Fludarabine/2 Gy TBI is Superior to 2 Gy TBI as Conditioning for HLA-Matched Related HCT: A Phase III Randomized Trial.

Fludarabine and 2-Gy TBI is Superior to 2 Gy TBI as Conditioning for HLA-Matched Related Hematopoietic Cell Transplantation: A Phase III Randomized Trial

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Abstract
The risks and benefits of adding fludarabine to a 2-Gy total body irradiation (TBI) nonmyeloablative regimen are unknown. For this reason, we conducted a prospective randomized trial comparing 2-Gy TBI alone, or in combination with 90 mg/m^2 fludarabine (FLU/TBI), before transplantation of peripheral blood stem cells from HLA-matched related donors. Eighty-five patients with hematological malignancies were randomized to be conditioned with TBI alone (n = 44) or FLU/TBI (n = 41). All patients had initial engraftment. Two graft rejections were observed, both in the TBI group. Infection rates, nonrelapse mortality, and graft-versus-host disease (GVHD) were similar between groups. Three-year overall survival was lower in the TBI group (54% versus 65%; hazard ratio [HR], .57; *P* = .09), with higher incidences of relapse/progression (55% versus 40%; HR, .55; *P* = .06), relapse-related mortality (37% versus 28%; HR, .53; *P* = .09), and a lower progression-free survival (36% versus 53%; HR, .56; *P* = .05). Median donor T cell chimerism levels were significantly lower in the TBI group at days 28 (61% versus 90%; *P* < .0001) and 84 (68% versus 92%; *P* < .0001), as was NK cell chimerism on day 28 (75% versus 96%; *P* = .0005). In conclusion, this randomized trial demonstrates the importance of fludarabine in augmenting the graft-versus-tumor effect by ensuring prompt and durable high-level donor engraftment early after transplantation.

During the development of the widely used nonmyeloablative conditioning regimen based on 2-Gy low-dose total body irradiation (TBI) and 90 mg/m^2 fludarabine (FLU), the first 44 patients in the initial clinical trial were conditioned with a regimen directly translated from our canine studies 1, 2 and 3. The regimen consisted of 2-Gy TBI alone, and although results were encouraging, a nonfatal graft rejection rate of 20% was observed [4]. To reduce the high rejection rate, FLU (30 mg/m^2/day for 3 days) was added to the 2-Gy TBI, which resulted in a decrease in rejections to 3% [5]. However, in a retrospective analysis of the first 176 patients with hematologic malignancies treated with nonmyeloablative hematopoietic cell transplantation (HCT) from HLA-identical related donors, higher nonrelapse mortality (NRM) was observed among patients conditioned with 2-Gy TBI and FLU (FLU/TBI) (FLU/TBI: 31% versus TBI:14% at 2 years; *P* = .02). The increased NRM was due to increased infectious events with or without graft-versus-host disease (GVHD) [5]. As rejections were mainly observed in patients who had not been treated with significant myelosuppressive chemotherapy [5] (myeloid malignancies or multiple myeloma) before allogeneic HCT, we hypothesized that in patients at a low risk of rejection, conditioning with 2-Gy TBI alone could be sufficient to allow stable long-term engraftment. These results needed to be taken with caution, however, as they were retrospective and not from concurrent transplantation cohorts. Therefore, we initiated this phase III trial, where patients at low or moderate risk of rejection were randomized between conditioning with 2-Gy TBI alone or in combination with FLU (30 mg/m^2/day for 3 days)/FLU/TBI) before transplantation with peripheral blood stem cells (PBSC) from human leukocyte antigen (HLA)-matched related donors.

Patients and Methods

The study was a randomized phase III trial including 9 transplantation centers: Fred Hutchinson Cancer Research Center (FHCRC), Medical College of Wisconsin, University of Leipzig, Oregon Health and Science University, VA Puget Sound Health Care System Huntsman Cancer Institute/University of Utah, University of Torino School of Medicine, LDS Hospital, University of Tuebingen, and University of Cologne. The FHCRC acted as the coordinating center. The study was approved by the institutional review board at each center, and written informed consent was obtained from all patients before the start of treatment.

Randomization, Study Endpoints, and Accrual

Patients were randomized equally between conditioning with 2-Gy TBI alone or in combination with FLU 30 mg/m^2/day for 3 days. The randomization was performed at the FHCRC and was stratified balanced over time for institution, disease risk (indolent versus aggressive) [6], and a history of prior high-dose HCT.
The initial primary endpoint was the comparison of 1-year NRM between arms. However, as accrual was slow, and it was unlikely that the enrollment goal of 200 patients would be met within a reasonable timeframe, the data safety monitoring board recommended a change of the primary objective to overall survival at 3 years to accommodate a lower accrual goal of 110 patients. Apart from including NRM at 1 year in the secondary objectives, the rest of the secondary objectives remained the same (disease progression, relapse-related mortality, graft rejection, grades II to IV acute and chronic GVHD, infections, and immune reconstitution).
The protocol was opened for accrual in December 2003 and closed by the principal investigator (with data safety-monitoring board approval) in May 2011, after accruing 85 patients, because of a difference in relapse and progression rates between the 2 arms.
The database was analyzed as of December 12, 2012.

Eligibility Criteria

Included in this study were patients with hematological malignancies treatable by allogeneic HCT who were not curable by high-dose conditioning with autologous stem cell support and ineligible for high-dose allogeneic HCT because of age or comorbidities. Donors were related and at least genotypically HLA identical at 1 haplotype and phenotypically or genotypically identical at the allele level at HLA-A, -B, -C, -DRB1 and -DQB1 for the second haplotype [7]. The hematological malignancies allowed were aggressive non-Hodgkin lymphomas (NHLs); low-grade NHL with <6 months' duration of complete remission (CR) between courses of therapy; mantle cell lymphoma; chronic lymphocytic leukemia (CLL) that did not meet the National Cancer Institute's Working Group criteria for CR, partial remission, or relapse within 12 months after FLU or other nucleoside analogue-containing therapy; failed FLU-cyclophosphamide-rituximab therapy, had 17p deletion; progressed to prolymphocytic leukemia or T cell CLL or prolymphocytic leukemia; Hodgkin lymphoma (HL) that had at least failed frontline therapy and were ineligible for or had failed high-dose conditioning with autologous stem cell support; multiple myeloma (MM) that was chemotherapy sensitive after failed high-dose conditioning with autologous stem cell support; acute myeloid leukemia (AML) or acute lymphoblastic leukemia beyond first CR; chronic myeloid leukemia (CML) beyond the first chronic phase after myelosuppressive therapy; myelodysplastic syndrome (MDS) or myeloproliferative disease after myelosuppressive therapy; or Waldenstrom's macroglobulinemia after failing 2 courses of therapy. Patients with CML, AML, acute lymphoblastic leukemia, MDS, or myeloproliferative disease required < 5% marrow blasts at the time of transplantation. Myelosuppressive therapy less than 3 weeks before conditioning or high-dose conditioning with stem cell support less than 6 months before allogeneic HCT was not allowed.

Patients were excluded from the trial if they were pregnant or breast-feeding; had rapidly progressing intermediate- or high-grade NHL, unless in minimal disease state; chronic myelomonocytic leukemia, leukemic blasts in the peripheral blood detected by standard pathology; central nervous system involvement refractory to intrathecal chemotherapy; infection with human immunodeficiency virus, bacterial, viral, or fungal infections unresponsive to therapy; decompensated liver disease; lung carbon monoxide diffusion capacity <30%, total lung capacity <30%, forced expiratory volume <30%, or dependency on supplementary oxygen; symptomatic coronary artery disease or cardiac ejection fraction <35%; poorly controlled hypertension; or a Karnofsky performance score <50%.

Treatment and Evaluations

Patients allocated to the TBI-only arm received 2 Gy at a rate of .07 Gy/min to .10 Gy/min from a linear accelerator on the day of HCT (day 0) with PBSC, whereas patients in the FLU/TBI arm in addition received FLU (30 mg/m^2/day) on days -4, -3, and -2 before 2-Gy TBI and HCT. PBSC were collected from related donors on days -1 and 0 ($CD34^+$ target cell dose was 5×10^6 cells/kg of recipient weight) after administration of granulocyte colony-stimulating factor (G-CSF) (16 μg/kg) on days -4 to 0. Postgrafting immunosuppression consisted of oral cyclosporine (CSP; 5 mg/kg twice daily from days -3 to +56, and, in the absence of GVHD, tapered by 6% weekly until day +180) and mycophenolate mofetil (15 mg/kg twice daily from day 0 to +27). CSP levels were monitored by immunoassay, and whole blood trough levels were targeted at 500 ng/mL for the first 28 days after transplantation and at 150 ng/mL to 450 ng/mL until the start of taper (Abbott, TDX, Abbott Park, IL). If there was evidence of persistent/progressive disease or relapse in the absence of GVHD on day 56 after transplantation, all immunosuppressive agents were rapidly tapered to allow graft-versus-tumor effects to occur. If relapse was observed, these cases were considered treatment failures, and the patients were taken off protocol. Donor lymphocyte infusion (DLI) was not offered on this protocol, and patients with low chimerism or disease progression were eligible for ongoing DLI protocols or treatment plans. For the purpose of survival analysis, patients were followed past the time point of relapse or DLI. Chimerism analysis was performed as previously described [8]. Peripheral blood $CD3^+$ T cell chimerism studies were performed on days +28, +84 and +365, and, if the patient had <50% donor chimerism on day 28, additional analyses were performed on days +56 and +180. If the patient was not >95% $CD3^+$ T cell donor chimerism at 1 year, analyses were repeated annually. Natural killer (NK) cell (CD56) and granulocyte (CD33) chimerisms were obtained on days +28 and +84, respectively. Full-donor chimerism was defined as >95% donor $CD3^+$ T cells, and graft rejection was defined as the inability to detect at least 5% donor $CD3^+$ T cells in peripheral blood. Toxicities were determined using the Common Toxicity Criteria, Version 2.0 [9].

All patients received standard prophylaxis against infections as previously published [10]. Diagnosis, clinical grading, and treatment of acute and chronic GVHD were performed by local investigators according to established criteria 11 and 12. Tumor responses were assessed using standard criteria and PCR, cytogenetics, fluorescein in situ hybridization, and flow cytometric-based methods as appropriate.

Analysis of Peripheral Blood Lymphocytes

Immunnophenotyping of peripheral blood lymphocytes was only performed in a subset of patient/donor pairs that underwent transplantation at the FHCRC. Peripheral blood was obtained from donors pre-G-CSF and from patients before transplantation and at days +28, +84, +180 and +365. Enumeration of mononuclear cell subsets [13] and immunophenotyping by flow cytometry for naïve and memory B cells, naïve and memory/effector $CD4^+$ and $CD8^+$ T cells,

monocytes, NK cells, and myeloid and plasmacytoid dendritic cells were performed as previously described 13 and 14.

Statistical Analysis

Survival was estimated by the Kaplan-Meier method. Cumulative incidence was estimated by standard methods in the competing risk setting. Nonrelapse death was a competing risk for the analysis of relapse/progression and relapse-related mortality, and, conversely, relapse/progression was a competing risk for the analysis of nonrelapse death. Death was a competing risk for the analysis of acute and chronic GVHD and infection. All statistical comparisons of time-to-event endpoints are based on hazard ratio (HR) analysis using Cox regression. Comparisons of chimerism are based on 2-sample Wilcoxon test. Comparisons of immune reconstitution are based on 2-sample *t*-test. All *P* values are 2 sided.

Results

Patients

Forty-four patients were accrued into the TBI-only arm, and 41 were accrued into the FLU/TBI arm. Patient demographics are summarized in Table 1. Overall median patient age was 55 (range, 17 to 73) years with a predominance of male gender (68%). Patients received G-CSF–mobilized PBSC containing a median of 7.9×10^6 (range, 1.9×10^6 to 22.7×10^6) $CD34^+$ cells/kg and 3.6×10^8 (range, 1.0×10^8 to 40.9×10^8) $CD3^+$ cells/kg. Underlying diseases were AML (n = 15), MDS (n = 4), NHL (n = 32), CLL (n = 9), MM (n = 9), and HL (n = 16) with slightly more patients with NHL compared with AML and lower relapse risk score [15] in the FLU/TBI arm, but this was not statistically different and within the limits expected by chance. Fifty-five percent of the patients had failed at least 1 high-dose HCT. As randomization was stratified upon failed prior high-dose HCT, the number of patients was evenly distributed between arms (TBI only, n = 26 [59%]; FLU/TBI, n = 21 [51%]). In the TBI arm, 3 of 26 patients had failed an allogeneic HCT (all from different HLA-matched siblings) compared with none in the FLU/TBI arm. There was no difference in median HCT comorbidity index between arms [16]. Four patients in the TBI arm and 3 in the FLU/TBI arm, all with refractory or relapsed $CD20^+$ B cell lymphomas, were concurrently enrolled on a protocol (ClinicalTrials.gov identifier: NCT00867529) studying the effects of peritransplantation rituximab (days -3, 10, 24 and 38).

Table 1.
Pretransplantation Demographics

Characteristic	TBI Only (n = 44)	Flu/TBI (n = 41)
Patient age, median (range), yr	54 (17-73)	56 (18-72)
Male patient gender	32 (73)	26 (63)
Donor age, median (range), yr	53 (17-73)	54 (15-71)
Sex of patient/donor		
Male/female	15 (34)	15 (37)
Other combinations	29 (66)	26 (63)
CMV serostatus of patient/donor		
Negative/negative	14 (32)	12 (29)
Other combinations	30 (68)	29 (71)
Prior failed high-dose HCT		
Autologous	23 (52)	21 (51)

Characteristic	TBI Only (n = 44)	Flu/TBI (n = 41)
Allogeneic	3 (7)	0
Number of previous regimens, median (range)	5 (1-17)	5 (1-19)
Diagnoses		
Non-Hodgkin lymphoma	14 (32)	18 (44)
Acute myeloid leukemia	10 (23)	5 (12)
Multiple myeloma	5 (11)	4 (10)
Chronic lymphocytic leukemia	5 (11)	4 (10)
Myelodysplastic syndrome	2 (5)	2 (5)
Hodgkin lymphoma	8 (18)	8 (20)
Relapse risk [15]		
Low	9 (20)	14 (34)
Standard	18 (41)	12 (29)
High	17 (39)	15 (37)
HCT comorbidity index		
0	13 (30)	6 (15)
1,2	6 (14)	13 (33)
3+	24 (56)	20 (51)
$CD34^+$ cells × 10^6/kg, median (range)	7.9 (2.6-19.8)	7.7 (1.9-22.7)
CD3 + cells × 10^8/kg, median (range)	3.7 (1.4-40.9)	3.5 (1.0-9.7)

Data presented as n (%) unless otherwise indicated.

Peripheral Blood Cell Changes, Rejections, and Chimerism

All patients had initial engraftment. Although no patients in the FLU/TBI arm rejected their grafts, 2 patients in the TBI arm, 1 with AML in second CR and 1 with MDS (refractory anemia with excess of blasts-2), experienced graft rejection 31 and 262 days after transplantation, respectively. The patient with AML had failed a prior allogeneic high-dose HCT from a different HLA-matched sibling, and after rejecting the graft from the current 2-Gy TBI-conditioned HCT, went on to receive a third allogeneic HCT from the same sibling and subsequently died from disease progression. The patient with MDS was in complete remission before transplantation, having received only 1 cycle of cytarabine and mitoxantrone. After rejecting the graft on this trial, the patient received a second myeloablative transplantation from a syngeneic donor. The patient was still alive and in remission at last follow-up.

Near-complete donor granulocyte chimerism was achieved promptly, with no significant differences between arms (Figure 1A). Median donor T cell chimerism levels were significantly higher in the FLU/TBI arm compared with the TBI arm at days +28 (90% versus 61%; $P < .0001$) and +84 (92% versus 68%; $P < .0001$) (Figure 1B). Median day +28 NK cell donor chimerism was also significantly higher in FLU/TBI-conditioned patients (FLU/TBI [n = 14], 96%; TBI [n = 15], 75%; $P = .0005$) and correlated with day +28 donor T cell chimerism (Pearson's correlation coefficient = .82; $P < .0001$).

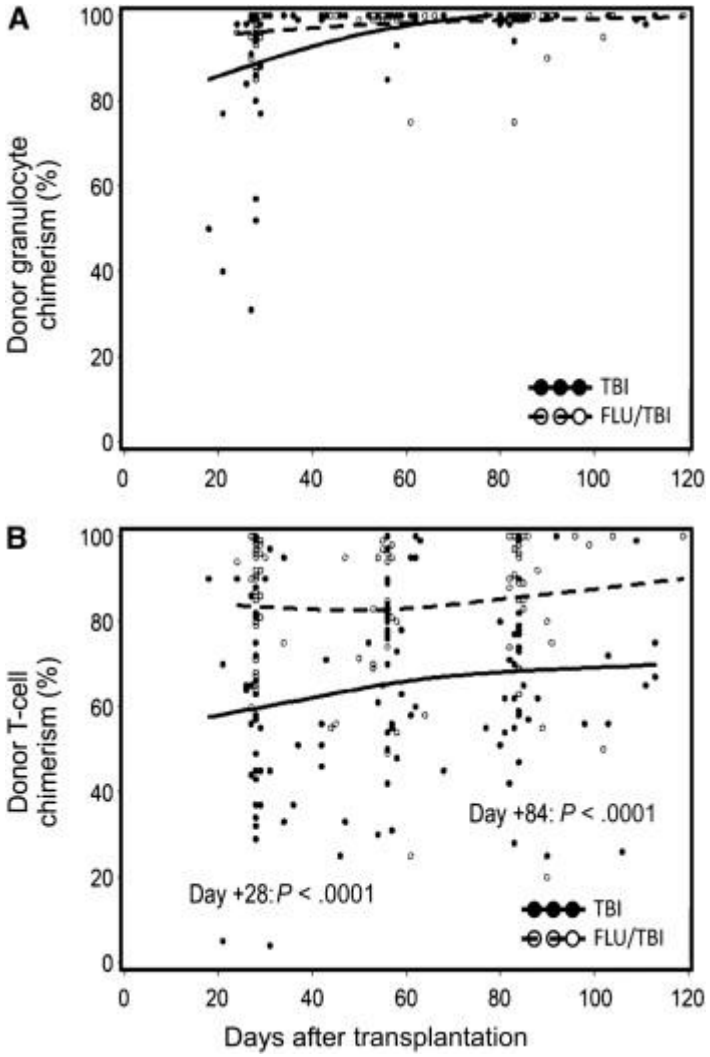

Figure 1.
Donor granulocyte and T cell chimerism. Percent donor granulocyte (A) and T cell chimerism (B) in patients conditioned with 2-Gy total body irradiation only (TBI, n = 44) or in combination with 90 mg/m^2 fludarabine (FLU/TBI, n = 41). Horizontal lines represent medians, and dots represent the individual data points. *P* values are 2 tailed.

Median absolute granulocyte count nadirs were similar in the 2 groups (TBI: 396 [range, 0 to 2340] cells/μL; FLU/TBI: 270 [0 to 1090] cells/μL; *P* = .32). However, the median number of days with absolute granulocyte counts below 500 cells/μL was significantly higher in the FLU/TBI group (TBI: 0 [range, 0 to 40] days; FLU/TBI: 4 [range, 0 to 17] days; *P* = .05), but the number of patients who required G-CSF treatment for prolonged neutropenia (persistence or development of granulocyte counts below 500 cell/μL past day +21 after transplantation) was similar (TBI: 12%; FLU/TBI: 18%; *P* = .45). Platelet nadirs (TBI: 59,000 [range, 6000 to 251,000] platelets/μL; FLU/TBI: 59,000 [range, 6000 to 209,000] platelets/μL; *P* = .62) and days below platelet counts of 20,000 platelets/μL (TBI: 0 [range, 0 to 24] days; FLU/TBI: 0 [range, 0 to 3] days; *P* = .63) were similar in both arms. The percentages of patients who needed red blood cell transfusions trended to be higher in the FLU/TBI arm (TBI: 43%; FLU/TBI: 63%; *P* = .06), whereas the percentages of patients who needed platelet transfusions were similar in both arms (TBI: 23%; FLU/TBI: 27%; *P* = .66).

DLI

None of the patients in the FLU/TBI arm received DLI. A total of 8 patients in the TBI arm received DLI: 2 patients because of low chimerism (both died from relapsed AML) and 6 patients because of relapse or progression (4 died from relapse, whereas 1 with HL and 1 with small lymphocytic lymphoma were alive at last follow-up).

Immune Reconstitution

In the TBI arm, samples for immunophenotyping were available from 16 patients at day 28 and from 8 patients at day 90, whereas in the FLU/TBI arm, samples were available from 7 and 13 patients at days 28 and 90, respectively.
Immunophenotyping showed that the median absolute number of NK cells was significantly higher at day +28 after transplantation in the FLU/TBI arm compared with the TBI arm (Figure 2). The difference disappeared by day +90. No differences between arms were observed for the median absolute number of $CD4^+$ cells at day +28, but at day +90, lower levels were observed in patients in the FLU/TBI arm compared with the TBI arm (significant for naïve $CD4^+$ cells and a trend for the CD4 memory/effector population [Figure 2]). There were no significant differences between arms in absolute counts of naïve and memory/effector $CD8^+$ T cells, naïve and memory B cells, monocytes, and myeloid and plasmacytoid dendritic cells at any posttransplantation time point and in the counts of NK cells and naïve and memory/effector CD4 T cells on day 180 and 365 (data not shown).

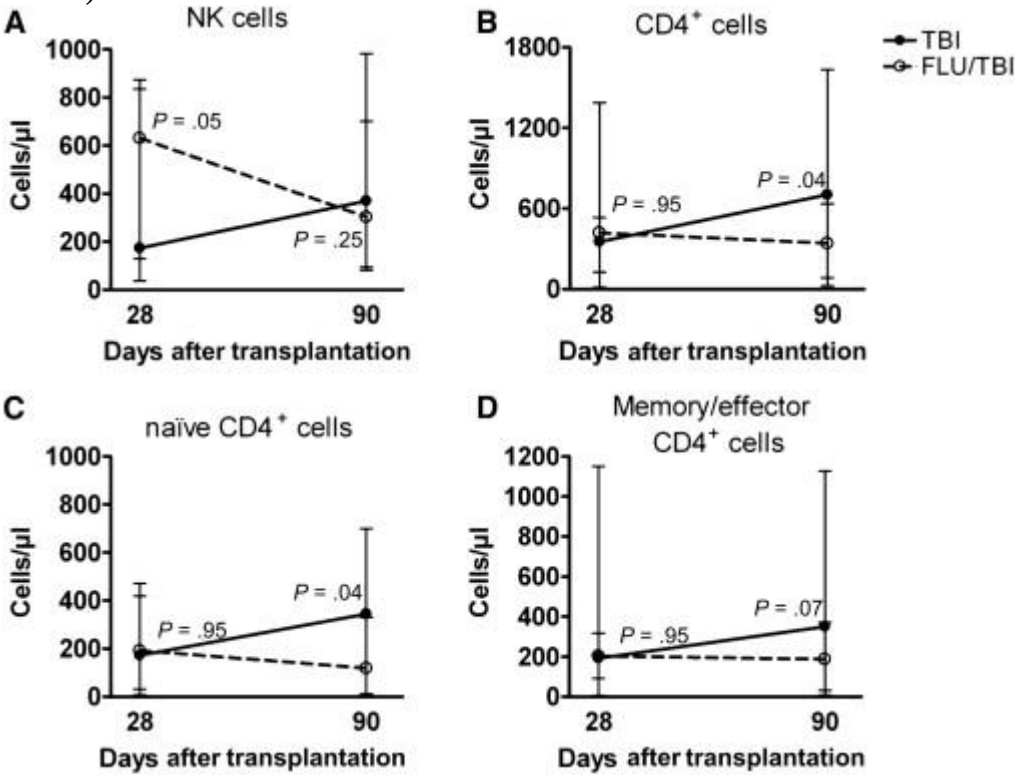

Figure 2.
Immune reconstitution. Mean absolute numbers of natural killer (NK) cells (A), $CD4^+$ (B), naïve $CD4^+$ (C), and memory/effector $CD4^+$ (D) cells at days 28 (TBI: n = 16; FLU/TBI: n = 7) and 90 (TBI: n = 8; FLU/TBI: n = 13) after transplantation in patients conditioned with 2-Gy total body irradiation only (TBI) or in combination with 90 mg/m^2 fludarabine (FLU/TBI). Bars represent standard error of the mean. *P* values are 2 tailed.

GVHD

The cumulative incidences of grades II to IV and grades III to IV acute GVHD at 120 days in the TBI arm were 32% and 11%, respectively, and in the FLU/TBI arm, 46% (grades I to IV acute GVHD: HR, 1.60 [95% confidence interval (CI), .8 to 3.1]; $P = .16$) and 7% (grades III to IV acute GVHD: HR, .66 [95% CI, .2 to 2.7]; $P = .56$), respectively (Figure 3A). Although the cumulative incidence of chronic GVHD was higher in the FLU/TBI arm at 3 years (72% versus 48%), the difference did not reach statistical significance (HR, 1.52 [.9 to 2.7]; $P = .14$) (Figure 3B).

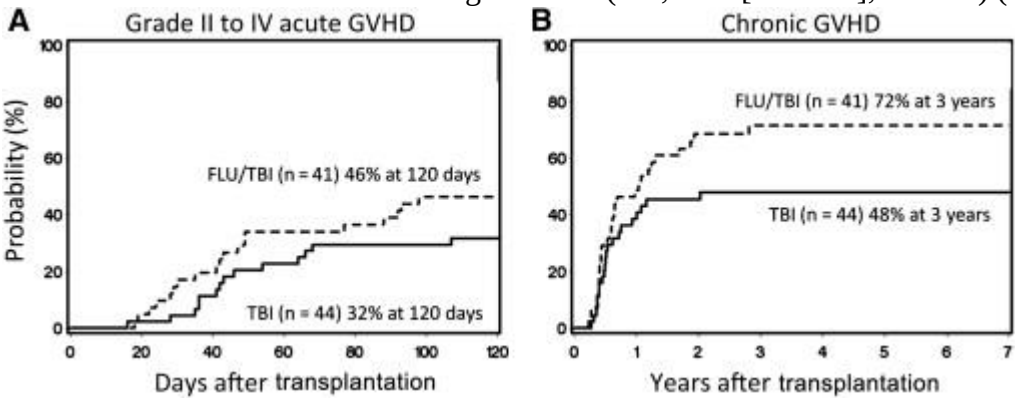

Figure 3.
Graft-versus-host disease. Cumulative incidences of grade II to IV acute (A) and chronic (B) GVHD among patients conditioned with 2-Gy total body irradiation only (TBI) or in combination with 90 mg/m^2 fludarabine (FLU/TBI).

Regimen-Related Toxicities, Infections, and NRM

The most common toxicities were, as expected, reversible neutropenia and thrombocytopenia. In general, toxicities unrelated to the blood and bone marrow were mild with 14 patients in both arms (TBI arm: 32%, FLU/TBI arm: 34%) experiencing 1 or more grade III to IV toxicities. No differences in distribution of toxicities were observed between arms (Supplementary Table 1). One patient in the FLU/TBI arm developed a squamous cell carcinoma 3.5 years after transplantation. The cumulative 3-year incidences of bacterial (TBI: 64%, FLU/TBI: 68%; $P = .52$), viral (TBI: 60%, FLU/TBI: 68%; $P = .27$), and fungal (TBI: 21%, FLU/TBI 22%; $P = .87$) infections were similar in the 2 arms. NRM at 3 years was 9% in the TBI arm and 7% in the FLU/TBI arm (HR, .67 [95% CI, .1 to 3.0]; $P = .59$) (Figure 4). Of the 4 NRM deaths in the TBI arm, 1 was due to chronic GVHD, and 1 was due to multiple pulmonary emboli and hemolytic uremic syndrome, whereas 2 were caused by GVHD complicated with severe sepsis on days 194 and 287 after transplantation. In the FLU/TBI arm, 3 NRM deaths were observed on days 9, 59, and 254, all associated with sepsis.

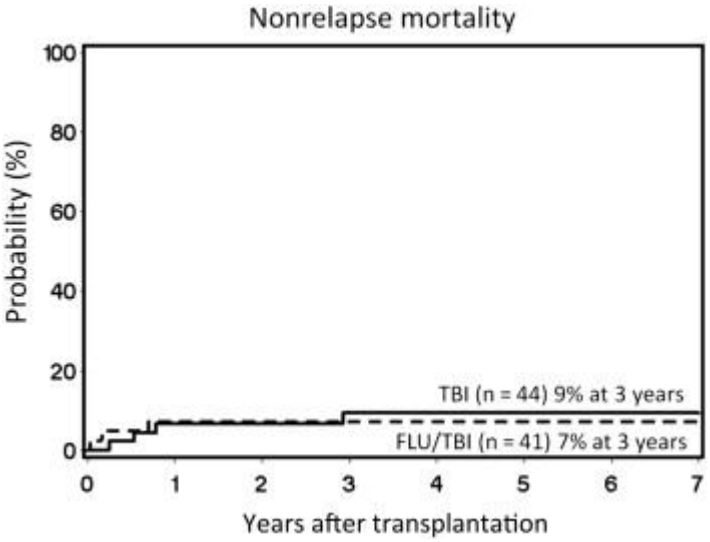

Figure 4.
Nonrelapse mortality. Cumulative incidence of nonrelapse mortality among patients conditioned with 2 Gy total body irradiation only (TBI) or in combination with 90 mg/m^2 fludarabine (FLU/TBI).

Relapse and Survival

At the time of analysis, the median follow-up of 48 surviving patients was 5 (range, 1.5 to 8) years. There was a trend for a higher progression/relapse rate in the TBI arm than in the FLU/TBI arm (55% versus 40% at 3 years; HR, .55 [95% CI, .3 to 1.0]; *P* = .06) (Figure 5A), which translated into a trend toward a higher relapse-related mortality (37% versus 28% at 3 years; HR, .53 [95% CI, .3 to 1.1]; *P* = .09) (Figure 5B) and worse progression-free survival (36% versus 53% at 3 years; HR, .56 [95% CI, .3 to 1.0]; *P* = .05) (Figure 6A). Because of limited sample size, it was not possible to evaluate if the effects of FLU vary according to disease; however, similar trends of increased relapse in the TBI arm were observed when patients with lymphoid and myeloid malignancies were analyzed separately (data not shown).

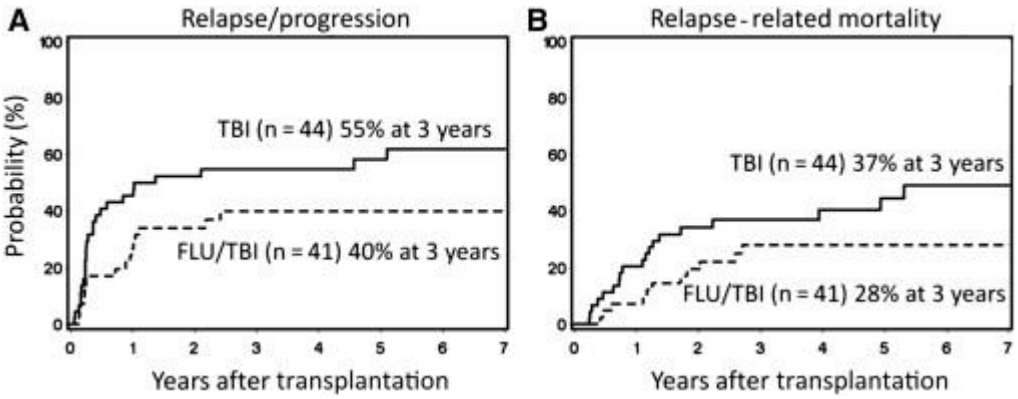

Figure 5.
Relapse or progression incidence and relapse-related mortality. Cumulative incidences of relapse or progression (A) and relapse-related mortality (B) among patients conditioned with 2-Gy total body irradiation only (TBI) or in combination with 90 mg/m^2 fludarabine (FLU/TBI).

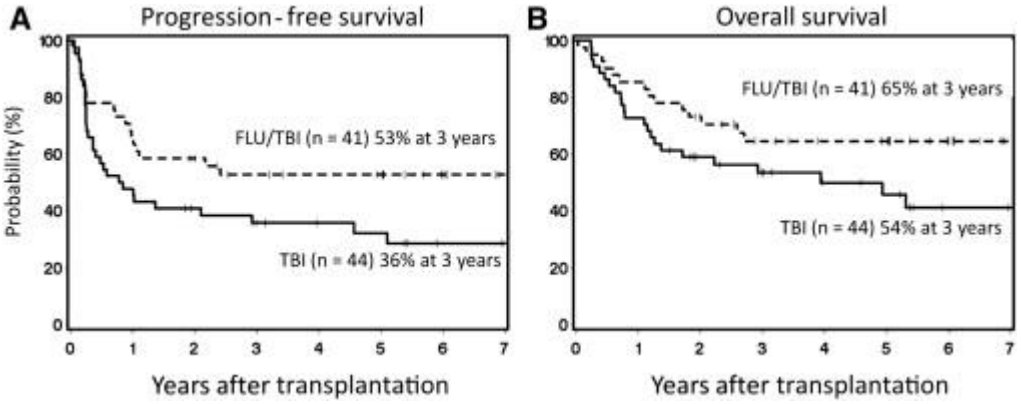

Figure 6.
Progression-free survival and overall survival. Cumulative incidences of progression-free survival (A) and overall survival (B) among patients conditioned with 2-Gy total body irradiation only (TBI) or in combination with 90 mg/m^2 fludarabine (FLU/TBI).

Twenty-six patients relapsed in the TBI arm. At the end of follow-up, 3 were alive with relapsed disease (HL, n = 2; NHL, n = 1), whereas 4 (HL, n = 1; NHL, n = 2; MM, n = 1) were brought back into remission. In the FLU/TBI arm, 16 patients relapsed, of whom 5 were alive at the end of follow-up. One of the 5 who had HL was brought back into remission, 3 had progressive disease, and 1 patient with CLL had stable disease.
Compared with the TBI arm, there was a trend toward higher overall survival in the FLU/TBI arm (54% versus 65% at 3 years; HR, .57 [95% CI, .3 to 1.1]; *P* = .09) (Figure 6B).

Discussion

Historically, the addition of FLU to 2-Gy TBI to the conditioning regimen successfully reduced the rejection rate from 20% to 3%, by augmenting the pretransplantation immunosuppression 4, 5 and 17. In a retrospective analysis of the first 176 patients, however, the question of whether adding FLU to 2-Gy TBI-exposed patients at low to moderate risk of rejection to unnecessary toxicity was raised [5]. In the current study, 85 patients at low to moderate risk of rejection were randomized to conditioning with either 2-Gy TBI alone or in combination with FLU. Baseline characteristics were balanced between groups, except for a slight imbalance toward more patients with NHL compared with AML in the FLU/TBI group. Although the number of prior treatment regimens and transplantations were similar in both groups, 21 of 41 patients in the FLU/TBI arm had failed autologous transplantations. In the TBI group, 23 of 44 patients failed autografts and 3 failed allografts. Only 2 rejections were observed in the trial, both in the TBI-only group. One had a prior allograft from a different donor, possibly indicating that donor cells given in the first transplantation were not adequately myelosuppressed with TBI only. For this reason, the current practice is to condition patients who have failed prior allografts with 3-Gy TBI in addition to FLU. The second patient who rejected the graft had only received 1 cycle of chemotherapy before transplantation, which possibly was insufficiently myelosuppressive.
All patients had initial engraftment, even the 2 who experienced rejection. Donor granulocyte chimerism was prompt, with no difference between arms. However, significant differences were observed in the rate of achieving donor T and NK cell chimerism. Patients conditioned with FLU/TBI had significantly higher levels of donor T cell chimerism at days 28 and 84 after transplantation. Day-28 NK cell chimerism was also higher in the FLU/TBI group. Low levels of donor T and NK cell chimerism have previously been associated with graft rejection in settings of nonmyeloablative, reduced-intensity and in high-dose conditioning. In a cohort of 38 patients who underwent transplantation after conditioning with FLU/TBI, Keil et al. [18] observed that donor T cell chimerism <90% at day 28 was associated with a higher rejection rate. Similarly, analyses by Baron et al. have demonstrated that day-14 donor T and NK cell chimerism levels <50%,

conditioning with 2-Gy TBI with or without FLU (90 mg/m^2) were associated with increased graft rejection 19 and 20. In a recent study of pediatric patients conditioned with a variety of high-dose and reduced-intensity regimens, early low donor T and NK cell chimerism levels were also associated with increased risk of graft rejection, regardless of conditioning intensity [21].
Although the addition of FLU was associated with an increased number of days with absolute granulocyte counts below 500 cells/μL and a trend toward increased red blood cell transfusion needs, no increases in NRM or bacterial, viral, or fungal infection rates were observed. However, it is possible that the 2 early septic deaths in the FLU/TBI arm could be related to FLU. NRM rates in both arms were lower than in the previous retrospective analysis [5] that prompted the current study, probably reflecting the recent years' overall improvement in supportive care, which has lowered NRM in general and, in particular, also negated the effect of FLU on NRM. Although 55% of the patients in the current study had failed a high-dose transplantation before entering the trial, NRM rates still compared favorably to previously published data on nonmyeloablative regimens, such as fludarabine/busulfan in AML/MDS (NRM 26% at 2 years) [22] and fludarabine/cyclophosphamide/rituximab in follicular lymphoma (NRM 15% at 5 years) [23]. A recent registry study investigating the outcome of low-intensity conditioning allogeneic transplantation in patients with NHL who had relapsed after autologous transplantations, reported an NRM of 44% at 3 years [24].
In this study, the relapse/progression incidence, relapse-related mortality, and progression-free survival were superior in the FLU/TBI arm, whereas only nonsignificant trends toward higher rates of acute and chronic GVHD were observed. It is possible that some of the effect on relapse may be due to the antineoplastic effects of the FLU, although this is unlikely in this patient population. The observed differences in outcome between arms in our study were likely due to differences in donor T and NK cell chimerism kinetics. Our results are in agreement with previous observations by both Keil et al. [18] and our own group [19], where donor T cell chimerism <90% (day 28 after transplantation) and <75% (day 84 after transplantation) were associated with a higher risk of relapse and lower progression-free survival. Although it is agreed that the graft-versus-tumor effects and GVHD after HLA-identical HCT are mainly a product of T cell activity [25], the roles of NK cells are far less explored. Killer cell immunoglobulin-like receptor genes are inherited independently from HLA, and will be mismatched in 75% of matched related transplantations [26]. In 2 studies by Baron et al., in which patients were conditioned with FLU/TBI, and NK cell chimerism was investigated along with T cell chimerism, T cell chimerism was mainly associated with the development GVHD, whereas rapid development of NK cell chimerism was associated with lower relapse rates and better progression-free survival 20 and 27. Notably, no association between NK cell chimerism and GVHD was observed [27]. As T and NK cell chimerism correlated closely at day +28 in our study, it is an open question whether the superior outcomes in the FLU/TBI arm were due to faster, more complete donor T cell chimerism, NK cell chimerism, or both.
Reconstitution of immune cells was similar between arms, except for NK and CD4$^+$ T cells. Why only CD4$^+$ T cell counts were affected in the current study is not clear, but these results are in line with findings by De Bock et al. that showed levels of CD4$^+$ cells, including naïve CD4$^+$, regenerated slower than other cell subsets after nonmyeloablative conditioning with fludarabine and low-dose TBI [28]. Although our findings should be interpreted with caution because of small sample size, it is possible that they represent an effect induced by fludarabine, as both CD8$^+$ and CD4$^+$ cells have been shown to be highly sensitive to depletion by fludarabine in CLL [29], whereas NK cells have been shown to be more resistant [30], explaining the higher NK and lower CD4$^+$ cell counts in the FLU/TBI arm. Conversely, with 2-Gy TBI alone conditioning killing fewer T (including CD4$^+$) cells, the higher levels of CD4$^+$ cells could be a consequence of a longer period with mixed chimerism.
In conclusion, the current study showed that in the setting of a randomized phase III clinical trial, the addition of FLU (30 mg/m^2) for 3 days to conditioning with 2-Gy TBI before allogeneic HCT

from HLA-matched related donors with CSP and mycophenolate mofetil as postgrafting GVHD prophylaxis is safe and efficacious. FLU did not increase NRM or the incidence of infections and was associated with lower relapse and better progression-free survival, probably because of the induction of higher levels of donor T and NK cell chimerism early after transplantation. Furthermore, the study suggests that donor engraftment in heavily pretreated patients is possible with only 2-Gy TBI, but that a lower conditioning intensity correlates to a slower speed of engraftment, which may be associated with a decrease in successful outcome.

Although NRM is low with the current nonmyeloablative regimens, relapse still represents a challenge. In the context of reduced-intensity regimens, where conditioning intensity and antineoplastic activity is increased compared with the nonmyeloablative regimens, the possible beneficial effects on relapse rates are counterbalanced by an increase in NRM at 19% to 25% 31, 32 and 33.

A possible solution to this problem could be further studies with minimal conditioning intensity, where the issue of slow development of donor chimerism could be approached by augmenting the antitumor effect by adding disease-specific agents, such as anti-CD20 antibodies [34] or receptor tyrosine kinase inhibitors [35], in the posttransplantation period, thereby allowing full-donor chimerism ample time to develop. A different approach could also include substituting external beam radiation with targeted α-emitter – labeled anti-CD45 – based radioimmunotherapy, which reduces off-target radiation toxicity and increases radiation dose selectively in cells responsible for rejection and tumor cells [36].

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