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ORIGINAL ARTICLE

# 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

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The impact of *in vivo* T-cell depletion on transplantation outcomes in patients transplanted with reduced-intensity conditioning (RIC) remains controversial. This study assessed the outcome of 1250 adult patients with *de novo* AML in first CR (CR1) given PBSC from HLA-identical siblings after chemotherapy-based RIC. A total of 554 patients did not receive any form of *in vivo* T-cell depletion (control group), whereas antithymocyte globulin (ATG) and alemtuzumab were given in 444 and 252 patients, respectively. The incidences of grade II-IV acute GVHD were 21.4, 17.6 and 10.2% in control, ATG and alemtuzumab patients, respectively ($P<0.001$). In multivariate analysis, the use of ATG and the use of alemtuzumab were each associated with a lower risk of chronic GVHD ($P<0.001$ each), but a similar risk of relapse, and of nonrelapse mortality, and similar leukemia-free survival and OS. Further, among patients given BU-based RIC, the use of $<6$ mg/kg ATG did not increase the risk of relapse (hazard ratio, HR = 1.1), whereas there was a suggestion for higher relapse risk in patients given $\geqslant 6$ mg/kg ATG (HR = 1.4, $P=0.08$). In summary, these data suggest that a certain amount of *in vivo* T-cell depletion can be safely used in the conditioning of AML patients in CR1 given PBSC after chemotherapy-based RIC.

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**Keywords:** reduced-intensity conditioning; AML; GVHD; ATG; alemtuzumab

## INTRODUCTION

The use of PBSC instead of BM in patients receiving grafts from HLA-matched donors after myeloablative conditioning has been associated with faster hematological recovery, lower relapse risk in patients with advanced disease (due to higher immune-mediated graft-versus-tumor (GVT) effects), but also higher incidence extensive chronic GVHD.[1–4] These observations prompted several groups of investigators to study *in vivo* T-cell depletion of the graft with antithymocyte globulin (ATG) or alemtuzumab as a way to reduce severe GVHD in patients given PBSC after high-dose myeloablative conditioning regimen.[5–7] These studies demonstrated that the use of ATG or alemtuzumab was successful at preventing severe GVHD without apparently increasing the relapse incidence (RI).[5–7] In contrast to patients given grafts after myeloablative conditioning who benefit from both the high-dose chemo/radiotherapy given as part of the conditioning regimen and the GVT effect for tumor eradication, patients given grafts after reduced-intensity conditioning regimen (RIC) rely mainly on the GVT effect for tumor eradication.[8–13] Thus, given the tight association between occurrence of GVHD and the GVT effect,[14–18] one might hypothesized that *in vivo* T-cell depletion in the RIC setting might be detrimental because of high risk of tumor relapse. In agreement with this hypothesis, a recent study from the Center for International Blood and Marrow Transplant Research (CIBMTR) observed that that *in vivo* T-cell depletion with ATG or alemtuzumab increased the RI and decreased disease-free survival in a cohort of 1676 patients with various hematological malignancies given BM or PBSC from HLA-matched or HLA-mismatched related or unrelated donors after chemotherapy-based RIC.[19] This study aimed to investigate these findings in a more homogeneous cohort of 1250 adult patients with *de novo* AML in first CR (CR1) given PBSC from HLA-identical siblings after chemotherapy-based RIC.

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## PATIENTS AND METHODS

### Data collection

This was a retrospective study performed by the Acute Leukemia Working Party (ALWP) of the European group for Blood and Marrow Transplantation (EBMT) group. 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 SCT and follow-up. The scientific board of the Acute Leukemia Working Party of EBMT approved this study. Data of adult *de novo* AML patients in CR1 at transplantation and given G-CSF-mobilized PBSC from HLA-identical siblings between 2000 and 2011 after chemotherapy-based RIC were included. Patients given *ex vivo* T-cell depleted grafts were excluded. Grading of acute and chronic GVHD was performed using established criteria.[20] For the purpose of this study, all necessary data were prospectively collected according to the EBMT guidelines and using the EBMT Minimum Essential Data forms. List of institutions reporting data included in this study is provided in the supplemental data.

### Statistical analysis

Data from all patients meeting the inclusion/exclusion criteria were included in the analyses. Start time was date of transplant for all end points. To evaluate probability of relapse (RI), patients dying either from direct toxicity of the procedure or from any other cause not related to leukemia were censored. The nonrelapse mortality (NRM) was defined as death while in CR. Patients were censored at the time of relapse or of the last follow-up. Cumulative incidence curves were used for RI and NRM in a competing risk setting, as death and relapse were competing together.[21]

For estimating of the cumulative incidence of chronic GVHD, death was considered as a competing event. OS and leukemia-free (LFS) survival rates (starting from date of transplant) were calculated using the Kaplan–Meier estimates. Univariate analyses were done using the Gray's test for cumulative incidence functions and the 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. 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. Factors included in the Cox models included the use of ATG in the conditioning (and dose of ATG in the models for patients given BU-based RIC), the use of alemtuzumab in the conditioning (and dose of alemtuzumab in the models for patients given melphalan-based RIC), patient age, year of transplantation $>$ median, time from diagnosis to transplantation $>$ median, female donor to male recipient versus other gender combinations, donor and recipient CMV serostatus, poor risk cytogenetic or presence of an internal-tandem duplication of Fms-like tyrosine kinase 3 (FLT3-ITD) and center activity. 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. The Bonferroni correction was applied for comparisons when ATG and alemtuzumab patients were simultaneously compared with controls. Statistical analyses were performed with SPSS 19 (SPSS Inc, Chicago, IL, USA) and R 2.13.2 (R Development Core Team, Vienna, Austria) software packages.

## RESULTS

### Patients and conditioning

Data from 1250 patients given PBSC from HLA-identical siblings were included in the current analysis (Table 1). Briefly, 554 patients (44%) did not receive any *in vivo* T-cell depletion (control patients), whereas ATG and alemtuzumab were given in 444 (36%) and 252 (20%) patients, respectively. Centers that used ATG were mainly located in France (231 patients (52%)), whereas centers that used alemtuzumab were mainly located in UK (212 patients (84%)). The proportion of patients with poor risk cytogenetics or Fms-like tyrosine kinase 3-internal-tandem duplication was higher in ATG patients (23%) than in control (17%) or alemtuzumab (18%) patients ($P=0.04$). RIC consisted of BU-containing regimens in 51% of control patients, 88% of ATG patients and 16% of alemtuzumab patients, whereas 24% of control patients, 6% of ATG patients and 83% of alemtuzumab patients received a melphalan-based RIC ($P<0.0001$). We did not have complete data on the use of pre-emptive DLI in the registry but at least 15 (5%) control patients, 39 (14%) ATG patients and 66 (36%) alemtuzumab patients received pre-emptive DLI (defined as DLI given before/without AML relapse). Finally, there were a higher proportion of patients transplanted in low-activity centers[22] (arbitrarily defined as centers that contributed for $\leqslant 10$ patients in the current study) among control patients (55%) than among ATG (43%) or alemtuzumab (44%) patients ($P<0.001$).

### Impact of type of chemotherapy-based RIC on allo-SCT outcomes among control patients

Given that the distribution of RIC regimens used varied among control, ATG and alemtuzumab patients, we first analyzed the impact of the type of conditioning regimen (melphalan-based versus BU-based versus other) on transplantation outcomes among control patients (not given *in vivo* T-cell depletion). Three-year incidences ($\pm$ s.e.) of RI and NRM were $27\pm4\%$ and $22\pm4\%$, respectively, in patients given melphalan-based RIC, $30\pm2\%$ and $17\pm3\%$, respectively, in those given BU-based RIC and $35\pm4\%$ and $13\pm3\%$, respectively, in those given other chemotherapy-based RIC (global *P*-values for RI and NRM: $P=0.13$ and $P=0.18$, respectively) (Figure 1). Three-year LFS and OS were $49\pm5\%$ and $52\pm5\%$, respectively, in patients given melphalan-based RIC $53\pm3\%$ and $55\pm3\%$, respectively, in those given BU-based RIC and $51\pm5\%$, and $60\pm5\%$, respectively, in those given other chemotherapy-based RIC (global *P*-values for LFS and OS: $P=0.99$ and $P=0.37$, respectively) (Figure 1).

### Impact of *in vivo* T-cell depletion in the general population

*Engraftment and GVHD*. Five control patients (0.9%), four ATG patients (0.9%) but no alemtuzumab patients failed to engraft ($P=0.32$). Secondary graft rejection occurred in 1 (0.18%), 1 (0.23%) and 1 (0.4%) control, ATG and alemtuzumab patients, respectively. The 100-day incidences of grade II-IV acute GVHD in control, ATG and alemtuzumab patients were 21.4%, 17.6% ($P=0.13$ in comparison with control patients) and 10.2% ($P<0.0001$ in comparison with control patients and $P=0.01$ in comparison with ATG patients), respectively. The 100-day incidences of grade III-IV acute GVHD in control, ATG and alemtuzumab patients were 9.6%, 8.8% ($P=0.67$ in comparison with control patients) and 1.6% ($P<0.0001$ in comparison with control patients and $P<0.0001$ in comparison with ATG patients), respectively. In multivariate analysis, the only factor significantly associated with a low risk of grade II-IV acute GVHD was the use of alemtuzumab (hazard ratio, HR = 0.4; 95% CI: 0.3–0.7, $P<0.001$). With a median follow-up of 28 months (range, 1–138 months), 3-year cumulative incidences of chronic and extensive chronic GVHD were $57\pm2\%$ and $38\pm3\%$, respectively, in control patients, $39\pm3\%$ ($P<0.001$ in comparison with control patients) and $19\pm2\%$ ($P<0.001$ in comparison with control patients), respectively, in ATG patients and $37\pm3\%$ ($P<0.001$ in comparison with control patients and $P=0.31$ in comparison with ATG patients) and $10\pm2\%$ ($P<0.001$ in comparison with control patients and $P=0.006$ in comparison with ATG patients), respectively, in alemtuzumab patients (Figure 2). In multivariate analysis, the use of ATG and the use of alemtuzumab were each associated with a lower risk of chronic GVHD (HR = 0.6 (95% CI, 0.5–0.8), $P<0.001$ and HR = 0.5 (95% CI, 0.4–0.6), $P<0.001$, respectively] and a lower risk of extensive chronic GVHD (HR = 0.5 (95% CI, 0.3–0.6), $P<0.001$ and HR = 0.2 (95% CI, 0.1–0.4), $P<0.001$, respectively). No other factors were associated with the risk of chronic GVHD, while longer time from diagnosis to transplantation was associated with a lower risk of extensive chronic GVHD.

*RI, NRM, OS and LFS*. Three-year cumulative incidences of RI and NRM were $31\pm2\%$ and $17\pm2\%$, respectively, in control patients, $35\pm2\%$ ($P=0.26$ in comparison with control group) and $13\pm2\%$

**Table 1.** Patients and transplant characteristics

| | *Control* (n = *554)* | *ATG* (n = *444)* | *Alemtuzumab* (n = *252)* | *Global* P-*value* [a] |
|---|---|---|---|---|
| Median patient age, y (range) | 56 (19–76) | 56 (19–74) | 54 (23–70) | 0.21 |
| Median year of SCT, y (range) | 2007 (00–11) | 2008 (00–11) | 2008 (00–11) | 0.01 |
| Recipient gender, % M | 56 | 53 | 47 | 0.05 |
| Donor gender, % M | 54 | 56 | 56 | 0.81 |
| Female donor/male recipient, % pts | 23.4 | 21.9 | 19.5 | 0.48 |
| Median WBC at diagn $\times 10^9$/L (range) | 12.9 (0.1–700) | 9.3 (0.2–879) | 13 (0.1–274) | 0.08 |
| Median time form diagn to SCT, d (range) | 154 (54–874) | 166 (63–762) | 158 (70–425) | 0.05 |
| Patient CMV seropositivity, *n* (%) | 194 (72) | 122 (66) | 64 (60) | 0.05 |
| Donor CMV seropositivity, *n* (%) | 186 (69) | 112 (61) | 60 (55) | 0.02 |
| *Cytogenetics* n (%) | | | | |
| Good risk[b] | 14 (2.5) | 15 (3.4) | 5 (2.0) | |
| Intermediate risk[c] | 219 (39.5) | 193 (43.5) | 110 (43.7) | |
| High risk or FLT3-ITD positive[d] | 95 (17.1) | 103 (23.2) | 44 (17.5) | 0.04 |
| Not reported | 226 (40.8) | 133 (30.0) | 93 (36.9) | |
| *Conditioning (RIC),* n *(%)*[e] | | | | *<0.0001* |
| Melphalan-based RIC | 134 (24) | 25 (6) | 209 (83) | |
| Fludarabine-melphalan | 133 (24) | 25 (6) | 207 (82) | |
| Other melphalan-based | 1 (0.2) | 0 | 2 (1) | |
| BU-based RIC | 285 (51) | 389 (88) | 40 (16) | |
| Fludarabine–BU | 272 (49) | 379 (85) | 39 (15) | |
| Other BU based | 13 (2) | 10 (2) | 1 (0.5) | |
| Other chemotherapy-based RIC | 135 (24) | 30 (7) | 3 (1) | |
| *In vivo T-cell depletion,* n (%) | | | | |
| None | 554 (100) | 0 | 0 | |
| ATG <6 mg/kg | 0 | 202 | 0 | |
| ATG ⩾6 mg/kg | 0 | 144 | 0 | |
| ATG dose unknown | 0 | 98 | 0 | |
| Alemtuzumab <80 mg | 0 | 0 | 124 | |
| Alemtuzumab ⩾80 mg | 0 | 0 | 33 | |
| Alemtuzumab dose unknown | 0 | 0 | 95 | |
| *Postgrafting immunosuppression,* n *(%)* | | | | *<0.0001* |
| Tacro or CSP + Mtx | 383 (70) | 90 (21) | 30 (12) | |
| Tacro or CSP + MMF | 110 (20) | 123 (29) | 20 (8) | |
| Tacro or CSP alone | 54 (10) | 217 (50) | 199 (80) | |
| Unknown | 7 | 14 | 3 | |
| *Center activity,* n *(%)* | | | | *<0.0001* |
| Higher (>10 patients in current survey) | 250 (45.1) | 252 (56.8) | 141 (56.0) | |
| Lower (⩽10 patients in current survey) | 304 (54.9) | 192 (43.2) | 111 (44.0) | |

Abbreviations: ATG = antithymocyte globulin; diagn = diagnosis; F = female; FLT3-ITD = Fms-like tyrosine kinase 3-internal-tandem duplication; M = male; MMF = mycophenolate mofetil; Pts = patients; RIC = reduced-intensity conditioning; tacro = tacrolimus; Y = year. [a]Calculated with the $\chi^2$ or the Kruskal–Wallis test. [b]Defined as t(8;21), t(15;17), inv or del (16), or acute promyelocytic leukemia, these abnormalities only or combined with others. [c]Defined as all cytogenetics not belonging to the good or high risk (including trisomias). [d]Defined as 11q23 abnormalities, complex karyotype and abnormalities of chromosomes 5 and 7. [e]Defined as use of fludarabine associated with BU ⩽8 mg/kg or other nonmyeloablative drugs.

($P = 0.37$ in comparison with control group), respectively, in ATG patients and 33 ± 3% ($P = 0.88$ in comparison with control group) and 15 ± 2% ($P = 0.28$ in comparison with control group), respectively, in alemtuzumab patients. Three-year LFS and OS were 51 ± 2% and 55 ± 2%, respectively, in control patients, 51 ± 3% ($P = 0.74$ in comparison with control group) and 59 ± 3% ($P = 0.74$ in comparison with control group), respectively, in ATG patients and 52 ± 3% ($P = 0.29$ in comparison with control group) and 58 ± 3% ($P = 0.24$ in comparison with control group), respectively, in alemtuzumab patients. Causes of death included relapse, GVHD, infection, graft rejection/failure or others in 111 (51.9%), 41 (19.2%), 31 (14.5%), 1 (0.5%) and 30 (14%) control patients, respectively, 90 (53.3%), 27 (16%), 25 (14.8%), 1 (0.6%) and 26 (15.4%) ATG patients, respectively, and 50 (56.2%), 11 (12.4%), 15 (16.9%), 1 (1.1%) and 12 (13.5%) alemtuzumab recipients, respectively.

In multivariate analysis, the use of ATG and the use of alemtuzumab were each associated with a similar risk of RI (HR = 1.1, $P = 0.40$ and HR = 1.0, $P = 0.75$, respectively), a similar risk of NRM (HR = 0.9, $P = 0.60$ and HR = 0.9, $P = 0.57$, respectively) and similar LFS (HR = 1.0, $P = 0.78$ and HR = 0.9, $P = 0.45$, respectively) and OS (HR = 0.9, $P = 0.60$ and HR = 0.9, $P = 0.31$, respectively). Other factors associated with allo-SCT outcomes included poor risk cytogenetics or Fms-like tyrosine kinase 3-internal-tandem duplication positivity associated with a higher risk of RI (HR = 1.8, $P < 0.001$) leading to worse LFS (HR = 1.4, $P = 0.001$) and OS (HR = 1.5, $P < 0.001$), high activity center associated with a lower RI (HR = 0.8, $P = 0.02$) and lower NRM (HR = 0.7, $P = 0.05$) leading to improved LFS (HR = 0.8, $P = 0.004$) and OS (HR = 0.8, $P = 0.006$), older patient age at transplantation associated with higher NRM (HR = 1.33 per 10 years, $P = 0.002$) leading to worse OS (HR = 1.1, $P = 0.04$), and female donors to male recipients associated with a higher risk of NRM (HR = 1.5, $P = 0.02$) leading to worse LFS (HR = 1.2, $P = 0.04$) and OS (HR = 1.3, $P = 0.02$) (Supplementary Table 1).

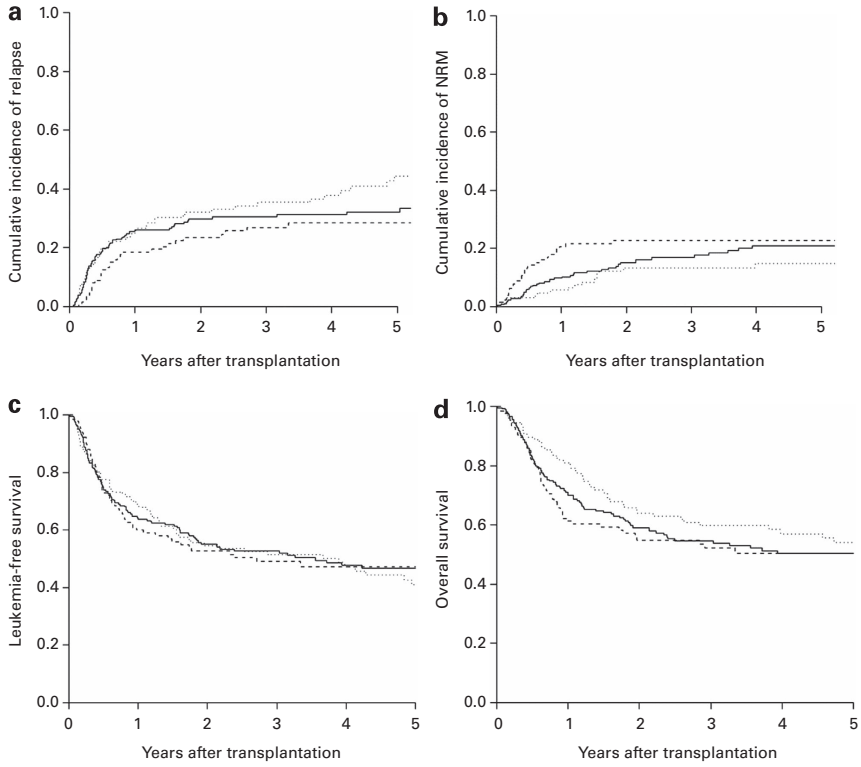


**Figure 1.** Cumulative incidence of relapse (global *P*-value = 0.13) (**a**), cumulative incidence of nonrelapse mortality (global *P*-value = 0.18) (**b**), leukemia-free survival (global *P*-value = 0.99) (**c**) and OS (global *P*-value = 0.37) (**d**) among patients conditioned with BU-based RIC ($n = 285$) (——), melphalan-based RIC ($n = 134$) (– – –) or other chemotherapy-based RIC ($n = 135$) (- - - -) and not given *in vivo* T-cell depletion (control patients).

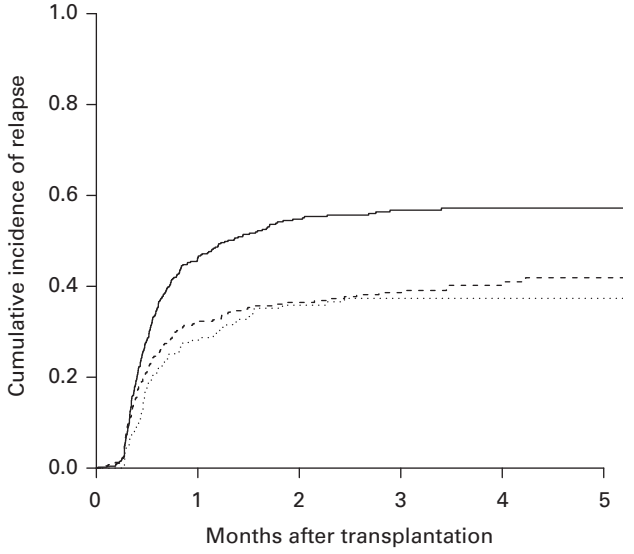


**Figure 2.** Cumulative incidence of chronic GVHD in control (——), ATG (– – –) and alemtuzumab patients (- - - -).

Given that most ATG recipients were given BU-based RIC and that most alemtuzumab recipients were given a melphalan-based RIC, we further assessed the impact of *in vivo* T-cell depletion by comparing allo-SCT outcomes between controls and ATG patients given a BU-based RIC and between control and alemtuzumab patients given a melphalan-based RIC.

### Impact of ATG among patients given BU-based RIC

Among patients given BU-based RIC ($n = 674$, excluding patients given alemtuzumab), acute GVHD of grade II, III and IV were observed in 29 (10%), 16 (6%) and 12 (4%) control patients ($n = 271$ with complete information), respectively, and in 36 (9%), 21 (5%) and 12 (3%) ATG patients ($n = 379$ with completed information) ($P = 0.35$ for the incidence of grade II-IV acute GVHD). Three-year cumulative incidences of chronic and extensive chronic GVHD were $55 \pm 4\%$ and $41 \pm 4\%$ in control patients, respectively, and $38 \pm 3\%$ ($P = 0.001$) and $18 \pm 2\%$ ($P < 0.0001$) in ATG patients, respectively. In univariate analysis, the use of ATG was associated with similar incidences of relapse ($32 \pm 4$ versus $27 \pm 4$ in control group at 3-year; $P = 0.25$), lower NRM ($13 \pm 2$ versus $22 \pm 4$ at 3-year in control group; $P = 0.02$), not significant difference for LFS ($55 \pm 4$ versus $49 \pm 5$ at 3-year; $P = 0.20$) and OS ($60 \pm 4$ versus $52 \pm 5$ at 3-year; $P = 0.08$) (Figure 3). In multivariate analyses, the use of ATG was associated with a lower incidence of chronic GVHD (HR = 0.6, $P = 0.002$ in patients given ATG at a dose $< 6$ mg/kg and HR = 0.6, $P = 0.03$ in patients given ATG at a dose $\geqslant 6$ mg/kg). Further, patients given $< 6$ mg/kg ATG had similar RI than those not given ATG (HR = 1.1, $P = 0.9$), while there was a suggestion for a higher RI in patients given ATG at a dose $\geqslant 6$ mg/kg (HR 1.4, 95% CI, 1.0–2.1, $P = 0.08$) (Table 2). Finally, ATG administration was not associated with NRM, LFS or OS.

### Impact of alemtuzumab among patients given melphalan-based RIC

Among patients given melphalan-based RIC ($n = 343$, excluding patients given ATG), acute GVHD of grade II, III and IV were observed in 19 (14%), 8 (6%) and 7 (5%) control patients ($n = 130$ with completed data), respectively, and in 16 (8%), 3 (1.5%) and 1 (0.5%) alemtuzumab patients ($n = 205$

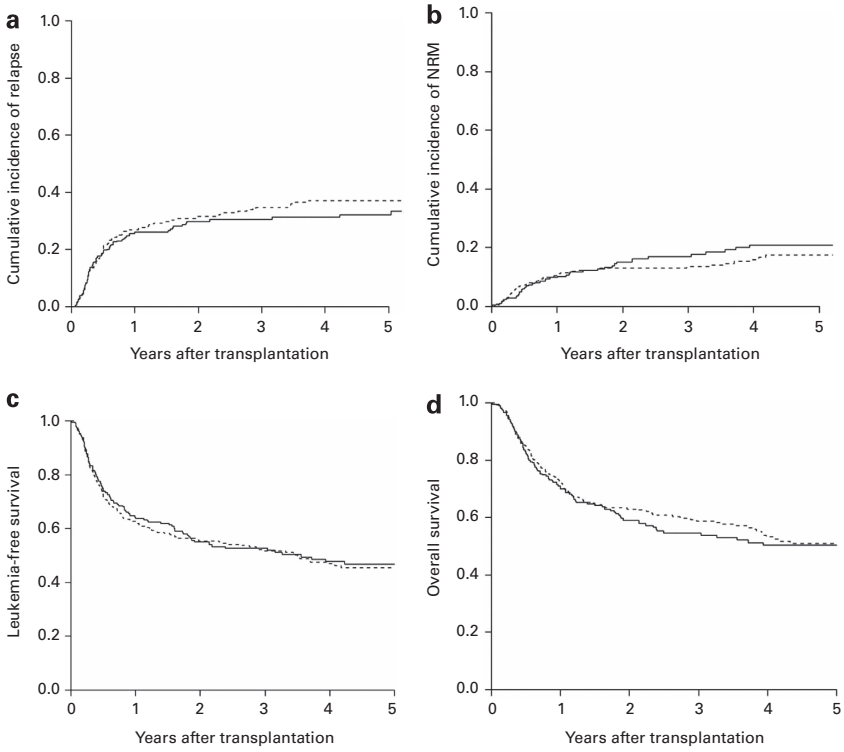


**Figure 3.** Cumulative incidence of relapse (**a**), cumulative incidence of nonrelapse mortality (**b**), leukemia-free survival (**c**) and OS (**d**) among control patients conditioned with BU-based RIC ($n = 285$) (——) versus ATG (– – –) patients given BU-based RIC ($n = 389$).

**Table 2.** Multivariate analyses of transplant outcomes among patients given BU-based RIC and no alemtuzumab ($n = 674$)

| | *cGVHD* | | *Progression/relapse* | | *Nonrelapse mortality* | | *LFS* | | *OS* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *HR*[a] *(95% CI)* | P-*value*[a] | *HR*[a] *(95% CI)* | P-*value*[a] | *HR*[a] *(95% CI)* | P-*value*[a] | *HR*[a] *(95% CI)* | P-*value*[a] | *HR*[a] *(95% CI)* | P-*value*[a] |
| *Use of ATG* | | | | | | | | | | |
| No ($n = 285$) | 1.0 | | 1.0 | | 1.0 | | 1.0 | | 1.0 | |
| 1–6 mg/kg ($n = 196$) | **0.6 (0.4–0.8)** | **0.002** | 1 (0.7–1.4) | 0.87 | 1.1 (0.6–1.8) | 0.82 | 1.0 (0.7–1.3) | 0.95 | 0.9 (0.7–1.3) | 0.60 |
| ⩾6mg/kg ($n = 111$) | **0.6 (0.4–1.0)** | **0.03** | 1.4 (1.0–2.1) | 0.08 | 0.9 (0.5–1.7) | 0.76 | 1.2 (0.9–1.7) | 0.20 | 0.9 (0.6–1.3) | 0.52 |
| Dose unknown ($n = 82$) | 0.7 (0.5–1.1) | 0.16 | 0.8 (0.5–1.3) | 0.36 | 0.7 (0.3–1.5) | 0.40 | 0.8 (0.5–1.2) | 0.21 | 0.8 (0.5–1.3) | 0.38 |
| Year of transplantation >median | 1.0 (0.7–1.3) | 0.76 | 1.2 (0.9–1.6) | 0.33 | 1.3 (0.8–2.0) | 0.29 | 1.2 (0.9–1.6) | 0.16 | 1.2 (0.9–1.6) | 0.26 |
| Patient age/10 years | 1.0 (0.9–1.2) | 0.58 | 1.0 (0.9–1.2) | 0.86 | **1.3 (1.1–1.8)** | **0.02** | 1.1 (1.0–1.2) | 0.15 | 1.1 (1.0–1.3) | 0.13 |
| Diagnosis to allo-SCT >median | 0.8 (0.6–1.1) | 0.14 | 0.8 (0.6–1.1) | 0.10 | 0.9 (0.6–1.3) | 0.46 | 0.8 (0.6–1.0) | 0.09 | 0.8 (0.6–1.1) | 0.17 |
| Female donor to male recipient ($n = 146$) | 1.1 (0.8–1.5) | 0.63 | 0.9 (0.7–1.3) | 0.69 | 1.4 (0.9–2.3) | 0.12 | 1.1 (0.8–1.4) | 0.61 | 1.1 (0.8–1.5) | 0.42 |
| Patient CMV seropositivity ($n = 463$) | 0.8 (0.6–1.1) | 0.17 | 0.9 (0.6–1.2) | 0.35 | 1.1 (0.7–1.9) | 0.62 | 0.9 (0.7–1.2) | 0.58 | 1.0 (0.7–1.4) | 0.96 |
| Donor CMV seropositivity ($n = 406$) | **1.5 (1.1–2.1)** | **0.007** | 0.9 (0.7–1.2) | 0.53 | 1.7 (1.0–2.8) | 0.05 | 1.1 (0.8–1.4) | 0.58 | 1.1 (0.8–1.5) | 0.55 |
| Poor cytogenetics or FLT3-ITD ($n = 139$) | 1.0 (0.7–1.4) | 0.88 | **1.8 (1.3–2.5)** | **<0.001** | 1.1 (0.6–1.9) | 0.75 | **1.6 (1.2–2.1)** | **0.001** | **1.7 (1.3–2.3)** | **<0.001** |
| High activity centers[b] ($n = 362$) | 1.1 (0.8–1.4) | 0.68 | 0.8 (0.6–1.1) | 0.17 | 0.8 (0.5–1.2) | 0.21 | 0.8 (0.6–1.0) | 0.06 | **0.8 (0.6–1.0)** | **0.05** |

Abbreviations: ATG = antithymocyte globulin; CI = confidence interval; FLT3-ITD = Fms-like tyrosine kinase 3-internal-tandem duplication; HR = hazard ratio; LFS = leukemia-free survival; RIC = reduced-intensity conditioning. [a]Statistically significant factors are in bold. [b]Arbitrarily defined as center that contributed for >10 patients in the current study.

with complete information), respectively ($P < 0.001$ for the incidence of grade II-IV acute GVHD). Three-year cumulative incidences of chronic and extensive chronic GVHD were $51 \pm 5\%$ and $32 \pm 5\%$ in control patients, respectively, and $36 \pm 4\%$ ($P = 0.01$) and $8 \pm 2\%$ ($P < 0.0001$) in alemtuzumab patients, respectively. In univariate analysis, the use of alemtuzumab was associated with a non-statistically significant suggestion of higher RI ($32 \pm 4$ versus $27 \pm 4$ at 3-year; $P = 0.25$) but also a lower NRM ($13 \pm 2$ versus $22 \pm 4$; $P = 0.02$), translating toward a slight trend for better LFS ($55 \pm 4$ versus $49 \pm 5$; $P = 0.20$) and a trend for better OS ($60 \pm 4$ versus $52 \pm 5$; $P = 0.08$) (Figure 4). In multivariate analyses, the use of alemtuzumab was associated with a lower incidence of chronic GVHD (HR = 0.4, $P < 0.001$ in patients given alemtuzumab at a dose < 80 mg/kg) but did not have a significant impact on RI, NRM, LFS or OS (Table 3).

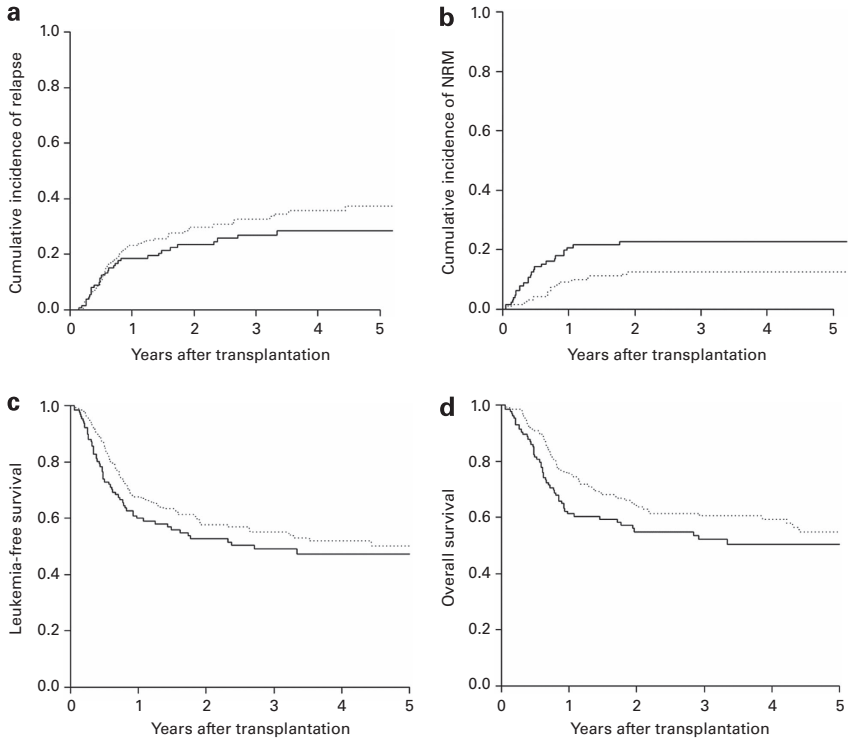


**Figure 4.** Cumulative incidence of relapse (**a**), cumulative incidence of nonrelapse mortality (**b**), leukemia-free survival (**c**) and OS (**d**) among control patients conditioned with melphalan-based RIC ($n = 134$) (——) versus alemtuzumab patients given melphalan-based RIC ($n = 209$) (- - - -).

**Table 3.** Multivariate analyses of transplant outcomes among patients given melphalan-based RIC and no ATG ($n = 343$)

| | *cGVHD* | | *Progression/relapse* | | *Nonrelapse mortality* | | *LFS* | | *OS* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *HR[a] (95% CI)* | P-*value*[a] | *HR[a] (95% CI)* | P-*value*[a] | *HR[a] (95% CI)* | P-*value*[a] | *HR[a] (95% CI)* | P-*value*[a] | *HR[a] (95% CI)* | P-*value*[a] |
| *Use of Alemtuzumab* | | | | | | | | | | |
| No ($n = 134$) | 1.0 | | 1.0 | | 1.0 | | 1.0 | | 1.0 | |
| $<80$ mg ($n = 108$) | **0.4 (0.2–0.6)** | **<0.001** | 1.2 (0.7–2.1) | 0.52 | 0.5 (0.2–1.1) | 0.09 | 0.8 (0.5–1.3) | 0.37 | 0.8 (0.5–1.2) | 0.26 |
| $>80$ mg ($n = 26$) | 0.4 (0.2–1.2) | 0.12 | 1.4 (0.6–3.3) | 0.50 | 0.6 (0.2–2.1) | 0.46 | 0.9 (0.5–1.9) | 0.85 | 0.9 (0.4–1.9) | 0.75 |
| Dose unknown ($n = 75$) | **0.6 (0.4–1.0)** | **0.04** | 1.2 (0.7–2.0) | 0.60 | 0.6 (0.3–1.2) | 0.13 | 0.8 (0.5–1.3) | 0.38 | 0.7 (0.4–1.1) | 0.13 |
| Year of transplantation $>$median | 0.8 (0.5–1.3) | 0.35 | 0.8 (0.4–1.4) | 0.39 | 0.9 (0.4–1.8) | 0.73 | 0.8 (0.5–1.3) | 0.36 | 0.8 (0.5–1.2) | 0.28 |
| Patient age/10 years | 1.1 (0.8–1.3) | 0.55 | 0.9 (0.7–1.2) | 0.66 | 1.1 (0.8–1.6) | 0.45 | 1.0 (0.8–1.2) | 0.83 | 1.1 (0.9–1.3) | 0.59 |
| Diagnosis to allo-SCT $>$median | 1.1 (0.8–1.7) | 0.58 | 1.0 (0.6–1.5) | 0.87 | 1.6 (0.9–2.9) | 0.11 | 1.1 (0.8–1.6) | 0.49 | 1.1 (0.8–1.6) | 0.55 |
| Female donor to male recipient ($n = 66$) | 1.6 (1.0–2.6) | 0.06 | 1.3 (0.8–2.2) | 0.34 | 1.2 (0.6–2.4) | 0.71 | 1.2 (0.8–1.9) | 0.39 | 1.3 (0.8–2.1) | 0.23 |
| Patient CMV seropositivity ($n = 223$) | 1.2 (0.8–1.9) | 0.44 | 1.1 (0.7–1.8) | 0.61 | 1.9 (0.8–4.2) | 0.13 | 1.3 (0.9–2.0) | 0.16 | 1.3 (0.9–2.1) | 0.17 |
| Donor CMV seropositivity ($n = 191$) | 0.7 (0.5–1.1) | 0.17 | 1.0 (0.6–1.5) | 0.88 | 1.5 (0.8–3.0) | 0.20 | 1.2 (0.8–1.7) | 0.44 | 1.2 (0.8–1.8) | 0.41 |
| Poor cytogenetics or FLT3-ITD ($n = 64$) | 0.8 (0.5–1.3) | 0.37 | **1.6 (1.0–2.7)** | **0.04** | 0.5 (0.2–1.3) | 0.16 | 1.2 (0.8–1.9) | 0.36 | 1.3 (0.9–2.1) | 0.19 |
| High-activity centers[b] ($n = 197$) | 0.9 (0.6–1.3) | 0.56 | 1.0 (0.6–1.5) | 0.86 | 0.6 (0.4–1.2) | 0.15 | 0.8 (0.6–1.2) | 0.33 | 0.7 (0.5–1.0) | 0.07 |

Abbreviations: CI = confidence interval; FLT3-ITD = Fms-like tyrosine kinase 3-internal-tandem duplication; HR = hazard ratio; LFS = leukemia-free survival; RIC = reduced-intensity conditioning. [a]Statistically significant factors are in bold. [b]Arbitrarily defined as center that contributed for $>10$ patients in the current study.

## DISCUSSION

Although GVHD has been linked to the GVT effect after RIC allo-SCT for AML,[15,17,18,23] only a limited or moderate form of chronic GVHD have been correlated with improved transplantation outcomes.[18] The current study indicates that 56% of AML patients in CR1 given PBSC from HLA-identical siblings after chemotherapy-based RIC in EBMT-affiliated centers have received *in vivo* T-cell depleting agents (that is, ATG or alemtuzumab) with the aim of preventing severe GVHD. However, a recent study from the CIBMTR observed that, among patients given RIC allo-SCT from related or unrelated donors for various hematological malignancies, *in vivo* T-cell depletion decreased disease-free survival due to a high incidence of disease relapse/progression.[19] This prompted us to perform the current study that aimed to assess the impact of *in vivo* T-cell depletion on transplantation outcomes in a more homogeneous population of AML patients in CR1 given PBSC from HLA-identical siblings.

Several observations could be drawn from this analysis. First, the use of *in vivo* T-cell depletion was successful at preventing GVHD, in line with previously published data.[5–7,19,24] Interestingly, acute GVHD prevention seemed stronger in patients receiving alemtuzumab than in ATG recipients.[25] This could be attributed to

the relatively low doses of ATG (<6 mg/kg) given in the majority of ATG recipients in the current survey as recent reports demonstrated a direct link between ATG dosage and GVHD incidence in patients given chemotherapy-based RIC.[26–28]

Second, ATG administration was not associated with a higher risk of disease relapse/progression in patients with *de novo* AML in first CR when the dose of ATG was <6 mg/kg (while there was a suggestion for an increased RI when the dose of ATG was ⩾6 mg/kg). This observation is in line with previous studies performed in patients given grafts after myeloablative conditioning showing that low or moderate doses of ATG did not significantly increase the RI,[5,7] but are in disagreement with the 'RIC *in vivo* T-cell depletion' CIBMTR study mentioned above.[19] Possible explanation for this discrepancy could be that a high proportion of patients (47%) in the CIBMTR study had lymphoid malignancies given that previous studies have observed a tight association between *in vivo* T-cell depletion of the graft and high RI in patients transplanted as treatment for multiple myeloma or lymphoma.[29,30] This observation is also in agreement with data from the French registry that showed poorer OS in patients given fludarabine and BU-based RIC in various hematological malignancies when the dose of ATG (Thymoglobuline) was ⩾10 mg/kg total dose,[31] whereas a recent single center study observed similar RI ($P = 0.6$) in patients given BU-based RIC for myeloid malignancies when the dose of ATG Thymoglobuline was increased from 2.5 mg/kg total dose to 5 mg/kg total dose.[28] Although we did not collect the brand of ATG used in the registry, Thymoglobulin is by far the most frequently used in Europe (and was most likely the ATG brand for all patients given <6 mg/kg ATG total dose), followed by ATG Fresenius, whereas horse-derived formulations of ATG are not available in Europe.

Interestingly, the use of alemtuzumab was not associated either with higher relapse risk. This is different to what we have previously observed in AML patients in CR1 given grafts from unrelated donors after chemotherapy-based RIC, where alemtuzumab patients had higher RI and lower LFS and OS than ATG patients,[32] and to what has been observed in a survey including patients given grafts from related or unrelated donors in CR1 or CR2 at transplantation but that excluded patients given pre-emptive DLI.[18] Possible explanations for these apparent discrepancies could be that the dose of alemtuzumab received was lower in related than in unrelated recipients or that it was possible to prevent relapse in alemtuzumab patients with persistent mixed chimerism and in those with minimal residual disease persistence by giving pre-emptive DLI,[33] a strategy that might have been less possible/successful in the unrelated donors setting.[34,35] Although we did not have complete data on pre-emptive DLI in the registry precluding us to rigorously analyse the impact of pre-emptive DLI in current patients, pre-emptive DLI were given more frequently in alemtuzumab patients (36%) than in control (5%) or ATG (14%) patients. Another strategy used to prevent AML relapse after alemtuzumab-based RIC has been based on the pre-emptive administration of azacitidine which can likely favor the GVT effect without excessive GVHD.[36,37]

Besides the impact of *in vivo* T-cell depletion on outcomes, this study also confirmed the negative impact of poor risk cytogenetics,[23,38,39] of being transplanted with a female donor in case of male recipients,[40] and of being transplanted in low-activity centers[18,22] on OS and PFS, as previously observed by our group[18,22,38,41] and by other groups of investigators.[39,40] Further, in contrast to what was observed in a recent CIBMTR study,[39] current data suggest that older patient age at transplantation is associated with higher NRM translating to worse LFS and OS in multivariate analyses.

There are limitations in this study including its retrospective design, and the fact that we did not have complete data on the use of pre-emptive DLI. We tried to limit as much as possible the impact of potential confounding factors by selecting a population as homogeneous as possible (only PBSC recipients (given the previously demonstrated lower incidence of cGVHD in marrow recipients), only related recipients (given that the vast majority unrelated PBSC recipients were given *in vivo* T-cell depletion in the registry), only chemotherapy-based RIC (given than very few TBI-based RIC recipients were given *in vivo* T-cell depletion), and only CR1 patients), and by performing multivariate analyses separately in patients given BU-based RIC and melphalan-based RIC.

In summary, these data suggest that *in vivo* T-cell depletion with <6 mg/kg ATG can be safely used in the conditioning of AML patients in CR1 given PBSC after BU-based RIC and that *in vivo* T-cell depletion with alemtuzumab did not alter OS/PFS in AML patients in CR1 given PBSC after melphalan-based RIC, at least when pre-emptive DLI were given in selected patients. Randomized prospective studies are needed to confirm these important data.

## CONFLICT OF INTEREST

MM has received lectures honoraria and research support from Genzyme and Fresenius whose products are discussed in this manuscript. FB has received lectures honoraria and research support from Genzyme. DB has received honoraria and research funding from Genzyme/Sanofi. AN has received research grants from Fresenius and Genzyme, took part in some of their clinical trials (Fresenius and Genzyme), participate in their investigator meeting and advisory board (Genzyme) and lecture for them (satellite meeting and special meeting + honorarium). RFD received lectures honoraria and research support from Genzyme and Sanofi.

## AUTHOR CONTRIBUTIONS

FB wrote the manuscript, designed the study and interpreted the data; ML designed the study, analyzed the data, interpreted the data and edited the manuscript; MM designed the study, interpreted the data and edited the manuscript; DB, LL-C, SV, CC, MA, PJ, HG, GS, PC, PB, AS, RFD, AN participated in study collection or design, provided clinical data and critically reviewed and gave final approval of the manuscript.

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Supplementary Information accompanies the paper on Bone Marrow Transplant website (http://www.nature.com/bmt)