Comparison of reduced-intensity conditioning regimens in patients with acute lymphoblastic leukemia > 45 years undergoing allogeneic stem cell transplantation – a retrospective study by the Acute Leukemia Working Party of EBMT

Zinaida Peric [1], Myriam Labopin [2], Christophe Peczynski[2], Emmanuelle Polge [2], Jan Cornelissen [3], Ben Carpenter[4], Mike Potter[5], Ram Malladi[6], Jenny Byrne [7], Harry Schouten[8], Nathalie Fegueux[9], Gerard Socié[10], Montserrat Rovira[11], Jurgen Kuball[12], Maria Gilleece[13], Sebastian Giebel[14], Arnon Nagler[15], Mohamad Mohty[16]

1 University Hospital Centre Zagreb, School of Medicine, University of Zagreb, Zagreb, Croatia
2 EBMT Paris study office / CEREST-TC, Saint Antoine Hospital, INSERM UMR 938, University Pierre et Marie Curie, Paris, France
3 Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands
4 University College London Hospital, London, United Kingdom
5 Royal Marsden Hospital, London, United Kingdom
6 University Hospital Birmingham NHS Trust, Queen Elizabeth Medical Centre, Edgbaston, Birmingham, United Kingdom
7 Nottingham University Hospital, Nottingham, United Kingdom
8 University Hospital Maastricht, Maastricht, The Netherlands
9 University Hospital Centre Lapeyronie, Montpellier, France
10 Hospital St. Louis, AP-HP, University of Paris, Paris, France
11 Hospital Clinic, Institute of Hematology & Oncology, Barcelona, Spain
12 University Medical Centre, Utrecht, The Netherlands
13 Leeds Teaching Hospitals Trust, Leeds, United Kingdom
14 Maria Sklodowska-Curie Institute – Oncology Center, Gliwice Branch, Gliwice, Poland
15 Hematology Division, Chaim Sheba Medical Centre, Tel-Hashomer, Israel and EBMT Paris study office/CEREST-TC, Saint Antoine Hospital, INSERM UMR 938, University Pierre et Marie Curie, Paris, France
16 Saint Antoine Hospital, University Pierre et Marie Curie, Paris, France

* Correspondence and reprint requests: Zinaida Peric, MD, PhD, University Hospital Centre Zagreb, School of Medicine, University of Zagreb, Kispaticeva 12, Zagreb, Croatia, phone number: +385 99 845 0771

e-mail address: zinaida.peric@mef.hr

Financial disclosure statement

All authors declare no support from any organization for the submitted work; no financial relationships with any organizations that might have an interest in the submitted work; no other relationships or activities that could appear to have influenced the submitted work.

Abstract

The optimal reduced-intensity conditioning (RIC) for patients with acute lymphoblastic leukemia (ALL) undergoing allogeneic stem cell transplantation (allo-HSCT) remains unclear. We retrospectively analyzed 417 patients > 45 years with ALL in first complete remission who underwent a matched-sibling or unrelated allo-HSCT and compared outcomes between fludarabine/busulfan (FLUBU, n=127), fludarabine/melphalan (FLUMEL, n=190) and fludarabine-TBI (FLUTBI, n=100) conditioning. At 2 years, there were no differences between the groups in terms of cumulative incidence (CI) of relapse (40% for FLUBU vs 36% for FLUMEL vs 41% for FLUTBI, p=0.21); transplant-related mortality (TRM) (18% for FLUBU, 22% for FLUMEL, 14% for FLUTBI, p=0.09); overall survival (OS) (55% for FLUBU, 50% for FLUMEL, 60% for FLUTBI, p=0.62) or leukemia-free survival (LFS) (43% for FLUBU, 42% for FLUMEL, 45% for FLUTBI, p=0.99), but GVHD-relapse-free survival (GFRS) was significantly lower in the FLUTBI group than FLUBU and FLUMEL group (18% vs 35% vs 28%, p=0.02). However, this difference was lost in the multivariate analysis when adjusted for the in vivo T-cell depletion. Finally, the FLUMEL regimen was shown to be an independent risk factor for a higher TRM (HR 1.97, 95% CI 1.05-3.72, p=0.04). We conclude that the 3 most popular RIC regimens yield similar transplant outcomes.

Key words: ALL, reduced-intensity, allo-HSCT, retrospective, outcome

Introduction

Long-term outcomes of older adults with acute lymphoblastic leukemia (ALL) remain poor, with an estimated 5-year leukemia-free survival (LFS) of approximately 30-40% (1-3). These results have been obtained with chemotherapy alone and are partly due to the inability of older adults to tolerate intensive regimens used in pediatric and young adult populations. The use of conventional myeloablative allogeneic hematopoietic stem cell transplantation (allo-HSCT) has been shown to improve survival rates in adults by 45-75% (4,5). However, transplant-related mortality (TRM) after myeloablative allo-HSCT is substantial, ranging between 33 and 58% (6), increases with age, and is higher for adults with impaired performance status (7,8). In such patients, reduced intensity conditioning (RIC) may offer the chance of a potentially curative strategy by obtaining a graft-versus-leukemia effect without the associated toxicities of myeloablative conditioning (MAC). On the other hand, the risk of relapse after RIC regimens may be greater than that after MAC regimens (8-10).

Although several RIC regimens have been developed over the last decades, their cytotoxic and immunosuppressive effects are different, and this may influence transplant outcome. However, to date there have been no large prospective studies comparing outcomes of different RIC regimens in patients with acute leukemias, and the optimal RIC regimen in allo-HSCT remains unclear. The most widely used RIC regimens are fludarabine with intermediate doses of busulfan (6.4 mg/kg), fludarabine with intermediate doses of melphalan (140 mg/m^2) and fludarabine with low-dose total-body irradiation (TBI, 2 Gy). Several retrospective studies have compared these regimens, but with contradictory results (11,12). This is probably due to small population numbers, different diseases being analyzed together and neither age limit for enrollment nor dosage of drugs in regimens being fixed. Furthermore, these studies focused mostly on acute myeloid leukemia and included only small numbers of ALL patients.

We therefore took advantage of the European Society for Blood and Marrow Transplantation (EBMT) dataset, and retrospectively compared outcomes of these three most popular RIC conditioning regimens following allo-HSCT from a matched sibling donor or an unrelated donor in a large homogeneous population of ALL patients aged 45 years or older undergoing transplant in first complete remission (CR1).

Patients and methods

Study design and data collection

This is a registry based retrospective study. Data were provided and the study design was approved by the Acute Leukemia Working Party (ALWP) of the EBMT group registry, in accordance with the EBMT guidelines for retrospective studies. The EBMT is a voluntary working group of more than 600 transplant centers which are required to report all consecutive stem cell transplantations and follow-ups once a year. Audits are routinely performed to determine the accuracy of the data. Since 1990, patients have been able to provide informed consent to authorize the use of their transplant information for research purposes. The ALWP of the EBMT granted ethical approval for this study.

Patient selection

Patients were selected according to the following criteria: (1) aged 45 years and older at the time of transplantation, (2) a diagnosis of ALL, with available data on the immunophenotype and Ph-positivity, (3) in CR1 (4) initial allo-HSCT between 2005 and June 2016, (4) HLA-matched related or unrelated donor (fully matched or mismatched at one HLA locus), (5) received peripheral blood hematopoietic stem cells (PBSC), (6) underwent the RIC conditioning regimen. Patients who received a previous allo-HSCT or T-depleted grafts were excluded. Indication for RIC allo-SCT depended on each center's policy. The RIC regimen was defined as the use of fludarabine associated with intermediate doses of intravenous busulfan (FLUBU, busulfan at 6.4 mg/kg), intermediate doses of melphalan (FLUMEL, melphalan at 140 mg/m^2) or low-dose total body irradiation (FLUTBI; TBI at 2 Gy).

Endpoints and definitions

The primary endpoint was overall survival (OS). Secondary endpoints were cumulative incidences (CI) of relapse, transplant-related mortality (TRM), acute and chronic graft-versus-host disease (GVHD), leukemia-free survival (LFS) and graft-versus-host disease free, relapse-free survival (GRFS). Acute and chronic GVHD were graded according to previously published criteria (13,14). OS was defined as the probability of survival, TRM as death without evidence of relapse, LFS as survival with no evidence of relapse or disease progression. GRFS was defined as survival with no previous grade III–IV acute GVHD, no severe chronic GVHD and no relapse.

Statistical analysis

The main patient characteristics were compared using the Mann-Whitney test for quantitative variables and chi-square test or Fisher's exact test for categorical variables. Probabilities of OS, LFS and GRFS were estimated using the Kaplan-Meier method, and the differences between groups were compared using the log-rank test. GVHD, relapse and TRM were calculated using the cumulative incidence method and analyzed in a time-dependent fashion. Differences between groups were compared using the Gray's test. For acute and chronic GVHD or relapse, death of the patient was considered as a competing risk of the event. For TRM, the competing event was relapse. Factors differing between the groups in terms of distribution and factors significantly associated with the outcome were included in the multivariate analysis. Multivariate analyses were performed using the Cox proportional-hazard model. All tests were two-sided and *P* values < 0.05 were considered as indicating a statistically significant association. Analyses were performed using the R statistical software version 3.2.3 (available online at http://www.R-project.org).

Results

Patient characteristics

A total of 417 patients was included in this study; 127 patients in the FLUBU group, 190 patients in the FLUMEL group and 100 patients in the FLUTBI group. Patient characteristics of each group are summarized in ***Table 1***. The median follow-up of patients was significantly longer (p=0.001) in the FLUTBI group (51 months, range, 34-69) than in the FLUBU group (35 months, range, 25-45) and FLUMEL group (23 months, range, 20-26). Patients in the FLUBU group were significantly older (median 59 years, range 45-71) than patients in the FLUMEL (median 54 years, range 45-74) and the FLUTBI (median 57 years, range 45-72) groups, (p=0.001). Incidence of Ph+ ALL was lower in the FLUMEL group compared to FLUBU or FLUTBI groups (52% vs 69%, p<0.001). Most patients in the FLUBU group received ATG (88%), while most of the FLUMEL patients received Campath (71%) as GVHD prophylaxis. Only 12% of the patients received in vivo T-cell depletion in the FLUTBI group (11 ATG and 1 Campath). The rest of the demographic and transplant characteristics were comparable between the 3 groups.

OS, LFS, relapse and TRM

At 2 years after transplantation, there was no significant differences in OS between the groups (***Figure 1A***, p=0.62) – namely; OS in the FLUBU group was 55%, (95%CI 45-65); 50% in the FLUMEL group (95%CI 42-59); and 60% in the FLUTBI group (95%CI 49-70). There was also no significant difference in LFS between the groups (p=0.99); (***Figure 1B***); 43% in the FLUBU group (95%CI 33-52); 42% in the FLUMEL group (95%CI 34-51) and 45% in the FLUTBI group (95%CI 35-56). Furthermore, there was no significant difference in the CI of relapse between the groups as shown in ***Figure 1C*** (p=0.21); it was 40 % in the FLUBU group (95%CI 30-49) at a median of 4.8 months (range, 1-49); 36% in the FLUMEL group (95%CI 28-44) at a median of 6 months (range, 2-32); and 41% in the FLUTBI group (95%CI 30-51) at a median of 3.7 months (range, 1-.31). Finally, TRM was also comparable between the groups (p=0.09) (***Figure 1D***); 18% in the FLUBU group (95%CI 11-26); 22% in the FLUMEL group (95%CI 16-29) and 14% in the FLUTBI group (95%CI, 8-22). The most frequent cause of death in all groups was relapse; 42% in the FLUBU group, 41% in the FLUMEL group and 60% in the FLUTBI group followed by GVHD; 28% in the FLUBU group, 14% in the FLUMEL group and 16% in the FLUTBI group. The CI of death associated with infection was highest in the FLUMEL group (11%, 95%CI, 7-16), followed by the FLUBU group (7%, 95% CI, 3-13) and lowest in the FLUTBI group (6%, 95% CI, 2-12).

Acute and chronic GVHD, GRFS

All groups had a similar CI of grade II-IV acute GVHD; 23% in the FLUBU group (95%CI 16-31), 27% in the FLUMEL group (95%CI 20-33) and 32% in the FLUTBI group (95%CI 23-42) (p=0.33). However, the CI of extensive chronic GVHD was significantly higher in the FLUTBI group (39%, 95%CI 29-50) in comparison to FLUBU (16%, 95%CI 9-23) and FLUMEL group (12%, 95%CI 7-18) (p=0.001) (***Figure 1E***). This difference resulted in significantly lower GFRS in the FLUTBI group (18%, 95%CI 10-26) compared to the FLUBU (35%, 95%CI 25-44) and the FLUMEL groups (28%, 95%CI 20-36) (p=0.02) (***Figure 1F***).

Multivariate analysis

The results of multivariate analysis are shown in ***Table 2***. On adjustment for patient-, disease- and transplant related-factors that were different among groups, a worse OS was associated only with older age (hazard ratio (HR) 1.56, 95% CI 1.21-2.03, p=0.0007) and female gender of patient (HR 0.67, 95% CI 0.49-0.93, p=0.01). Furthermore, decreased LFS was associated only with older age of patient (HR 1.57, 95% CI 1.23-2.00, p=0.0003). The CI of relapse was increased in older patients (HR 1.4, 95% CI 1.05-1.87, p=0.02) and CMV positive patients. (HR 0.66, 95% CI 0.45-0.97, p=0.03). Finally, the TRM was higher in the FLUMEL group (HR 1.97, 95% CI 1.05-3.71, p=0.04), as well as in older patients (HR 2.08, 95% CI 1.37-3.15, p=0.0006) and patients receiving a transplant from an unrelated donor (HR 2.22, 95% CI 1.23-4.01, p=0.008). On multivariate analysis, there were no differences in CI of chronic GVHD and GRFS between the 3 conditioning regimens when adjusting for the use of in vivo T-cell depletion. The CI of chronic GVHD was higher with the use of unrelated donors (HR 2.00, 95% CI 1.33-3.02, p=0.0008), while lower for transplants from CMV positive donors (HR 0.66, 95% CI 0.45-0.98, p=0.04) and with the use of T-cell depletion (HR 0.44, 95% CI 0.27-0.73, p=0.001). Finally, the only significant factor associated with lower GRFS was older age of the patient (HR 1.53, 95% CI 1.23-1.90, p=0.0001).

Discussion

To our knowledge, this is the first study comparing outcomes of the most used RIC conditioning regimens in adults with ALL. We compared RIC allo-HSCT after FLUBU, FLUMEL and FLUTBI conditioning in 417 ALL patients in CR1 and found similar transplantation outcomes in terms of OS, LFS and relapse. However, lack of in-vivo T-cell depletion with the FLUTBI regimen yielded more cGVHD and a lower GRFS, while FLUMEL emerged as an independent predictor of TRM in the multivariate analysis.

Allo-HSCT in CR1 is still often offered to older adults with ALL who are not treated with pediatric-inspired regimens. These patients are usually not eligible for MAC either, therefore many older adults standardly undergo RIC allo-HSCT. This strategy is supported by several large retrospective studies, which compared RIC vs MAC allo-HSCT in ALL patients and found a reduction of TRM in the RIC group (7,8,15-17). Unfortunately, this did not translate into a significant difference in OS, due to the higher risk of relapse in the RIC group. However, these studies included heterogeneous patient populations and a wide variety of conditioning regimens which could confound true differences between conditioning regimen intensity. This also raises the question of whether the choice of a RIC regimen could impact long-term leukemic control differently and improve outcomes.

So far, the answer to this question has been based mostly on single institution studies reporting their outcomes with RIC allo-HSCT (18-22). These studies were rather heterogeneous, included only a small number of ALL patients or had looked at a variety of conditioning regimens, making results difficult to interpret. However, two of these studies are worth mentioning as they reported impressive outcomes, both with FLUMEL conditioning. The first study from the City of Hope group reported a 2-year OS of 61.5% in 24 ALL patients aged over 50 years, with compromised organ function or prior allo-HSCT, while the Korean group reported a 3-year OS of 64% in 37 ALL patients with similar characteristics (18, 19). Interestingly, this is in concordance with the results from a prospective UK NCRI UKALL14 study, reporting a 2-year OS of 63% in 186 patients aged 40 years or older after a FLU-MEL-alemtuzumab conditioning (23). We, on the other hand, analyzed a similarly large FLUMEL group of 190 patients and found a 2-year OS of 50%, lower than OS in the FLUTBI (60%) and FLUBU group (55%) (p=0.62). Better outcomes in previous studies are probably related to more uniformity in terms of conditions and better selection of patients.

Previous retrospective comparisons between different RIC regimens were done mostly between FLUMEL and FLUBU conditioning and almost exclusively in AML patients (24,25). In these large cooperative group studies, relapse incidence was lower in FLUMEL conditioning, but again with significantly higher TRM which led to similar OS in comparison to the FLUBU group. The only available previous study including ALL patients that has compared RIC regimens is a subgroup analysis of the MAC and RIC allo-HSCT comparison done by ALWP (8). Mohty et al. analyzed 43 FLUTBI, 23 FLUBU and 25 FLUMEL allo-HSCT in the RIC subgroup and reported comparable TRM and relapse at 2 years (23 vs. 18 vs. 23%, respectively for TRM, and 55 vs. 45 vs. 48%, respectively for relapse, p = NS). The incidences of TRM were comparable in our study in the univariate analysis (14% vs 18% vs 22% in FLUTBI vs FLUBU vs FLUMEL, respectively, p=0.09) but FLUMEL conditioning emerged as a risk factor for higher TRM in the multivariate analysis.

One criticism of RIC regimens is that many of them do not include TBI, which is thought to reduce the risk of CNS relapse in ALL (26). This finding is mostly based on MAC and RIC comparisons, where TBI is usually added to MAC regimens (16, 26). Moreover, a recent large CIBMTR study comparing myeloablative TBI- and busulfan-based regimens confirmed a protective role of TBI for relapse in a multivariate analysis (27). Furthermore, a multi-centric study coordinated by the Fred Hutchinson Cancer Research Center evaluated a FLUTBI RIC regimen in patients older than 50 years, with comorbidities or prior transplantation and found a remarkable 3-year OS of 62% for patients in CR1 with relapse ranging from 15% to 32% depending of the Ph+ status (20). This contrasts with our study where the addition of TBI did not provide better anti-leukemic control since there was no significant difference in relapse incidence between the FLUTBI group in comparison to FLUBU and FLUMEL groups (41% vs 40% vs 36%, p=0.21). However, the low dose of TBI used in this study (2Gy) may have been insufficient to protect against CNS relapse and also we have previously shown that there is wide variation in TBI delivery among the centers which leads to potential obstacles when analyzing TBI data (28,29).

PBSC is a common source of stem cells in RIC allo-HSCT and all patients in our study received PBSC. Previous data comparing BM and PBSC in ALL RIC patients are lacking and the only data available are from the AML setting or from analysis of AML and ALL together, with contradictory results. A large Centre for International Blood and Marrow Transplant Research (CIBMTR) study in AML patients found no differences between BM and PBSC outcomes in RIC allo-HSCT (30). On the contrary, a previous EBMT study of RIC-allo HSCT in AML and ALL patients, found higher OS, LFS and relapse incidence but at the expanse of more chronic GVHD after the use of PBSC compared to BM (31). In our study, the only significant difference between RIC regimens was found in the incidence of chronic GVHD (significantly higher in the FLUTBI compared to FLUBU and FLUMEL group; (39% vs 16% vs 12%, p=0.001). This led to a significantly lower GRFS in the FLUTBI group but the difference was lost on multivariate analysis when adjusted for the use of ATG or Campath, traditionally used in the FLUBU and FLUMEL conditioning. Most of the patients in our study who received the FLUTBI regimen (88%) did not receive ATG or Campath, and this highlights the importance of in-vivo T-cell depletion in RIC regimens, particularly when PBSCs are used.

It is generally accepted that old age itself is not a contraindication for RIC allo-HSCT in patients with good performance status. However, large registry studies have shown that, when stratified by age, patients older than 66 years have higher rates of TRM and decreased OS (32). Of course, the older population also has a worse performance status and more comorbidities which makes it difficult to discern whether age or performance status contribute more to poorer outcomes. Nevertheless, in our study increasing age emerged as the main risk factor for worse outcomes; it independently predicted higher rates of TRM and relapse and lower OS, LFS and GRFS. Therefore, our results support the finding that in older adults, age may still modify the impact of poor performance status, and transplant, even with RIC, should be undertaken with caution.

Despite comparable outcomes between RIC regimens, the outcomes reported in our study are still unsatisfactory, with comparable LFS of less than 50% in all groups (43% in FLUBU vs 42% in FLUMEL vs 45% in FLUTBI, p=0.99). This highlights the importance of developing strategies for preventing relapse after allo-HSCT. Minimal residual disease (MRD) has been shown to be the strongest predictor of outcome after allo-HSCT (33-37). Strategies to improve allo-HSCT outcome in MRD-positive patients include pre-transplant elimination of MRD with potent new drugs such as blinatumomab (38), pre-transplant adjustment of ATG doses based on lymphocyte counts (39), as well as post-transplant pre-emptive donor-lymphocyte infusion (DLI) (40). A step further is the prevention of relapse in MRD-negative high-risk patients and includes tyrosine kinase inhibitor (TKI) maintenance therapy in Ph-positive (41-43), or prophylactic DLI in Ph-negative patients. In relapsed patients, major improvements have been made with bispecific and drug-conjugated antibodies (blinatumomab and inotuzumab ozogamicin), while exciting new strategies include genetically-enginereed T-lymphocytes - the chimeric antigen receptor T-cells (CAR-T cells) (44-46).

Our analysis has some limitations, mainly due to its retrospective design and some significant differences between populations' characteristics. Furthermore, it was not possible to provide the details of comorbidities nor further information on MRD in patients before transplant, which could have affected transplant outcomes. Nevertheless, this is the largest study of ALL patients receiving RIC allo-HSCT reported so far, leading to some important conclusions.

In summary, the three most popular RIC preparative regimens (FLUBU, FLUMEL and FLUTBI) yield similar transplantation outcomes in adults with ALL. However, FLUMEL conditioning seems to be associated with higher transplant-related toxicity, while more chronic GVHD in the FLUTBI group is mainly related to the low use of in-vivo T-cell depletion.

Acknowledgements: Not applicable.

Financial disclosure: There are no financial disclosures to report.

Conflict of interest statement: The authors have no conflicts of interests to report.

Authors' contributions: Z.P. assembled and analyzed data and wrote the first version of the manuscript. A.N., S.G. and M.M. designed the study, supervised research, analyzed data, and helped with writing the manuscript. E.P., M.L. and C.P. assembled the data, performed statistical analysis and commented on the manuscript. All other co-authors collected data, recruited patients and helped with writing the manuscript. All authors approved submission of the manuscript for publication.

References

1. Swaika A, Frank RD, Yang D, Finn LE, Jiang L, Advani P, et al. Second primary acute lymphoblastic leukemia in adults: a SEER analysis of incidence and outcomes. *Cancer Med*. 2018;7(2):499–507.
2. Storring JM, Minden MD, Kao S, Gupta V, Schuh AC, Schimmer AD, et al. Treatment of adults with BCR-ABL negative acute lymphoblastic leukaemia with a modified paediatric regimen. *Br J Haematol*. 2009;146(1):76–85.
3. Huguet F, Leguay T, Raffoux E, Thomas X, Beldjord K, Delabesse E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. *J Clin Oncol*. 2009;27(6):911–918.
4. Goldstone AH, Richards SM, Lazarus HM, Tallman MS, Buck G, Fielding AK, et al. In adults with standard-risk acute lymphoblastic leukemia, the greatest benefit is achieved from a matched sibling allogeneic transplantation in first complete remission, and an autologous transplantation is less effective than conventional consolidation/maintenance chemotherapy in all patients: final results of the International ALL Trial (MRC UKALL XII/ECOG E2993). *Blood*. 2008;111(4):1827–1833.
5. Gokbuget N, Kneba M, Raff T, Trautmann H, Bartram CR, Arnold R, et al. Adult patients with acute lymphoblastic leukemia and molecular failure display a poor prognosis and are candidates for stem cell transplantation and targeted therapies. *Blood*. 2012;120(9):1868–1876.
6. Marks DI, Perez WS, He W, Zhang MJ, Bishop MR, Bolwell BJ, et al. Unrelated donor transplants in adults with Philadelphia-negative acute lymphoblastic leukemia in first complete remission. *Blood*. 2008;112(2):426–434.
7. Roth-Guepin G, Canaani J, Ruggeri A, Labopin M, Finke J, Cornelissen JJ, et al. Allogeneic stem cell transplantation in acute lymphoblastic leukemia patients older than 60 years: a survey from the acute leukemia working party of EBMT. *Oncotarget*. 2017;8(68):112972-112979.
8. Mohty M, Labopin M, Volin L, Gratwohl A, Socie G, Esteve J, et al. Reduced-intensity versus

conventional myeloablative conditioning allogeneic stem cell transplantation for patients with acute lymphoblastic leukemia: a retrospective study from the European Group for Blood and Marrow Transplantation. *Blood.* 2010;116(22):4439–4443.

9. Aoudjhane M, Labopin M, Gorin NC, Shimoni A, Ruutu T, Kolb HJ, 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 (12):2304-2312.
10. Martino R, Iacobelli S, Brand R, Jansen T, van Biezen A, Finke J, et al. Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA- identical sibling donors in myelodysplastic syndromes. *Blood.* 2006; 108 (3):836-846.
11. Shimoni A, Hardan I, Shem-Tov N, Rand A, Herscovici C, Yerushalmi R, et al. Comparison between two fludarabine-based reduced-intensity conditioning regimens before allogeneic hematopoietic stem-cell transplantation: fludarabine/ melphalan is associated with higher incidence of acute graft-versus-host disease and non-relapse mortality and lower incidence of relapse than fludarabine/busulfan. *Leukemia*. 2007;21(10):2109-2116.
12. Damlaj M, Alkhateeb HB, Hefazi M, Partain DK, Hashmi S, Gastineau DA, et al. Fludarabine-busulfan reduced-intensity conditioning in comparison with fludarabine-melphalan is associated with increased relapse risk in spite of pharmacokinetic dosing. *Biol Blood Marrow Transplant.* 2016; 22 (8):1431-1439.

13. Glucksberg H, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, et al. Clinical manifestations of graft- versus-host disease in human recipients of marrow from HL-A- matched sibling donors. Transplantation. 1974; 18 (4):295-304.
14. Filipovich AH, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, 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(12):945-956.
15. Marks DI, Wang T, Perez WS, Antin JH, Copelan E, Gale RP, et al. The outcome of full-intensity and reduced-intensity conditioning matched sibling or unrelated donor transplantation in adults

with Philadelphia chromosome-negative acute lymphoblastic leukemia in first and second complete remission. *Blood*. 2010;116(3):366– 374.

16. Bachanova V, Marks DI, Zhang MJ, Wang H, de Lima M, Aljurf MD, et al. Ph+ ALL patients in first complete remission have similar survival after reduced intensity and myeloablative allogeneic transplantation: impact of tyrosine kinase inhibitor and minimal residual disease. *Leukemia*. 2014;28(3):658–665.
17. Tanaka J, Kanamori H, Nishiwaki S, Ohashi K, Taniguchi S, Eto T, et al. Reduced-intensity vs myeloablative conditioning allogeneic hematopoietic SCT for patients aged over 45 years with ALL in remission: a study from the Adult ALL Working Group of the Japan Society for Hematopoietic Cell Transplantation (JSHCT). *Bone Marrow Transplant*. 2013;48(11):1389–1394.
18. Stein AS, Palmer JM, O'Donnell MR, Kogut NM, Spielberger RT, Slovak ML, et al. Reduced-intensity conditioning followed by peripheral blood stem cell transplantation for adult patients with high- risk acute lymphoblastic leukemia. *Biol Blood Marrow Transplant*. 2009;15(11):1407–1414.
19. Cho BS, Lee S, Kim YJ, Chung NG, Eom KS, Kim HJ, et al. Reduced-intensity conditioning allogeneic stem cell transplantation is a potential therapeutic approach for adults with high-risk acute lymphoblastic leukemia in remission: results of a prospective phase 2 study. *Leukemia*. 2009;23(10):1763–1770.
20. Ram R, Storb R, Sandmaier BM, Maloney DG, Woolfrey A, Flowers MED, et al. Non-myeloablative conditioning with allogeneic hematopoietic cell transplantation for the treatment of high-risk acute lymphoblastic leukemia. *Haematologica*. 2011;96(8): 1113–1120.
21. Massenkeil G, Nagy M, Neuburger S, Tamm I, Lutz C, le Coutre P, et al. Survival after reduced-intensity conditioning is not inferior to standard high-dose conditioning before allogeneic haematopoietic cell transplantation in acute leukaemias. *Bone Marrow Transplant*. 2005;36(8):683–689.
22. Hamaki T, Kami M, Kanda Y, Yuji K, Inamoto Y, Kishi Y, et al. Reduced-intensity stem-cell transplantation for adult acute lymphoblastic leukemia: a retrospective study of 33 patients. *Bone Marrow Transplant*. 2005;35(6):549–556.
23. Okasha D, Kirkwood AA, Wrench B, Lawrie E, Zuborne Alapi K, Clifton-Hadley L, et al. Post-induction MRD predicts high relapse risk following reduced intensity conditioned allogeneic stem cel transplantation: a prospective study of adult ALL (UKALL 14, ISRCTN 66541317).

Haematologica. 2017;102 Suppl 2:326 (S802).

24. Baron F, Labopin M, Peniket A, Jindra P, Afanasyev B, Sanz MA, et al. 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 (7):1048-1055.
25. Kawamura K, Kako S, Mizuta S, Ishiyama K, Aoki J, Yano S, et al. Comparison of Conditioning with Fludarabine/Busulfan and Fludarabine/Melphalan in Allogeneic Transplantation Recipients 50 Years or Older. *Biol Blood Marrow Transplant*. 2017; 23(12):2079-2087.
26. Granados E, de La Camara R, Madero L, Diaz MA, Martin-Regueira P, Steegmann JL, et al. Hematopoietic cell transplantation in acute lymphoblastic leukemia: better long-term event-free survival with conditioning regimens containing total body irradiation. *Haematologica.* 2000;85(10):1060 – 1067.
27. Kebriaei P, Anasetti C, Zhang MJ, Wang HL, Aldoss I, de Lima M, et al. Intravenous busulfan compared with total body irradiation pretransplant conditioning for adults with acute lymphoblastic leukemia. *Biol Blood Marrow Transplant*. 2018; 24 (4):726-733.
28. Giebel S, Miszcyzk L, Slosarek K, Moukhtari L, Ciceri F, Esteve J, et al. Extreme heterogeneity of myeloablative total body irradiation techniques in clinical practice: a survey of the Acute Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Cancer*. 2014; 120(17):2760-2765.
29. Belkacemi Y, Labopin M, Giebel S, Loganadane G, Miszczyk L, Michallet M, et al. Single-Dose Daily Fractionation Is Not Inferior to Twice-a-Day Fractionated Total-Body Irradiation Before Allogeneic Stem Cell Transplantation for Acute Leukemia: A Useful Practice Simplification Resulting From the SARASIN Study. *Int J Radiat Oncol Biol Phys*. 2018;102(3):515-526.
30. Eapen M, Logan BR, Horowitz MM, Zhong X, Perales MA, Lee SJ, et al. Bone marrow or peripheral blood for reduced-intensity conditioning unrelated donor transplantation. *J Clin Oncol.* 2015;33(4):364–369.
31. Savani BN, Labopin M, Blaise D, Niederwieser D, Ciceri F, Ganser A, et al. Peripheral blood stem cell graft compared to bone marrow after reduced intensity conditioning regimens for acute leukemia: a report from the ALWP of the EBMT. *Haematologica*. 2016; 101(2):256-262.
32. Rosko A, Wang HL, de Lima M, Sandmaier B, Khoury HJ, Artz A, et al. Reduced intensity conditioned allograft yields favorable survival for older adults with B-cell acute lymphoblastic

leukemia. *Am J Hematol*. 2017;92(1):42–49.

33. Bassan R, Spinelli O, Oldani E, Intermesoli T, Tosi M, Peruta B, et al. Improved risk classification for risk-specific therapy based on the molecular study of minimal residual disease (MRD) in adult acute lymphoblastic leukemia (ALL). *Blood*. 2009;113(18):4153–4162.
34. Lussana F, Intermesoli T, Gianni F, Boschini C, Masciulli A, Spinelli O, et al. Achieving molecular remission before allogeneic stem cell transplantation in adult patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: impact on relapse and long-term outcome. *Biol Blood Marrow Transplant*. 2016; 22 (11):1983–1987.
35. Nishiwaki S, Imai K, Mizuta S, Kanamori H, Ohashi K, Fukuda T, et al. Impact of MRD and TKI on allogeneic hematopoietic cell transplantation for Ph+ ALL: a study from the adult ALL WG of the JSHCT. *Bone Marrow Transplant.* 2016;51(1):43–50.
36. Patel B, Rai L, Buck G, Richards SM, Mortuza Y, Mitchell W, et al. Minimal residual disease is a significant predictor of treatment failure in non T-lineage adult acute lymphoblastic leukaemia: final results of the international trial UKALL XII/ECOG2993. *Br J Haematol.* 2010;148(1):80–89.
37. Zhou Y, Slack R, Jorgensen JL, Wang SA, Rondon G, de Lima M, et al. The effect of peritransplant minimal residual disease in adults with acute lymphoblastic leukemia undergoing allogeneic hematopoietic stem cell transplantation. *Clin Lymphoma Myeloma Le*uk. 2014;14(4):319–326.

38. Gokbuget N, Zugmaier G, Klinger M, Kufer P, Stelljes M, Viardot A, et al. Long-term relapse-free survival in a phase 2 study of blinatumomab for the treatment of patients with minimal residual disease in B-lineage acute lymphoblastic leukemia. Haematologica. 2017;102(4):132–135.
39. Admiraal R, Nierkens S, de Witte MA, Petersen EJ, Fleurke GJ, Verrest L, et al. Association between anti-thymocyte globulin exposure and survival outcomes in adult unrelated haemopoietic cell transplantation: a multicentre, retrospective, pharmacodynamic cohort analysis. *Lancet Haematol*. 2017;4 (4):183-191.
40. Matsue K, Tabayashi T, Yamada K, Takeuchi M. Eradication of residual bcr-abl-positive clones by inducing graft-versus-host disease after allogeneic stem cell transplantation in patients with Philadelphia chromosome-positive acute lympho- blastic leukemia. *Bone Marrow Transplant.* 2002; 29(1):63-66.

41. Ribera JM, Oriol A, Morgades M, Montesinos P, Sarrà J, González- Campos J, et al. Treatment of high-risk Philadelphia chromosome- negative acute lymphoblastic leukemia in adolescents and

adults according to early cytologic response and minimal residual disease after consolidation assessed by flow cytometry: final results of the PETHEMA ALL-AR-03 trial. *J Clin Oncol.* 2014;32(15):1595– 1604.

42. Pfeifer H, Wassmann B, Bethge W, Dengler J, Bornhauser M, Stadler M, et al. Randomized comparison of prophylactic and minimal residual disease-triggered imatinib after allogeneic stem cell transplantation for BCR-ABL1-positive acute lymphoblastic leukemia. *Leukemia*. 2013;27(6):1254–1262.
43. Giebel S, Czyz A, Ottmann O, Baron F, Brissot E, Ciceri F, et al. Use of tyrosine kinase inhibitors to prevent relapse after allogeneic hematopoietic stem cell transplantation for patients with Philadelphia chromosome-positive acute lymphoblastic leukemia: a position statement of the Acute Leukemia Working Party of the European Society for Blood and Marrow Transplantation. *Cancer.* 2016;122(19):2941–2951.
44. Kantarjian H, Stein A, Gokbuget N, Fielding AK, Schuh AC, Ribera JM, et al. Blinatumomab versus chemotherapy for advanced acute lymphoblastic leukemia. *N Engl J Med*. 2017;376(9):836–847.
45. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leuke- mia. *N Engl J Med*. 2016;375(8):740–53.
46. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*. 2014;371(16):1507–1517.

TABLES

Table 1. Study population characteristics

Table 2. Multivariate analysis

FIGURES

Figure 1. Overall survival at 24 months **(A);** 55% (95%CI 45-65) in the FLUBU group; 50% (95%CI 42-59) in the FLUMEL group; and 60% (95%CI 49-70) in the FLUTBI group (p=0.62);

Leukemia-free survival at 24 months (**B);** 43% (95%CI 33-52) in the FLUBU group; 42% (95%CI 34-51) in the FLUMEL group and 45% (95%CI 35-56) in the FLUTBI group (p=0.99);

Cumulative incidence of relapse at 24 months (**C);** 40% (95%CI 30-49) in the FLUBU group; 36% (95%CI 28-44) in the FLUMEL group; and 41% (95%CI 30-51) in the FLUTBI group (p=0.21);

Cumulative incidence of transplant-related mortality **(D);** 18% (95%CI 11-26) in the FLUBU group; 22% (95%CI 16-29) in the FLUMEL group and 14% (95%CI, 8-22) in the FLUTBI group (p=0.09);

Cumulative incidence of extensive chronic GVHD **(E)**; 16% (95%CI 9-23) in the FLUBU group, 12% (95%CI 7-18) in the FLUMEL group and 39%, (95%CI 29-50) in the FLUTBI group (p=0.001).

GVHD-free-relapse-free survival at 24 months (**F);** 35% (95%CI 25-44) in the FLUBU group; 28% (95%CI 20-36) in the FLUMEL group and 18% (95%CI 10-26) in the FLUTBI group (p=0.01);

Characteristic	FLUBU group n=127	FLUMEL group n=190	FLUTBI group n=100	p value
Median follow-up in months (range)	35 (25-45)	2 (20-26)	51 (34-69)	**0.001**
Patient age median (range)	59 (45-71)	54 (45-74)	57 (45-72)	**<0.001**
Year of Tx_median (range)	2012 (2007-2016)	2013.5 (2006-2016)	2011 (2005-2016)	**<0.001**
Time from diagnosis to Tx in months, median (range)	6 (3-17)	6 (1-18)	6 (3-18)	**0.17**
Diagnosis				
B Ph-neg ALL	31 (24%)	48 (25%)	23 (23%)	
B Ph-pos ALL	88 (69%)	98 (52%)	66 (66%)	
T ALL	8 (6%)	44 (23%)	11 (11%)	**<0.001**
Donor				
Matched sibling	56 (49%)	71 (51%)	50 (54%)	
Unrelated 10/10	45 (40%)	52 (38%)	32 (35%)	
Unrelated 9/10	12 (11%)	15 (11%)	10 (11%)	
missing	14	52	8	**0.97**
Karnofsky score				
<90	37 (31%)	42 (24%)	32 (39%)	
>=90	83 (69%)	130 (76%)	51 (61%)	
missing	7	18	17	**0.06**
Patient gender				
male	50 (39%)	95 (50%)	50 (50%)	
female	77 (61%)	95 (50%)	50 (50%)	**0.14**
Donor gender				
male	71 (57%)	115 (61%)	51 (51%)	
female	54 (43%)	72 (39%)	48 (49%)	
missing	2	3	1	**0.26**
Patient CMV status				
negative	27 (28%)	74 (40%)	48 (38%)	
positive	71 (72%)	113 (60%)	79 (62%)	
missing	0	3	2	**0.12**
Donor CMV status				
negative	63 (51%)	107 (58%)	47 (47%)	
positive	60 (49%)	79 (42%)	52 (53%)	
missing	4	4	1	**0.24**
T-cell depletion in-vivo				
no	8 (6%)	34 (18%)	88 (88%)	

ATG	112 (88%)	21 (11%)	11 (11%)	
Campath	7 (6%)	135 (71%)	1 (1%)	**<0.001**

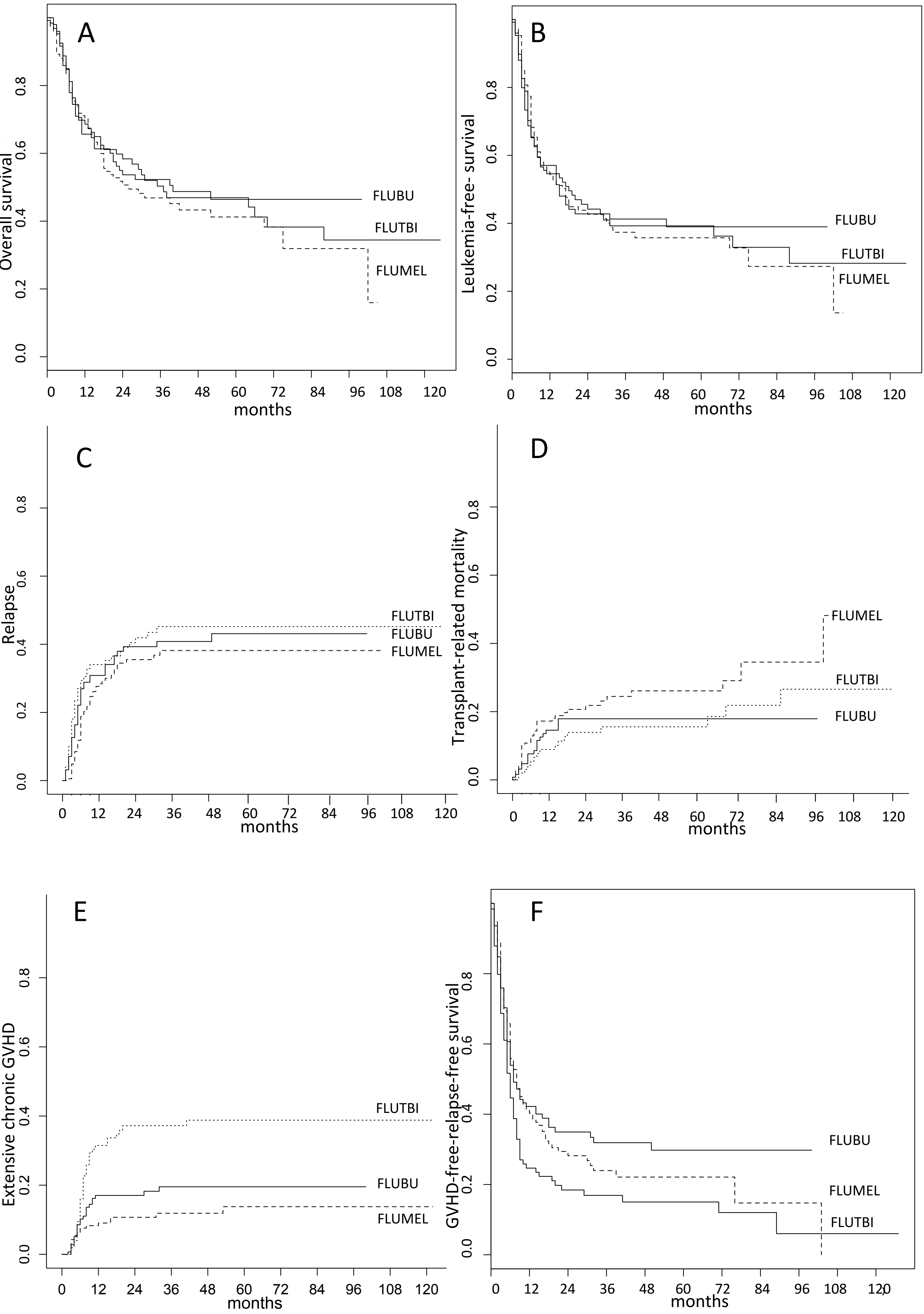
ALL-acute lymphoblastic leukemia, ATG-antithymocyte globulin

CMV-cytomegalovirus, TX -transplantation

Outcome	Variable	Hazard Ratio	95% Confidence interval	p-value
Overall survival	FLUBU (reference)	1		
	FLUMEL	1.33	0.85-2.08	0.21
	FLUTBI	0.87	0.46-1.66	0.67
	Age (per 10 years)	1.56	1.21-2.03	**0.0007**
	Year of Tx	1.01	0.95-1.07	0.87
	Time from diagnosis	0.99	0.94-1.06	0.88
	UD vs MSD	1.35	0.94-1.93	0.11
	Patient female	0.67	0.49-0.93	**0.01**
	Donor female	0.89	0.63-1.24	0.48
	Patient CMV positive	0.92	0.64-1.31	0.63
	Donor CMV positive	1.31	0.93-1.85	0.12
	TCD in-vivo	0.74	0.45-1.23	0.25
	centre			0.09
Leukemia-free survival	FLUBU (reference)	1		
	FLUMEL	1.23	0.82-1.85	0.31
	FLUTBI	1.06	0.59-1.92	0.85
	Age (per 10 years)	1.57	1.23-2.01	**0.0003**
	Year of Tx	0.98	0.93-1.03	0.42
	Time from diagnosis	0.99	0.93-1.05	0.74
	UD vs MSD	1.05	0.76-1.45	0.78
	Patient female	0.82	0.61-1.1	0.19
	Donor female	0.88	0.65-1.2	1.43
	Patient CMV positive	0.78	0.57-1.08	0.14
	Donor CMV positive	1.36	0.99-1.86	0.06
	TCD in-vivo	0.91	0.57-1.45	0.69
	centre			0.12
Cumulative incidence of relapse	FLUBU (reference)	1		
	FLUMEL	0.96	0.62-1.48	0.86
	FLUTBI	1.12	0.59-1.13	0.72
	Age (per 10 years)	1.4	1.05-1.87	**0.02**
	Year of Tx	0.98	0.92-1.05	0.57
	Time from diagnosis	1.01	0.94-1.08	0.86
	UD vs MSD	0.77	0.52-1.13	0.18
	Patient female	0.9	0.63-1.27	0.54
	Donor female	0.93	0.65-1.34	0.69
	Patient CMV positive	0.66	0.45-0.97	**0.03**
	Donor CMV positive	1.43	0.97-2.12	0.07
	TCD in-vivo	0.98	0.57-1.69	0.93
	centre			0.23

Cumulative incidence of transplant-related mortality	FLUBU (reference)	1		
	FLUMEL	1.97	1.05-3.72	**0.04**
	FLUTBI	0.9	0.36-2.25	0.81
	Age (per 10 years)	2.08	1.37-1.52	**0.0006**
	Year of Tx	0.97	0.88-1.06	0.52
	Time from diagnosis	0.93	0.84-1.05	0.23
	UD vs MSD	2.22	1.23-4.01	**0.008**
	Patient female	0.67	0.41-1.10	0.11
	Donor female	0.96	0.57-1.61	0.88
	Patient CMV positive	1.16	0.67-2.014	0.59
	Donor CMV positive	1.39	0.82-2.34	0.22
	TCD in-vivo	0.87	0.43-1.79	0.71
	centre			0.27
GVHD-free-relapse-free survival	FLUBU (reference)	1		
	FLUMEL	1.23	0.86-1.75	0.25
	FLUTBI	1.25	0.77-2.02	0.37
	Age (per 10 years)	1.53	1.23-1.90	**0.0001**
	Year of Tx	0.98	0.93-1.03	0.41
	Time from diagnosis	0.98	0.93-1.03	0.46
	UD vs MSD	1.11	0.82-1.50	0.49
	Patient female	0.82	0.63-1.06	0.12
	Donor female	0.95	0.72-1.25	0.73
	Patient CMV positive	0.85	0.64-1.13	0.27
	Donor CMV positive	1.03	0.77-1.37	0.86
	TCD in-vivo	0.73	0.50-1.07	0.11
	centre			0.22

CMV-cytomegalovirus, GVHD-graft.-versus-host disease, MSD-matched sibling donor
Tx-transplantation, UD-unrelated donor, TCD-T-cell depletion


A
Overall survival
months
FLUBU
FLUTBI
FLUMEL
B
Leukemia-free- survival
months
FLUBU
FLUTBI
FLUMEL
C
Relapse
months
FLUTBI
FLUBU
FLUMEL
D
Transplant-related mortality
months
FLUMEL
FLUTBI
FLUBU
E
Extensive chronic GVHD
months
FLUTBI
FLUBU
FLUMEL
F
GVHD-free-relapse-free survival
months
FLUBU
FLUMEL
FLUTBI