with > 3.2 x10⁷ TNC/Kg (HR = 1.4, 95% CI: 1.0-1.9). Further, interestingly, when patients were stratified according to the type of conditioning regimen used, there was a suggestion for worse LFS with RIC both in patients conditioned with the TCF regimen (HR = 1.4, 95% CI: 1.0-2.1), and in patients conditioned with other regimens (HR = 1.3, 95% CI: 1.0-1.7).

Finally, RIC and MAC patients had comparable OS in each transplantation variable subgroup (Figure 3B).

DISCUSSION

The impact of dose intensity on outcomes in AML patients has been the focus of many studies since the development of non-myeloablative/RIC regimens in the last 2 decades [13-18]. These studies have focused mainly on patients given PBSC as stem cell source. Large registry studies observed that the use of RIC regimen was associated with a higher risk of relapse, but also a lower incidence of NRM translating to similar OS and LFS [13-16]. More recently, the BMT-CTN performed a randomized study comparing RIC versus MAC in patients with MDS (N = 54) or AML (N = 218) (18-65 years, HCT-specific comorbidity index score [19] ≤ 4) who had < 5% marrow myeloblasts at allo-HCT [20]. As observed in registry studies, the use of RIC regimen was associated with higher risk of relapse (48 versus 14% at 18 months, P < 0.01) but also lower NRM (4% versus 16% at 18-months, P = 0.02). However, because the incidence of NRM was already low in MAC recipients, the amplitude of the reduction of NRM with RIC was insufficient to offset its negative impact on relapse, translating to significantly worse LFS in RIC recipients (47% vs 68% at 18 months, P < 0.01).

In contrast to allo-HCT using other stem cell source where relapse is usually the first cause of transplant failure in AML patients, NRM has remained the leading cause of death in UCB recipients given myeloablative conditioning [21]. Further, recent findings in humanized mice suggest that graft-versus-tumor effects might be higher with UCB than with PBSC [22], confirming prior clinical observations [23]. This could suggest that dose intensity might be less important following UCBT than following PBSC in regards to risk of relapse. Based on these findings, we hypothesized that the use of RIC versus MAC might be beneficial in the UCBT setting.

This prompted us to perform a retrospective study in the EBMT/Eurocord registries comparing UCBT outcomes in patients administered a RIC or a MAC regimen. Several observations were made.

First, despite prior evidence of strong graft-versus-tumor effects after UCBT [23], RIC UCBT recipients had a significantly higher risk of relapse than MAC UCBT recipients (HR = 1.6, 95% CI: 1.2-2.2). This confirms the results of a prior single center study [24] and demonstrates the importance of dose intensity for preventing AML relapse even in the setting of UCBT. As expected, NRM was lower after RIC than after MAC UCBT. As a net result, LFS, GRFS and OS were comparable in RIC and MAC recipients, rejecting our hypothesis that RIC regimens would provide better outcomes than MAC in AML patients undergoing UCBT.

The current study also confirmed a detrimental impact of ATG on NRM as recently reported in a study including data from patients given UCB after MAC conditioning [25] or RIC in the double UCBT setting [26]. Further, despite ATG not only induces in vivo T-cell depletion of the graft but also promotes the generation of regulatory T cells [27, 28], ATG failed to prevent GVHD in the current study. However ATG had no impact either on relapse incidence, in agreement with recent observations in the PBSC transplantation setting [29, 30]. Further, as previously observed in the UCB setting [5, 11], older age was associated with worse LFS and OS.

There are some limitation in our study including its design (retrospective registry survey), and the imbalance of the two groups for risk factors known to be associated with outcome: RIC patients were almost 2 decades older and were more often given UCB with ≥2 HLA-mismatches, but they received more often double UCBT and consequently received more cells while they were given less frequently ATG. These differences were carefully adjusted for in multivariate analysis while where forest plots demonstrated comparable OS with RIC and with MAC in each pre-transplant subgroups. Interestingly, a trend for better LFS was observed in MAC recipients in the subgroup of patients transplanted in first CR, in those who received ATG, and in patients infused with > 3.2 x10⁷ TNC/Kg.

Finally, RIC and MAC patients had comparable OS in each transplantation variable subgroup (Figure 3B).
Figure 3: Forest plot analysis of leukemia-free survival (A) and overall survival (B). HR and 95% confidence intervals were computed using univariate Cox analyses.
recent advances in the field of UCBT such as optimization of myeloablative regimen for UCBT, expansion of UCB hematopoietic stem/progenitor cells, and post-transplant administration of chimeric antigen receptor T cells are likely to improve outcomes of UCBT both in the RIC and in the MAC setting [31-33].

PATIENTS AND METHODS

Data collection

This survey is a retrospective study performed by the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT) and by Eurocord. EBMT registry is a voluntary working group of more than 500 transplant centers, participants of which are required once a year to report all consecutive stem cell transplantations and follow-up. Eurocord collects data on UCBT performed in > 50 countries worldwide and > 500 transplant centers, mainly EBMT centers. Population selection criteria included adult recipients (defined as ≥ 18 years old at UCBT), primary AML, first allogeneic stem cell transplantation, single or double unit UCBT performed from 2004 to 2013. Grading of acute and chronic GVHD was performed using established criteria [34]. HLA-compatibility was based on antigenic level typing for HLA-A and -B, and allele-level typing for HLA-DRB1. For the purpose of this study, all necessary data were collected according to EBMT and Eurocord guidelines. RIC was defined as use of fludarabine (Flu) associated with < 6 Gy total-body irradiation (TBI), or busulfan ≤ 8 mg/kg, melphalan ≤ 140 mg/m² or other nonmyeloablative drugs, as previously reported [14, 35-37]. Specifically, the combination of total body irradiation, cyclophosphamide and fludarabine (TCF regimen) was classified as RIC when the TBI dose was < 6 Gy (most RIC patients were given 2 Gy TBI) and as MAC when the TBI dose was ≥ 6 Gy (most MAC patients were given > 10 Gy TBI). Similarly, the association of thiopeta, busulfan and fludarabine (TBF regimen) was classified as RIC or MAC based on the dose of busulfan received (≤ 8 mg/kg or > 8 mg/kg, respectively).

Ethics

The scientific boards of the ALWP of EBMT and of Eurocord approved this study.

Statistical analyses

Data from all patients meeting the inclusion/exclusion criteria were included in the analyses. Start time was date of transplant for all endpoints. Neutrophil engraftment was defined as first of 3 consecutive days with a neutrophil count of at least 0.5 x 10⁹/L.

To evaluate the relapse incidence, patients dying either from direct toxicity of the procedure or from any other cause not related to leukemia were censored. NRM was defined as death without experiencing disease recurrence. Patients were censored at the time of relapse or of the last follow-up. Cumulative incidence functions (CIF) were used for relapse incidence and NRM in a competing risk setting, since death and relapse were competing together.

For estimating the cumulative incidence of chronic GVHD, death was considered as a competing event. OS and LFS were estimated using the Kaplan-Meier estimates. GVHD-free, relapse-free survival (GRFS) was defined as being alive with neither grade III-IV acute GVHD, severe chronic GVHD nor disease relapse [38]. Univariate analyses were done using Gray’s test for CIF and log rank test for OS and LFS. Associations of patient and graft characteristics with grade II-IV acute GVHD were evaluated using multivariate logistic regression. Variables introduced in the multivariate logistic regression included conditioning intensity (RIC versus MAC), single or double UCBT, gender combination, use of ATG and HLA-compatibility. Associations of patient and graft characteristics with other outcomes (chronic GVHD, relapse, NRM, LFS and OS) were evaluated in multivariable analyses, using Cox proportional hazards. Variables introduced in the Cox models included conditioning intensity (RIC versus MAC), conditioning type (thiotepa, busulfan and fludarabine (TBF) versus other and TBI, Flu and cyclophosphamide (TCF) versus other), the use of ATG or not, recipient age, recipient gender, years of transplantation and disease status at transplantation. Exploratory analyses of the heterogeneity of RIC vs MAC among pre-transplant subgroups for OS and LFS were performed using Cox models. The results of these Cox models were presented graphically using forest plots [39]. Heterogeneity according to age group for LFS was assessed by calculating the I² = (Qstatistic-degre of freedom)/Qstatistic x 100.

All tests were two sided. The type I error rate was fixed at 0.05 for determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS 19 (SPSS Inc, Chicago, IL), and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

ACKNOWLEDGMENTS

We thank Emmanuelle Polge and Audrey Mailhol from the office of the ALWP of EBMT. FB is Senior Research Associate at the National Fund for Scientific Research (FNRS) Belgium.
CONFLICTS OF INTERESTS

None.

GRANT SUPPORT

FBa is senior research associate at the F.R.S.-FNRS - Fonds de la Recherche Scientifique, Belgium.

Author contributions

FBa wrote the manuscript, designed the study, and interpreted the data; AR, EB and ML designed the study, analyzed and interpreted the data, and edited the manuscript; EG and AN designed the study, interpreted the data and edited the manuscript; MM, CS, SG, NG, JE, FC and BS helped in the study design and edited the manuscript; GS, NM, MM, AG, GS, DD, JS, JJC, MM reviewed the manuscript and provided clinical data. All authors approved the final version of the manuscript.

Editorial note

This paper has been accepted based in part on peer-review conducted by another journal and the authors’ response and revisions as well as expedited peer-review in Oncotarget.

REFERENCES

1. Suciu S, Mandelli F, de Witte T, Zittoun R, Gallo E, Labar B, De Rosa G, Belhabri A, Giustolisi R, Delarue R, Liso V, Mirto S, Leone G, Bourhis JH, Fioritoni G, Jehn U, 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; 102:1232-1240.

2. Dohner H, Estey EH, Amadori S, Appelbaum FR, Buchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D, Larson RA, Lo-Coco F, Niedierwieser D, Ossenkoppele GJ, Sanz MA, Sielra J, et al. Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukaemiaNet. Blood. 2010; 115:453-474.

3. Sureda A, Bader P, Cesarso S, Dreger P, Duarte RF, Dufour C, Falkenburg JH, Farge-Bancel D, Gennery A, Kroger N, Lanza F, Marsh JC, Nagler A, Peters C, Velardi A, Mohty M, et al. Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. Bone marrow transplantation. 2015; 50:1037-1056.

4. Weisdorf D, Eapen M, Ruggeri A, Zhang MJ, Zhong X, Brunstein C, Ustun C, Rocha V and Gluckman E. Alternative donor transplantation for older patients with acute myeloid leukemia in first complete remission: a center for international blood and marrow transplant research-eurocord analysis. Biol Blood Marrow Transplant. 2014; 20:816-822.

5. Ruggeri A, Sanz G, Bittencourt H, Sanz J, Rambaldi A, Volt F, Yakoub-Agha I, Ribera JM, Mannone L, Sierra J, Mohty M, Solano C, Nabhan S, Arcese W, Gluckman E, Labopin M, et al. Comparison of outcomes after single or double cord blood transplantation in adults with acute leukemia using different types of myeloablative conditioning regimen, a retrospective study on behalf of Eurocord and the Acute Leukemia Working Party of EBMT. Leukemia. 2014; 28:779-786.

6. Barker JN, Weisdorf DJ, Defor TE, Blazar BR, Miller JS and Wagner J. Rapid and complete donor chimerism in adult recipients of unrelated umbilical cord blood transplantation after reduced-intensity conditioning. Blood. 2003; 102:1915-1919.

7. Baron F and Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders (Review). Molecular Therapy. 2006; 13:26-41.

8. Brunstein CG, Fuchs EJ, Carter SL, Karanes C, Costa LJ, Wu J, Devine SM, Wingard JR, Aljitawi OS, Cutler CS, Jagasia MH, Ballen KK, Eapen M and O’Donnell PV. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. Blood. 2011; 118:282-288.

9. Somers JA, Braakman E, van der Holt B, Petersen EJ, Marji EW, Huisman C, Sintnicolaas K, Oudshoorn M, Groenendijk-Sijnke ME, Brand A and Cornelissen JJ. Rapid induction of single donor chimerism after double umbilical cord blood transplantation preceded by reduced intensity conditioning: results of the HOVON 106 phase II study. Haematologica. 2014; 99:1753-1761.

10. Rio B, Chevret S, Vigouroux S, Chevallier P, Furst S, Sirvent A, Bay JO, Socie G, Ceballos P, Huynh A, Cornillon J, Francoise S, Legrand F, Yakoub-Agha I, Michel G, Maillard N, et al. Decreased nonrelapse mortality after unrelated cord blood transplantation for acute myeloid leukemia using reduced-intensity conditioning: a prospective phase II multicenter trial. Biol Blood Marrow Transplant. 2015; 21:445-453.

11. Tucunduva L, Ruggeri A, Sanz G, Furst S, Socie G, Michallet M, Arcese W, Milpied N, Yakoub-Agha I, Linkesch W, Cornelissen J, Mannone L, Iori AP, Ribera JM, Sanz J, Montesinos P, et al. Risk factors for outcomes after unrelated cord blood transplantation for adults with acute lymphoblastic leukemia: a report on behalf of Eurocord and the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone marrow transplantation. 2014; 49:887-894.
12. Devillier R, Harbi S, Forst S, Crocchiolo R, El-Cheikh J, Castagna L, Etienne A, Calmes B, Lemarie C, Prebet T, Granata A, Charbonnier A, Rey J, Chabannon C, Faucher C, Vey N, et al. Poor outcome with nonmyeloablative conditioning regimen before cord blood transplantation for patients with high-risk acute myeloid leukemia compared with matched related or unrelated donor transplantation. Biol Blood Marrow Transplant. 2014; 20:1560-1565.

13. Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb HJ, Frassoni F, Boiron JM, Yin JL, Finke J, Shouten H, Blaise D, Falda M, Fauser AA, Esteve J, Polge E, 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.

14. Ringden O, Labopin M, Ehninger G, Niederwieser D, Olsson R, Basara N, Finke J, Schwertdfeger R, Eder M, Bunjes D, Gorin NC, Mohty M and Rocha V. Reduced intensity conditioning compared with myeloablative conditioning using unrelated donor transplants in patients with acute myeloid leukemia. Journal of Clinical Oncology. 2009; 27:4570-4577.

15. Luger SM, Ringden O, Zhang MJ, Perez WS, Bishop MR, Bornhauser M, Bredeson CN, Cairo MS, Copelan EA, Gale RP, Giralt SA, Gulbas Z, Gupta V, Hale GA, Lazarus HM, Lewis VA, et al. Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS. Bone marrow transplantation. 2012; 47:203-211.

16. Passweg JR, Labopin M, Cornelissen J, Volin L, Socie G, Huynh A, Tabrizi R, Wu D, Caddock C, Schaap N, Kuball J, Chevallier P, Cahn JY, Blaise D, Ghavamzadeh A, Bilger K, et al. Conditioning intensity in middle-aged patients with AML in first CR: no advantage for myeloablative regimens irrespective of the risk group-an observational analysis by the Acute Leukemia Working Party of the EBMT. Bone marrow transplantation. 2015; 50:1063-1068.

17. Rubio MT, Savani BN, Labopin M, Piemontese S, Polge E, Ciceri F, Bacigalupo A, Arcese W, Koc Y, Beelen D, Gulbas Z, Wu D, Santarone S, Tischer J, Afanasyev B, Schmid C, et al. Impact of conditioning intensity in T-replete haplo-identical stem cell transplantation for acute leukemia: a report from the acute leukemia working party of the EBMT. Journal of hematology & oncology. 2016; 9:25.

18. Savani BN, Labopin M, Kroger N, Finke J, Ehninger G, Niederwieser D, Schwertdfeger R, Bunjes D, Glass B, Socie G, Ljungman P, Caddock C, Baron F, Ciceri F, Gorin NC, Esteve J, et al. Expanding transplant options to patients over 50 years- Improved outcome after reduced intensity conditioning mismatched-unrelated donor transplantation for patients with acute myeloid leukemia: A report from the Acute Leukemia Working Party of the EBMT. Haematologica. 2016. In press.

19. Sorror ML, Maris MB, Storb R, Baron F, Sandmaier BM, Maloney DG and Storer B. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005; 106:2912-2919.

20. Scott BL, Pasquini MC, Logan B, Wu J, Devine S, Porter DL, Maziarz RT, Warlick E, Fernandez HF, Alyea EP, Hamadani M, Bashey A, Giralt SA, Leifer E, Geller N, Le-Rademacher J, 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 Clinical Trials Network (BMT CTN) 0901. Blood. 2015; 126.

21. Baron F, Labopin M, Ruggeri A, Mohty M, Sanz G, Milpied N, Bacigalupo A, Rambaldi A, Bonifazi F, Bosi A, Sierra J, Yakoub-Agha I, Santasusana JM, Gluckman E and Nagler A. Unrelated cord blood transplantation for adult patients with acute myeloid leukemia: higher incidence of acute graft-versus-host disease and lower survival in male patients transplanted with female unrelated cord blood-a report from Eurocord, the Acute Leukemia Working Party, and the Cord Blood Committee of the Cellular Therapy and Immunobiology Working Party of the European Group for Blood and Marrow Transplantation. Journal of hematology & oncology. 2015; 8:107.

22. Hiwarkar P, Qasim W, Ricciardelli I, Gilmour K, Quezada S, Saudemont A, Amrolia P and Veys P. Cord blood T cells mediate enhanced antitumor effects compared with adult peripheral blood T cells. Blood. 2015; 126:2882-2891.

23. Barker J and Hanash A. Cord blood T cells are “completely different”. Blood. 2015; 126:2778-2779.

24. Oran B, Wagner JE, DeFor TE, Weisdorf DJ and Brunstein CG. Effect of conditioning regimen intensity on acute myeloid leukemia outcomes after umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2011; 17:1327-1334.

25. Pascal L, Mohty M, Ruggeri A, Tucunduva L, Milpied N, Chevallier P, Tabrizi R, Labalette M, Gluckman E, Labopin M and Yakoub-Agha I. Unrelated cord blood transplantation for adult patients transplanted with female unrelated cord blood-a report from the Acute Leukemia Working Party (ALWP) of the European Group for Blood and Marrow Transplantation (EBMT). Journal of hematology & oncology. 2015; 8:107.

26. Pascal L, Tucunduva L, Ruggeri A, Blaise D, Ceballos P, Chevallier P, Cornelissen J, Maillard N, Tabrizi R, Petersen E, Linkesch W, Sengelov H, Kenzey C, Pagliuca A, Holler E, Einsele H, et al. Impact of ATG-containing myeloablative conditioning regimens on the outcome of patients undergoing unrelated single-unit cord blood transplantation for hematological malignancies. Bone marrow transplantation. 2015; 50:45-50.

27. Shimony O, Nagler A, Gellman YN, Refaeli E, Rosenblum N, Eshkar-Sebban L, Yerushalmi R, Shimoni A, Lytton
SD, Stanevsky A, Or R and Naor D. Anti-T lymphocyte globulin (ATG) induces generation of regulatory T cells, at least part of them express activated CD44. J Clin Immunol. 2012; 32:173-188.

28. Ehx G, Hannon M, Beguin Y, Humblet-Baron S and Baron F. Validation of a multicolor staining to monitor phosphoSTAT5 levels in regulatory T-cell subsets. Oncotarget. 2015; 6: 43255-43266. doi: 10.18632/oncotarget.6486.

29. Socie G, Schmoor C, Bethge WA, Ottinger HD, Stelljes M, Zander AR, Volin L, Ruutu T, Heim DA, Schwerdtfeger R, Kolbe K, Mayer J, Maertens JA, Linkesch W, Holler E, Koza V, et al. Chronic graft-versus-host disease: long-term results from a randomized trial on graft-versus-host disease prophylaxis with or without anti-T-cell globulin ATG-Fresenius. Blood. 2011; 117:6375-6382.

30. Baron F, Labopin M, Blaise D, Lopez-Corral L, Vigouroux S, Craddock C, Attal M, Jindra P, Goker H, Socie G, Chevallier P, Browne P, Sandstedt A, Duarte RF, Nagler A and Mohty M. Impact of in vivo T-cell depletion on outcome of AML patients in first CR given peripheral blood stem cells and reduced-intensity conditioning allo-SCT from a HLA-identical sibling donor: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Bone marrow transplantation. 2014; 49:389-396.

31. Munoz J, Shah N, Rezvani K, Hosing C, Bollard CM, Oran B, Olson A, Popat U, Molldrem J, McNiece IK and Shpall EJ. Concise review: umbilical cord blood transplantation: past, present, and future. Stem cells translational medicine. 2014; 3:1435-1443.

32. Baron F, Ruggeri A and Nagler A. Methods of ex vivo expansion of human cord blood cells: challenges, successes and clinical implications. Expert review of hematology. 2016:1-18.

33. Mehta RS, Di Stasi A, Andersson BS, Nieto Y, Jones R, de Lima M, Hosing C, Popat U, Kebrina P, Oran B, Alousy A, Rezvani K, Qazilbash M, Bashir Q, Bollard C, Cooper L, et al. The development of a myeloablative, reduced-toxicity, conditioning regimen for cord blood transplantation. Clinical lymphoma, myeloma & leukemia. 2014; 14:e1-5.

34. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, Lerner KG and Thomas ED. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974; 18:295-304.

35. Baron F, Labopin M, Niederwieser D, Vigouroux S, Cornelissen JJ, Malm C, Vindelov LL, Blaise D, Janssen JJ, Petersen E, Socie G, Nagler A, Rocha V and Mohty M. Impact of graft-versus-host disease after reduced-intensity conditioning allogeneic stem cell transplantation for acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European group for blood and marrow transplantation. Leukemia. 2012; 26:2462-2468.

36. Baron F, Labopin M, Peniket A, Jindra P, Afanasyev B, Sanz MA, Deconinck E, Nagler A and Mohty M. Reduced-intensity conditioning with fludarabine and busulfan versus fludarabine and melphalan for patients with acute myeloid leukemia: a report from the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. Cancer. 2015; 121:1048-1055.

37. Czerw T, Labopin M, Schmid C, Cornelissen JJ, Chevallier P, Blaise D, Kuball J, Vigouroux S, Garban F, Lioure B, Fegueux N, Clement L, Sandstedt A, Maertens J, Guillerm G, Bordessoule D, et al. High CD3+ and CD34+ peripheral blood stem cell grafts content is associated with increased risk of graft-versus-host disease without beneficial effect on disease control after reduced-intensity conditioning allogeneic transplantation from matched unrelated donors for acute myeloid leukemia - an analysis from the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. Oncotarget. 2016:doi: 10.18632/oncotarget.8463.

38. Ruggeri A, Labopin M, Ciceri F, Mohty M and Nagler A. Definition of GvHD-free, relapse-free survival for registry-based studies: an ALWP-EBMT analysis on patients with AML in remission. Bone marrow transplantation. 2016; 51:610-611.

39. Lewis S and Clarke M. Forest plots: trying to see the wood and the trees. BMJ. 2001; 322:1479-1480.