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Allogeneic – Adult

# Reduced-Intensity Conditioning with Fludarabine, Melphalan, and Total Body Irradiation for Allogeneic Hematopoietic Cell Transplantation: The Effect of Increasing Melphalan Dose on Underlying Disease and Toxicity

George L. Chen[1,*], Theresa Hahn[1], Gregory E. Wilding[2], Adrienne Groman[3], Alan Hutson[3], Yali Zhang[1], Usman Khan[1], Hong Liu[4], Maureen Ross[1], Barbara Bambach[5], Meghan Higman[5], Vishala Neppalli[6], Sheila Sait[7], AnneMarie W. Block[7], Paul K. Wallace[8], Anurag K. Singh[9], Philip L. McCarthy[1]

[1] *BMT Program, Department of Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
[2] *Department of Biostatistics, School of Public Health and Health Professions, University at Buffalo, Buffalo, New York*
[3] *Department of Biostatistics and Bioinformatics, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
[4] *Department of Oncology, Buffalo Medical Group, Buffalo, New York*
[5] *Department of Pediatrics, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
[6] *Department of Pathology, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
[7] *Cytogenetics Laboratory, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
[8] *Flow Cytometry Laboratory, Roswell Park Comprehensive Cancer Center, Buffalo, New York*
[9] *Department of Radiation Medicine, Roswell Park Comprehensive Cancer Center, Buffalo, New York*

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A B S T R A C T

Disease relapse and toxicity are the shortcomings of reduced-intensity conditioning (RIC) for allogeneic hematopoietic cell transplantation (alloHCT). We hypothesized that adding total body irradiation (TBI) to and decreasing melphalan (Mel) from a base RIC regimen of fludarabine (Flu) and Mel would increase cytoreduction and improve disease control while decreasing toxicity. We performed a phase II trial of Flu 160 mg/m$^2$, Mel 50 mg/m$^2$, and TBI 400 cGy (FluMelTBI-50, n = 61), followed by a second phase II trial of Flu 160 mg/m$^2$, Mel 75 mg/m$^2$, and TBI 400 cGy (FluMelTBI-75, n = 94) as RIC for alloHCT. Outcomes were compared with a contemporaneous cohort of 162 patients who received Flu 125 mg/m$^2$ and Mel 140 mg/m$^2$. Eligibility criteria were equivalent for all 3 regimens. All patients were ineligible for myeloablative/intensive conditioning. The median (range) follow-up for all patients was 51 (15 to 103) months. Day 100 donor lymphoid chimerism and transplant-related mortality, neutrophil and platelet engraftment, acute and chronic graft versus host disease incidence, overall survival (OS), and progression-free survival (PFS) were equivalent between FluMel, FluMelTBI-50, and FluMelTBI-75. Stomatitis was decreased for FluMelTBI versus FluMel ($P < .01$). PFS for patients not in complete remission on alloHCT was improved for FluMelTBI-75 versus FluMel ($P = .03$). On multivariate analysis, OS ($P = .05$) and PFS ($P = .05$) were significantly improved for FluMelTBI-75 versus FluMel. FluMelTBI-75 is better tolerated than FluMel, with improved survival and disease control.



## INTRODUCTION

Reduced-intensity conditioning (RIC) regimens for allogeneic hematopoietic cell transplantation (alloHCT) were developed on the basis of the hypothesis that hematologic malignancies could be controlled by allograft-derived immunologic antitumor effects without intensive cytoreductive chemotherapy or radiation and their toxicities. RIC regimens, however, may not control active disease caused by less upfront cytoreduction and disease progression that occurs before the onset of graft-versus-tumor effects. Some RIC regimens have incorporated more intensive cytoreduction to control the underlying disease but at the cost of increased regimen-related toxicity (RRT). The optimum RIC regimen has not been defined.

Fludarabine and melphalan (FluMel) and fludarabine and total body irradiation (FluTBI) are standard RIC regimens [1-3].

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* Correspondence and reprint requests: George L. Chen, MD, Department of Medicine, Roswell Park Comprehensive Cancer Center, Elm & Carlton Streets, Buffalo, NY 14263.
*E-mail address:* george.chen@roswellpark.org (G.L. Chen).

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Both are adequately immunosuppressive and can induce full donor chimerism after alloHCT. Fludarabine (Flu) is a potent immunosuppressive nucleoside analog. It is a radiosensitizer and can synergistically enhance the cytotoxic effect of melphalan by inhibiting DNA polymerase function which can repair the damage caused by melphalan (Mel) induced DNA cross-linking [4-7]. We hypothesized that combining Flu, Mel, and total body irradiation (TBI) would result in a RIC regimen that would be adequately immunosuppressive to enable full donor chimerism. At the same time, Flu, Mel, and TBI would synergize, resulting in increased cytoreduction and better disease control while allowing a lower melphalan dose with less RRT and transplant related-mortality (TRM).

Starting from a base regimen of Flu 125 mg/m$^2$ and Mel 140 mg/m$^2$ (FluMel), we reasoned that a reduction in toxicity, primarily mucositis, could be achieved at the cost of increased relapse due to reduced regimen intensity by decreasing the dose of melphalan to 50 mg/m$^2$ [1]. To compensate for the reduced regimen intensity from the lower Mel dose, we increased the Flu dose from 125 to 160 mg/m$^2$ and added 400 cGy TBI. Flu has previously been used in RIC in doses ranging from 125 to 200 mg/m$^2$. The dose of TBI was justified on the basis of a previous study showing that TRM after RIC with Flu and 200 cGy TBI versus Flu and 400 cGy was not significantly different while relapse was decreased but not significantly different [8].

We tested the combination of Flu 160 mg/m$^2$, Mel 50 mg/m$^2$, and TBI 400 cGy (FluMelTBI-50) in a phase II clinical trial. Although FluMelTBI-50 effectively induced full donor chimerism and had an acceptable TRM, the incidence of disease progression was significant. Therefore, we increased the Mel dose to 75 mg/m$^2$ in a second phase II clinical trial, FluMelTBI-75, to increase cytoreduction and potentially improve disease control. In this report, we present the clinical outcomes from the FluMelTBI-50 and FluMelTBI-75 trials. To better evaluate the short- and long-term potential of these new regimens, we compared the clinical outcomes with those from a contemporaneous patient cohort treated at our center with the base FluMel regimen.

## METHODS

### *Patients*

All patients age ≥4 and ≤75 years with a hematologic disease requiring alloHCT ineligible for myeloablative conditioning were evaluated for RIC. These patients were suboptimal myeloablative alloHCT candidates because of HLA mismatch, prior autologous hematopoietic cell transplant (autoHCT) or alloHCT, Karnofsky performance score (KPS) ≤80, age ≥60 years, and intermediate or high-risk Hematopoietic Cell Transplant Specific Comorbidity Index (HCT-CI). Patients with insurance coverage who agreed to participate in a clinical trial received FluMelTBI-50 or FluMelTBI-75. Patients who were unable or unwilling to participate in these trials received nonprotocol therapy with FluMel (Figure 1). Eligibility criteria for FluMelTBI-50, FluMelTBI-75, and FluMel were equivalent as follows: Patients with any underlying hematologic malignancy not eligible for myeloablative conditioning were eligible, including those who received prior HCT. Patients were excluded for uncontrolled central nervous system disease, Karnofsky performance score (KPS) ≤50, hemoglobin-corrected carbon monoxide lung-diffusing capacity <40%, cardiac ejection fraction <40%, bilirubin, liver alkaline phosphatase, and transaminases ≥3x the upper limit of normal, Child's class B/C liver failure, creatinine clearance <40 mL/min, HIV infection, pregnancy, and uncontrolled diabetes mellitus, cardiovascular disease, serious infection, or other potentially harmful medical condition.

Related donors were HLA matched at ≥5/6 loci at A, B, or DRB1. Unrelated donors were HLA matched at ≥8/10 loci at A, B, C, DRB1, or DQB1.

Patients and donors provided written informed consent or assent for all studies. Parental permission for children was obtained when appropriate. Unrelated donors were procured and consented through the National Marrow Donor Program. All studies were approved by the institutional review board. FluMelTBI-50 and FluMelTBI-75 were registered at ClinicalTrials.gov as NCT00856388 and NCT01529827. All authors had access to the primary clinical trial data, which was analyzed by G.L.C., T.H., G.W., A.G., A.H., Y.Z., and P.L.M.

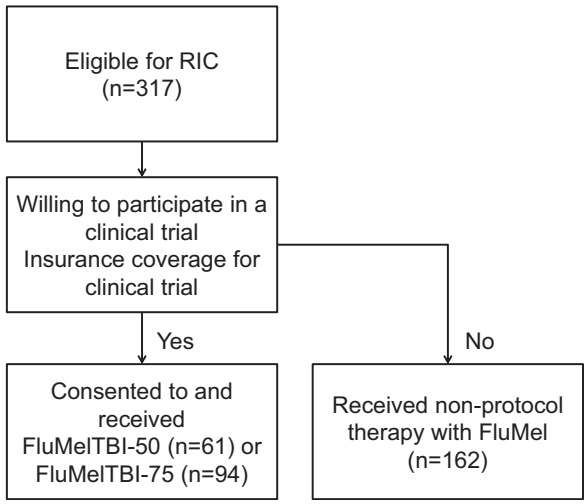


**Figure 1.** Consort diagram. All patients eligible for a RIC regimen received FluMel, FluMelTBI-50, or FluMelTBI-75.

### *Treatment Plan*

*FluMelTBI-50 and FluMelTBI-75*

From March 2009 to April 2012, 61 patients were enrolled onto a phase II trial (FluMelTBI-50) of RIC with fludarabine 40 mg/m$^2$ intravenously on days −5, −4, −3, and −2 before donor graft infusion, melphalan 50 mg/m$^2$ intravenously on day −2, and TBI 200 cGy × 2 fractions on day −1. Preliminary analysis demonstrated that the regimen induced full donor chimerism with acceptable TRM but with a higher than desired relapse rate. Thereafter, patients were enrolled onto a second phase II trial (FluMelTBI-75) that used the same Flu and TBI doses but an increased melphalan dose (75 mg/m$^2$) with the intent to reduce relapse. From May 2012 to February 2015, 94 patients were treated with FluMelTBI-75.

*FluMel*

From September 2007 to November 2014, 162 patients received nonprotocol therapy with FluMel, which consisted of Flu 25 mg/m$^2$ intravenously on days −6, −5, −4, −3, −2 and Mel 70 mg/m$^2$ intravenously on days −3, −2.

Flu and Mel dosing used body surface area based on actual body weight. TBI was delivered as previously described [9].

*Supportive Care*

Acute graft-versus-host disease (GvHD) prophylaxis consisted of tacrolimus, methotrexate, and mycophenolate as previously described [10]. Antibacterial, antifungal, and antiviral prophylaxes were given according to institutional standards. Cytomegalovirus antigenemia was monitored weekly and preemptively treated with ganciclovir.

*Definitions*

Comorbidities were classified according to the HCT-CI [11]. Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count ≥0.5 × 10$^6$ cells/mL. Platelet engraftment was defined as the first day with a platelet count ≥20 × 10$^6$ platelets/mL without platelet transfusions in the preceding 7 days. Toxicity, acute GvHD, and chronic GvHD data were graded according to established criteria [12-16]. Donor chimerism was determined by short tandem repeat (STR) analysis [17]. Full donor chimerism was defined as ≥95% of lymphoid cells with donor STRs.

### *Statistical Methods*

*FluMelTBI-50*

Sample size calculation was based on the expected confidence interval corresponding to the estimated TRM rate of 25% based on the historical rate of 15% to 20% for patients receiving RIC with fludarabine and cyclophosphamide and the expected increased TRM with FluMelTBI-50. The calculated sample size was 50 evaluable subjects with 61 subjects accrued. Planned interim analyses of patient safety (day 100 TRM) was performed when 10, 20, and 30 patients were accrued. Early stopping rules for excess toxicity were based on a 1-sided null hypothesis test of the true TRM rate being ≤30%, at a nominal significance level of 0.05, with an O'Brien-Fleming adjustment for multiple testing [18]. The stopping rule was not triggered for FluMelTBI-50. Preliminary analysis of FluMelTBI-50 outcomes demonstrated a day 100 TRM of 11% and a disease progression rate of 11%. Therefore, Mel dosing was increased to 75 mg/m$^2$ to improve disease control in the subsequent FluMelTBI-75 phase II trial.

*FluMelTBI-75*

Sample size was based on detecting a true day 100 TRM >20%. This was set higher than the 11% observed in FluMelTBI-50 due to the higher Mel dose given. The calculated sample size was 81 evaluable subjects, defined as subjects observed ≥100 days without disease progression, with 94 subjects were accrued. Enrollment followed a Kepner Chang type II phase II design. A planned interim analysis of patient safety (day 100 TRM) was performed after 41 evaluable patients were accrued. With this design, the probability was 80.9% of detecting a true TRM rate of 10% and 4.9% to falsely conclude the true TRM rate was <20%.

Descriptive statistics were used to summarize baseline patient characteristics, disease history, treatment related variables, and the rate of complete donor chimerism. The cumulative incidence function was used to estimate day +100 TRM, acute GvHD, and chronic GvHD. Relapse was a competing risk for TRM. Disease progression and death were competing risks for acute and chronic GvHD. Gray's test was used to determine statistical significance. Regimen-related toxicities were analyzed as $2 \times 3$ contingency tables with Fisher's exact test. The Kaplan-Meier method was used to estimate time-to-event distributions for overall survival (OS) and progression-free survival (PFS). OS was defined as time from alloHCT to death from any cause. PFS was defined as time from alloHCT to disease progression or death from any cause. Patients who had not died or progressed were censored at the date of last follow-up. GvHD and death were competing risks for disease progression. Associations between potential confounders (age, disease risk, HLA matching, disease, pre-alloHCT disease status, KPS, prior HCT, graft source) and OS and PFS were evaluated in univariate and multivariate Cox proportional hazards models. Multivariate analysis adjusted for all statistically significant risk factors in the univariate analysis. The proportionality assumption was met for all multivariate analyses. Confidence intervals were calculated using log-log transformations [19]. *P* values ≤.05 were considered significant. All statistical analyses and plots were performed using SAS software, version 9.3 or higher (SAS Institute, Cary, NC), or R (R Foundation for Statistical Computing, Vienna, Austria) [20]. Clinical outcome data were prospectively gathered by trained data managers. Study endpoints were determined by a clinical epidemiologist and reviewed and confirmed by attending physicians.

## RESULTS

### *Patient and Disease Characteristics*

Patient demographics are presented in Table 1. Median (range) follow-up was 65 (18 to 103) and 67 (48 to 86) months for FluMel and FluMelTBI-50. Median follow-up was the shortest for FluMelTBI-75 at 33 months because trial enrollment was sequenced after FluMelTBI-50 enrollment. Median follow-up was greater than the 1- and 2-year and almost equal to the 3-year time points used in our analysis.

Time from diagnosis to alloHCT, disease status on alloHCT, donor and graft characteristics, and prior HCT status were evenly distributed among the 3 treatment regimens, but age, underlying disease, HCT-CI, and KPS were not. Patients receiving FluMelTBI-50 or FluMelTBI-75 were significantly older (>60 years) than those receiving FluMel. Twice as many patients receiving FluMelTBI-75 as FluMel or FluMelTBI-50 were diagnosed with myelodysplastic syndrome. A higher proportion of patients receiving FluMelTBI-50 or FluMelTBI-75

**Table 1**
Patient Demographics*

| | FluMel (n = 162) | FluMelTBI-50 (n = 61) | FluMelTBI-75 (n = 94) | *P* Value |
|---|---|---|---|---|
| Median age, yr (range) | 54.5 (8-72) | 57 (5-72) | 60 (12-73) | .001 |
| Median time from diagnosis to alloHCT, mo (range) | 9 (2-277) | 10 (2-315) | 8 (3-237) | NS |
| Age, yr | | | | .02 |
| ≤39, N (%) | 24 (15)† | 7 (12)‡ | 11 (12)§ | |
| 40-59, N (%) | 89 (55) | 27 (44) | 35 (37) | |
| ≥60, N (%) | 49 (30) | 27 (44) | 48 (51) | |
| Disease | | | | .03 |
| ALL, N (%) | 12 (7) | 5 (8) | 5 (5) | |
| AML, N (%) | 76 (47) | 27 (44) | 40 (43) | |
| HL/NHL, N (%) | 32 (20) | 10 (16) | 12 (13) | |
| MDS/MPN, N (%) | 25 (15) | 10 (16) | 32 (34) | |
| Other, N (%) | 17 (11) | 9 (15) | 5 (5) | |
| Disease status before transplant | | | | NS |
| CR, N (%) | 76 (47) | 27 (44) | 42 (45) | |
| Not in CR, N (%) | 86 (53) | 34 (56) | 52 (55) | |
| Donor | | | | NS |
| Related, N (%) | 41 (25)¶ | 21 (34) | 25 (27) | |
| Unrelated, N (%) | 121 (75) | 40 (66) | 69 (73) | |
| Graft source | | | | NS |
| Peripheral blood, N (%) | 139 (86) | 49 (80) | 79 (84) | |
| Bone marrow, N (%) | 23 (14) | 12 (20) | 15 (16) | |
| HCT-CI status | | | | .02 |
| Low, N (%) | 66 (41) | 20 (33) | 21 (22) | |
| Intermediate, N (%) | 31 (19) | 8 (13) | 24 (26) | |
| High, N (%) | 65 (40) | 33 (54) | 49 (52) | |
| HLA matching | | | | NS |
| RD, N (%) | 41 (25)¶ | 21 (34)# | 25 (27) | |
| 10/10 URD, N (%) | 90 (56) | 24 (39) | 52 (55) | |
| Mismatched URD, N (%) | 31 (19) | 16 (26) | 17 (18) | |
| KPS | | | | .01 |
| 50-70, N (%) | 71 (44) | 22 (36) | 54 (57) | |
| 80, N (%) | 69 (43) | 23 (38) | 25 (27) | |
| 90 or 100, N (%) | 22 (14) | 16 (26) | 15 (16) | |
| Prior hematopoietic cell transplant, N (%) | 40 (25) | 15 (25) | 14 (15) | NS |

ALL indicates acute lymphoblastic leukemia; AML, acute myeloid leukemia; HL, Hodgkin lymphoma; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin lymphoma; RD, related donor; URD, unrelated donor.

* Percentages have been rounded.

† Includes one 8-year-old subject.

‡ Includes two 5- and 12-year-old subjects.

§ Includes one 12-year-old subject.

¶ Includes 3 syngeneic donors.

# Includes 1 single antigen mismatched related donor.

had high HCT-CI. Compared with FluMel, a smaller proportion of FluMelTBI-50 patients had a KPS ≤80 whereas a larger proportion of FluMelTBI-75 patients had a KPS ≤80. In general, patients who received FluMelTBI-75 tended to have more adverse prognostic factors.

### *Engraftment and Chimerism*

The proportion of patients achieving full peripheral blood donor lymphoid chimerism by days 30 and 100 was not significantly different between FluMel, FluMelTBI-50, and FluMelTBI-75 (Table 2). The time of neutrophil and platelet engraftment was not significantly different among the 3 regimens (Table 2).

### *TRM, RRT, and Acute and Chronic GvHD*

Day 100 TRM was not significantly different between FluMel, FluMelTBI-50, and FluMelTBI-75 although the event kinetics differed by group (Table 2, Figure 2). The onset of FluMel events began early before day 40, whereas FluMelTBI-75 and FluMelTBI-50 events began progressively later. The most frequent cause of death within the first year after alloHCT was disease relapse for FluMel (14%) and FluMelTBI-50 (17%), and GvHD ± infection for FluMelTBI-75 (18%, Table 3).

The most common RRT observed after FluMel, FluMelTBI-50, and FluMelTBI-75 conditioning was stomatitis (Table 4). Clinically relevant stomatitis (Bearman or World Health Organization grade ≥2) was significantly reduced for FluMelTBI-50 or FluMelTBI-75 compared with FluMel ($P < .01$ and $P = .05$, respectively). The incidence of stomatitis was higher in FluMelTBI-75 than FluMelTBI-50 as expected from the higher dose of Mel. Renal toxicity was decreased ($P = .05$) and cardiac toxicity increased ($P = .01$) for FluMelTBI-50 and FluMelTBI-75 compared with FluMel. Renal toxicities were creatinine elevation to more than twice the baseline (N = 3) or requiring dialysis (N = 2) within 1 week of receiving melphalan. Cardiac toxicities consisted of atrial fibrillation (N = 5), decreased ejection fraction (N = 5), supraventricular tachycardia (N = 1), pericarditis (N = 2), cardiac arrest (N = 1), and myocardial infarction (N = 1). The incidence of other toxicities did not significantly differ between regimens.

The day 100 cumulative incidence of grade II-IV and III-IV acute GvHD was not significantly different between the three conditioning regimens (Table 2, Figure 3A,B), although the incidence of grade II acute GvHD was decreased from days 20 to 60 for FluMelTBI-50 and FluMelTBI-75 compared with FluMel. No significant differences in the cumulative incidence of National Institutes of Health (NIH) moderate/severe chronic GvHD were observed among the 3 regimens at 3 years (Table 2, Figure 3C).

### *Disease Responses*

We hypothesized that TBI would synergize with Mel to improve disease response and control in patients conditioned with FluMelTBI. Malignant disease not in complete remission (CR) on alloHCT is an adverse prognostic factor and the operating characteristics of RIC regimens are more fully revealed when disease response and durability are measurable [21]. For patients not in CR on alloHCT, those receiving FluMelTBI-75 were significantly more likely to achieve a CR after alloHCT (relative risk = 1.28, 95% confidence interval [95% CI] 1.03 to 1.61, $P = .04$) than FluMel. In contrast, subjects receiving FluMelTBI-50 were not more likely to achieve a CR after alloHCT compared with those receiving FluMel (relative risk = 1.01, 95% CI 0.74 to 1.39, $P$ = NS).

**Table 2**
Unadjusted Univariate AlloHCT Outcomes

| Characteristic | FluMel (N = 162) | FluMelTBI-50 (N = 61) | *P* Value | FluMeTBI-75 (N = 94) | *P* Value |
|---|---|---|---|---|---|
| Evaluable patients achieving full peripheral blood donor lymphoid chimerism, % (95% CI) | | | | | |
| 30 d (%) | 97 (92-99) | 89 (76-96) | | 95 (88-99) | NS |
| 100 d (%) | 96 (91-99) | 94 (83-99) | | 96 (89-99) | NS |
| Time to neutrophil engraftment, median (range) d | 15 (9-35) | 16 (10-39) | | 16 (10-29) | NS |
| Time to platelet engraftment, median (range) d | 17 (10-50) | 17 (10-74) | | 17 (9-64) | NS |
| TRM, % cumulative incidence (95% CI) | | | | | |
| 100 d | 10.5 (6.2-16) | 8.8 (3.2-18) | | 8.7 (4-15.6) | NS |
| Relapse/disease progression, % cumulative incidence (95% CI) | | | | | |
| 100 d | 14.0 (8.0-20) | 13.6 (6.3-23.8) | | 6.8 (2.7-13.3) | |
| Acute GvHD grade at day 100, % cumulative incidence (95% CI) | | | | | |
| II-IV | 53 (44-60) | 52 (39-64) | | 56 (45-65) | NS |
| III-IV | 26 (18-33) | 31 (19-44) | | 26 (17-36) | NS |
| NIH moderate/severe chronic GvHD, % cumulative incidence (95% CI) | | | | | |
| 1 yr | 12.3 (7.8-17.9) | 11.5 (5.0-20.9) | | 13.8 (7.7-21.6) | |
| 2 yr | 18.6 (13.0-25.0) | 21.3 (12.0-32.4) | | 21.2 (13.3-30.3) | |
| 3 yr | 23.3 (17.0-30.1) | 23.0 (13.3-34.2) | | 24.9 (15.8-35.0) | |
| OS, % (95% CI) | | | | | NS |
| 1 yr | 61 (53-68) | 64 (51-75) | | 62 (51-71) | |
| 2 yr | 50 (42-57) | 46 (33-58) | | 46 (36-56) | |
| 3 yr | 45 (37-52) | 46 (33-58) | | 45 (34-55) | |
| PFS, % (95% CI) | | | | | NS |
| 1 yr | 57 (46-67) | 61 (45-73) | | 61 (48-73) | |
| 2 yr | 50 (37-63) | 61 (45-73) | | 61 (48-73) | |
| 3 yr | 47 (32-60) | 53 (33-70) | | 61 (48-73) | |
| OS, % (95% CI), patients not in CR at alloHCT | | | NS* | | NS* |
| 1 yr | 45 (34-55) | 56 (38-71) | | 54 (39-66) | |
| 2 yr | 34 (24-44) | 41 (24-57) | | 41(28-55) | |
| 3 yr | 26 (17-36) | 41 (24-57) | | 41 (28-55) | |
| PFS, % (95% CI), patients not in CR at alloHCT | | | NS* | | .03* |
| 1 yr | 41 (25-57) | 47 (26-65) | | 55 (36-71) | |
| 2 yr | 34 (17-52) | 47 (25-65) | | 55 (36-71) | |
| 3 yr | 34 (17-55) | 47 (25-65) | | 55 (36-71) | |

* Pairwise comparison with FluMel.

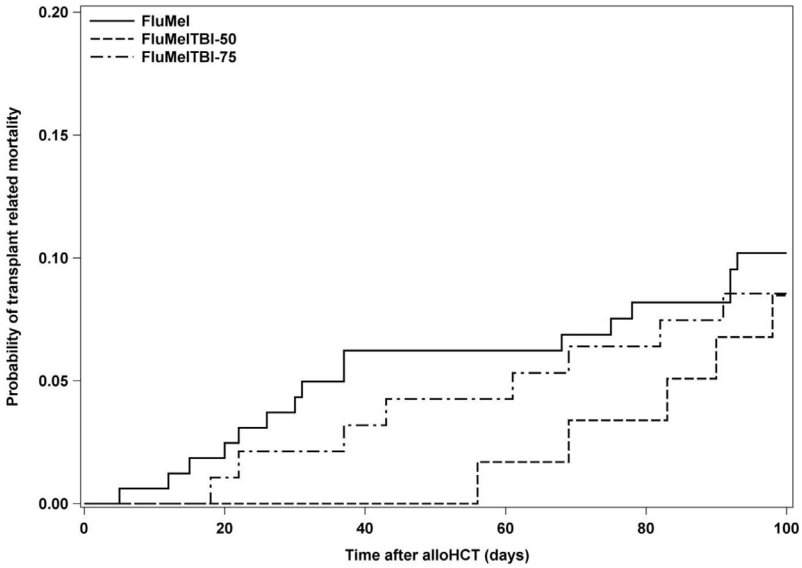


**Figure 2.** Transplant-related mortality. The day 100 cumulative incidence of transplant-related mortality was not significantly different among FluMel, FluMelTBI-50, and FluMelTBI-75. The kinetics of TRM events differed among groups, however, with the majority of FluMel events occurring between days 0 and 40, FluMelTBI-50 events after day 50, and FluMelTBI-75 events in between. Cumulative incidence was calculated using the competing risks of relapse and death.

**Table 3**
Causes of Death*

| | FluMel (N = 162) N (%) | FluMelTBI-50 (N = 61) N (%) | FluMelTBI-75 (N = 94) N (%) |
|---|---|---|---|
| On or before day +100 | | | |
| Disease relapse | 12 (7) | 3 (5) | 1 (1) |
| GvHD, GvHD/infection | 5 (3) | 2 (3) | 5 (5) |
| Infection | 2 (1) | 2 (3) | 1 (1) |
| Organ failure, graft failure/infection | 3 (2) | 1 (2) | 1 (1) |
| RRT, RRT/infection | 6 (4) | 1 (2) | 1 (1) |
| Day +101 to 1 year | | | |
| Disease relapse | 12 (7) | 7 (12) | 7 (7) |
| GvHD, GvHD/infection | 12 (7) | 6 (10) | 12 (13) |
| Infection | 5 (3) | 2 (3) | 4 (4) |
| Organ failure, graft failure/infection | 3 (2) | 1 (2) | 0 (0) |
| RRT (including HUS) | 0 (0) | 1 (2) | 2 (2) |
| Secondary cancer | 3 (2) | 0 (0) | 0 (0) |
| Unrelated | 3 (2) | 0 (0) | 2 (2) |
| >1 year | | | |
| Disease relapse | 14 (9) | 4 (7) | 3 (3) |
| GvHD, GvHD/infection | 7 (4) | 7 (11) | 7 (7) |
| Infection | 6 (4) | 2 (3) | 3 (3) |
| Organ failure, graft failure/infection | 1 (1) | 0 (0) | 0 (0) |
| RRT (including HUS) | 1 (1) | 0 (0) | 1 (1) |
| Secondary cancer | 2 (1) | 1 (2) | 0 (0) |
| Unrelated | 4 (2) | 3 (5) | 1 (1) |

HUS indicates hemolytic uremic syndrome.
* Percentages have been rounded.

### *OS and PFS*

Unadjusted OS and PFS were not significantly different between FluMel, FluMelTBI-50, and FluMelTBI-75, although the incidence of PFS at 3 years was higher for FluMelTBI-75 than FluMel due to a plateau in disease progression events after 1 year (Table 2).

Based on our univariate analysis (Table 5), we developed multivariate models for OS and PFS adjusted for conditioning regimen, age, donor match, disease status at alloHCT, and HCT-CI (Table 6). PFS was also adjusted for prior blood or marrow transplantation. KPS and underlying malignancy were not included in the final models because they correlated with HCT-CI and disease status at the time of alloHCT, respectively. Patients conditioned with FluMelTBI-50 had a lower but nonsignificant risk of death (OS) and disease progression (PFS) compared with FluMel. In contrast, patients conditioned with FluMelTBI-75 had significant reductions in the risk of death (30%, HR 0.70, 95% CI 0.49 to 1.00, $P = .05$) and disease progression (29%, HR 0.71, 95% CI 0.50 to 1.00, $P = .05$) compared with FluMel. Use of a mismatched unrelated donor, underlying disease not in CR at the time of alloHCT, higher HCT-CI, and prior HCT all negatively affected OS or PFS.

We performed a sensitivity analysis of OS and PFS for patients not in CR on alloHCT to investigate the durability of disease control after a specific RIC regimen. Unadjusted OS in patients with disease not in CR on alloHCT was improved but not statistically significant for FluMelTBI-75 versus FluMel (Figure 4A). Unadjusted PFS at 3 years in patients with disease

**Table 4**
Regimen-Related Toxicities*

| Toxicity | FluMel N (%) | FluMelTBI-50 N (%) | FluMelTBI-75 N (%) | *P* Value |
|---|---|---|---|---|
| Bladder | | | | NS |
| Grade 0-1 | 161 (99.4) | 61 (100) | 94 (100) | |
| Grade 2 | 1 (0.6) | 0 (0) | 0 (0) | |
| Cardiac | | | | .01 |
| Grade 0-1 | 159 (98) | 54 (89) | 89 (95) | |
| Grade 2-3 | 3 (2) | 7 (12) | 5 (5) | |
| Central nervous system | | | | NS |
| Grade 0-1 | 160 (99) | 60 (98) | 93 (99) | |
| Grade 2-3 | 2 (1) | 1 (2) | 1 (1) | |
| Gastrointestinal | | | | NS |
| Grade 0-1 | 156 (96) | 61 (100) | 91 (97) | |
| Grade 2-3 | 6 (4) | 0 (0) | 3 (3) | |
| Hepatic | | | | NS |
| Grade 0-1 | 161 (99.3) | 60 (98.4) | 92 (98) | |
| Grade 2-3 | 1 (0.7) | 1 (2) | 2 (2) | |
| Pulmonary | | | | NS |
| Grade 0-1 | 154 (95) | 57 (93) | 92 (98) | |
| Grade 2-4 | 8 (5) | 4 (7) | 2 (2) | |
| Renal | | | | .05 |
| Grade 0-1 | 157 (97) | 61 (100) | 94 (100) | |
| Grade 2-3 | 6 (4) | 0 (0) | 0 (0) | |
| Stomatitis (Bearman) | | | | <.01 |
| Grade 0-1 | 133 (76) | 58 (95) | 80 (85) | |
| Grade 2-4 | 39 (24) | 3 (5) | 14 (15) | |
| Stomatitis (WHO) | | | | .05 |
| Grade 0-1 | 117 (72) | 53 (87) | 75 (80) | |
| Grade 2-4 | 45 (28) | 8 (13) | 19 (20) | |
| Hepatic veno-occlusive disease | 2 (1) | 2 (3) | 1 (1) | NS |

No grade 3 bladder toxicities occurred. No grade 4 bladder, cardiac, central nervous system, gastrointestinal, hepatic, or renal toxicities occurred.
WHO indicates World Health Organization.
* Percentages have been rounded except between 99-100 and 0-1.

not in CR on alloHCT was significantly greater for FluMelTBI-75 than FluMel (55% [95% CI 36% to 71%] versus 34% [95% CI 17% to 55%], respectively, $P = .03$, Figure 4B). No significant difference was seen in OS or PFS when FluMelTBI-50 was compared with FluMel.

Our observations indicate that FluMel, FluMelTBI-50, and FluMelTBI-75 have equivalent engraftment, donor chimerism, day 100 TRM, and acute and chronic GvHD. FluMelTBI-50 and FluMelTBI-75 are acceptable RIC regimens that can induce full donor chimerism. FluMelTBI-50 and FluMelTBI-75 may be better tolerated with less mucositis and sensitivity to the effects of pre-transplant comorbidity. OS and PFS were improved in patients who received FluMelTBI-75, with the greatest benefit seen in those patients not in CR at the time of alloHCT.

## DISCUSSION

Toxicity and disease relapse have been the shortcomings of RIC regimens. In this study, we hypothesized that modification of the standard FluMel RIC by adding 400 cGy TBI and decreasing the Mel dose would result in improved disease response and decreased toxicity. We demonstrated in our regimens that evolved from FluMel to FluMelTBI-50 and finally FluMelTBI-75 that disease response improved in a dose-dependent manner as TBI was added and Mel increased from 50 to 75 mg/m$^2$ after an initial reduction from 140 mg/m$^2$. Toxicity was significantly decreased in FluMelTBI with mitigation of many baseline adverse prognostic characteristics. Our observations suggest a synergistic cytoreductive effect from the combination of Flu, Mel, and TBI that more completely induced CR after transplantation in patients with disease not in CR on alloHCT. These responses appeared to be durable and translated into increased survival and less relapse. Thus FluMelTBI, especially with 75 mg/m$^2$ of melphalan, may be of utility in less robust patients with residual malignant disease.

This is the largest report to date of FluMelTBI and the first to compare FluMelTBI with a contemporary cohort of FluMel. FluMelTBI has been reported as a myeloablative and haplo-cord transplantation regimen [22-26]. It has also been reported as RIC in 14 patients using Flu 200 mg/m$^2$, Mel 140 mg/m$^2$, and TBI 400 cGy (FluMelTBI-140) [27]. These doses of fludarabine and melphalan were higher than those used in FluMelTBI-75. As expected from the greater regimen intensity, more toxicity was observed with Bearman grade 2 to 4 mucositis occurring in 49% versus 15% of patients conditioned with FluMelTBI-140 versus FluMelTBI-75. OS and PFS at 1 year were comparable: 57% and 57% for FluMelTBI-140 versus 61% and 62% for FluMelTBI-75. These results suggest that in regimens combining FluMel with 400 cGy TBI, disease response plateaus at Mel 75 mg/m$^2$, whereas toxicity continues to increase with Mel dose. Therefore, FluMelTBI-75 may be the optimum dosing for RIC because it achieves the maximum disease response while maintaining acceptable toxicity.

The primary limitation of these 2 phase II trials was their single-arm design, which did not allow comparison with a standard RIC regimen such as FluMel to determine suitability for phase III testing. Comparisons of single-arm phase II trials to historical controls have high false-positive rates for activity due to substantial improvements in the clinical care of patients [28]. The original reports of FluMel and FluTBI were published >11 years ago, making comparison of our results with the original outcomes less informative [1-3,29,30]. To provide a proper context for interpreting the outcome data, we used as a comparator a contemporaneous (rather than historic) cohort treated with FluMel during the same time period as the FluMelTBI subjects. Despite this, there were still significant differences in age, underlying disease, and HCT-CI among the 3 groups, with median age and HCT-CI being greater in FluMelTBI-75. These differences in age and HCT-CI would have

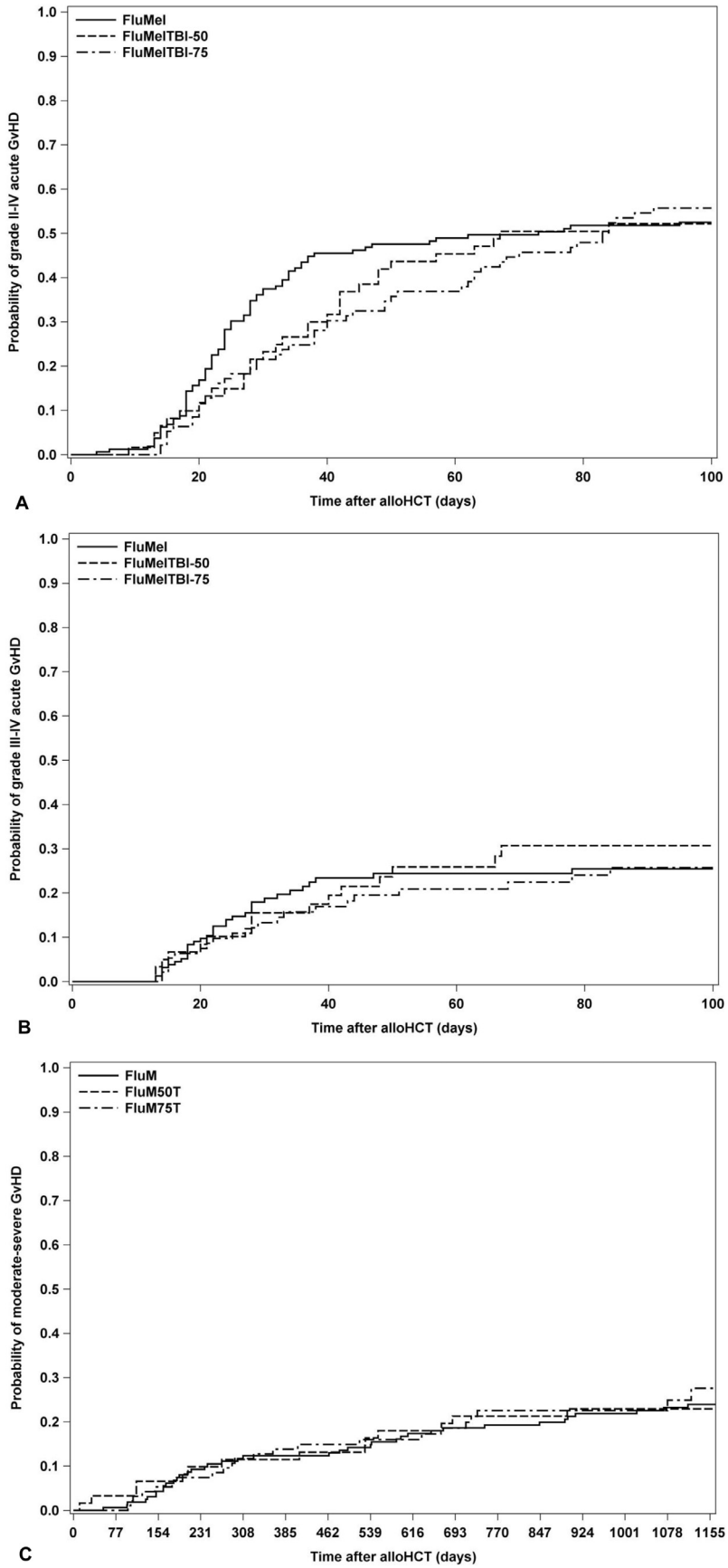


**Figure 3.** GvHD. All cumulative incidences were calculated using the competing risks of relapse and death. (A) Cumulative incidence of grade II to IV acute GvHD. Although the day 100 cumulative incidence is not significantly different, the rate of acute GvHD development is decreased for FluMelTBI-50 and FluMelTBI-75. (B) Cumulative incidence of grade III to IV acute GvHD. Day 100 cumulative incidence and rate of grade III to IV acute GvHD was not significantly different among the 3 regimens. (C) Cumulative incidence of NIH moderate/severe chronic GvHD. The cumulative incidence is not significantly different among FluMel, FluMelTBI-50, and FluMelTBI-75. All cumulative incidences were calculated using the competing risks of relapse and death.

**Table 5**
Univariate Analysis of Overall Survival and Progression-Free Survival by Conditioning Regimen

| Explanatory Variable | FluMel | | | FluMelTBI-50 | | | FluMelTBI-75 | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95% CI | P | HR | 95% CI | *P* Value | HR | 95% CI | *P* Value |
| Overall survival | | | | | | | | | |
| Age, yr | | | | | | | | | |
| ≤39 | 1 | | | 1 | | | 1 | | |
| 40-50 | 1.17 | 0.65-2.15 | NS | 1.82 | 0.53-6.21 | NS | 0.96 | 0.38-2.41 | NS |
| ≥60 | 1.25 | 0.65-2.4 | NS | 2.36 | 0.7-8.03 | NS | 1.06 | 0.44-2.56 | NS |
| Disease status at alloHCT | | | | | | | | | |
| CR | 1 | | | 1 | | | 1 | | |
| Not in CR | 2.92 | 1.9-4.5 | <.001 | 1.19 | 0.6-2.3 | NS | 1.44 | 0.8-2.54 | NS |
| HCT-CI | | | | | | | | | |
| Low | 1 | | | 1 | | | 1 | | |
| Intermediate | 3.28 | 1.81-5.93 | <.001 | 3.68 | 1.32-10.26 | 0.01 | 1.66 | 0.65-4.23 | NS |
| High | 3.98 | 2.40-6.60 | <.001 | 2.54 | 1.11-5.79 | 0.03 | 2.78 | 1.22-6.33 | .02 |
| KPS | | | | | | | | | |
| 90-100 | 1 | | | 1 | | | 1 | | |
| 80 | 1.29 | 0.65-2.58 | NS | 1.51 | 0.64-3.58 | NS | 1.10 | 0.41-2.92 | NS |
| 50-70 | 2.08 | 1.05-4.12 | .04 | 1.90 | 0.81-4.46 | NS | 2.06 | 0.86-4.93 | NS |
| Prior HCT | | | | | | | | | |
| None | 1 | | | 1 | | | 1 | | |
| AlloHCT/autoHCT | 1.87 | 1.22-2.86 | .004 | 0.75 | 0.36-1.60 | NS | 0.96 | 0.43-2.13 | NS |
| Progression-free survival | | | | | | | | | |
| Age, yr | | | | | | | | | |
| ≤39 | 1 | | | 1 | | | 1 | | |
| 40-50 | 1.01 | 0.57-1.79 | NS | 1.33 | 0.45-3.97 | NS | 0.45 | 0.2-1.01 | NS |
| ≥60 | 1.05 | 0.57-1.94 | NS | 1.88 | 0.64-5.53 | NS | 0.52 | 0.25-1.11 | NS |
| Disease status at alloHCT | | | | | | | | | |
| CR | 1 | | | 1 | | | 1 | | |
| Not in CR | 2.67 | 1.8-4.0 | <.001 | 1.51 | 0.8-2.8 | NS | 1.33 | 0.8-2.3 | NS |
| HCT-CI | | | | | | | | | |
| Low | 1 | | | 1 | | | 1 | | |
| Intermediate | 3.47 | 1.97-6.09 | <.001 | 0.88 | 0.2-3.90 | NS | 0.26 | 0.06-1.15 | NS |
| High | 3.87 | 2.39-6.27 | <.001 | 0.82 | 0.18-3.73 | NS | 0.55 | 0.13-2.36 | NS |
| KPS | | | | | | | | | |
| 90-100 | 1 | | | 1 | | | 1 | | |
| 80 | 1.30 | 0.67-2.52 | NS | 1.27 | 0.55-2.91 | NS | 1.05 | 0.42-2.64 | NS |
| 50-70 | 2.16 | 1.12-4.16 | .02 | 1.84 | 0.82-4.13 | NS | 1.94 | 0.86-4.37 | NS |
| Prior HCT | | | | | | | | | |
| None | 1 | | | 1 | | | 1 | | |
| AlloHCT/autoHCT | 2.12 | 1.41-3.20 | <.001 | 0.87 | 0.42-1.78 | NS | 1.12 | 0.53-2.38 | NS |

HR indicates hazard ratio; NS, not significant.

**Table 6**
Multivariate Analysis of OS and PFS for All Patients Studied

| Explanatory Variable | OS | | | PFS | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | *P* Value | HR | 95% CI | *P* Value |
| RIC regimen | | | | | | |
| FluMel | 1 | | | 1 | | |
| FluMelTBI-50 | 0.77 | 0.52-1.15 | NS | 0.82 | 0.56-1.20 | NS |
| FluMelTBI-75 | 0.70 | 0.49-1.00 | .05 | 0.71 | 0.50-1.00 | .05 |
| Age, yr | | | | | | |
| ≤39 | 1 | | | 1 | | |
| 40-59 | 1.19 | 0.74-1.91 | NS | 0.90 | 0.58-1.40 | NS |
| ≥60 | 1.60 | 0.98-2.60 | NS | 1.29 | 0.80-2.07 | NS |
| Donor match | | | | | | |
| RD | 1 | | | 1 | | |
| MMUD | 1.77 | 1.16-2.71 | .01 | 1.57 | 1.03-2.40 | .04 |
| MUD | 1.10 | 0.77-1.57 | NS | 1.01 | 0.71-1.42 | NS |
| Disease status | | | | | | |
| CR | 1 | | | 1 | | |
| Not in CR | 2.12 | 1.56-2.88 | <.001 | 2.02 | 1.50-2.72 | <.001 |
| HCTCI | | | | | | |
| Low | 1 | | | 1 | | |
| Intermediate | 2.90 | 1.84-4.59 | <.001 | 2.42 | 1.57-3.73 | <.001 |
| High | 3.68 | 2.49-5.43 | <.001 | 3.04 | 2.12-4.36 | <.001 |
| Prior HCT | | | | | | |
| No HCT | | | | 1 | | |
| AlloHCT | | | | 1.51 | 0.82-2.76 | NS |
| AutoHCT | | | | 1.59 | 1.08-2.34 | .02 |

MUD indicates matched unrelated donor; MMUD, mismatched unrelated donor; HR, hazard ratio; NS, not significant; RD, related donor.

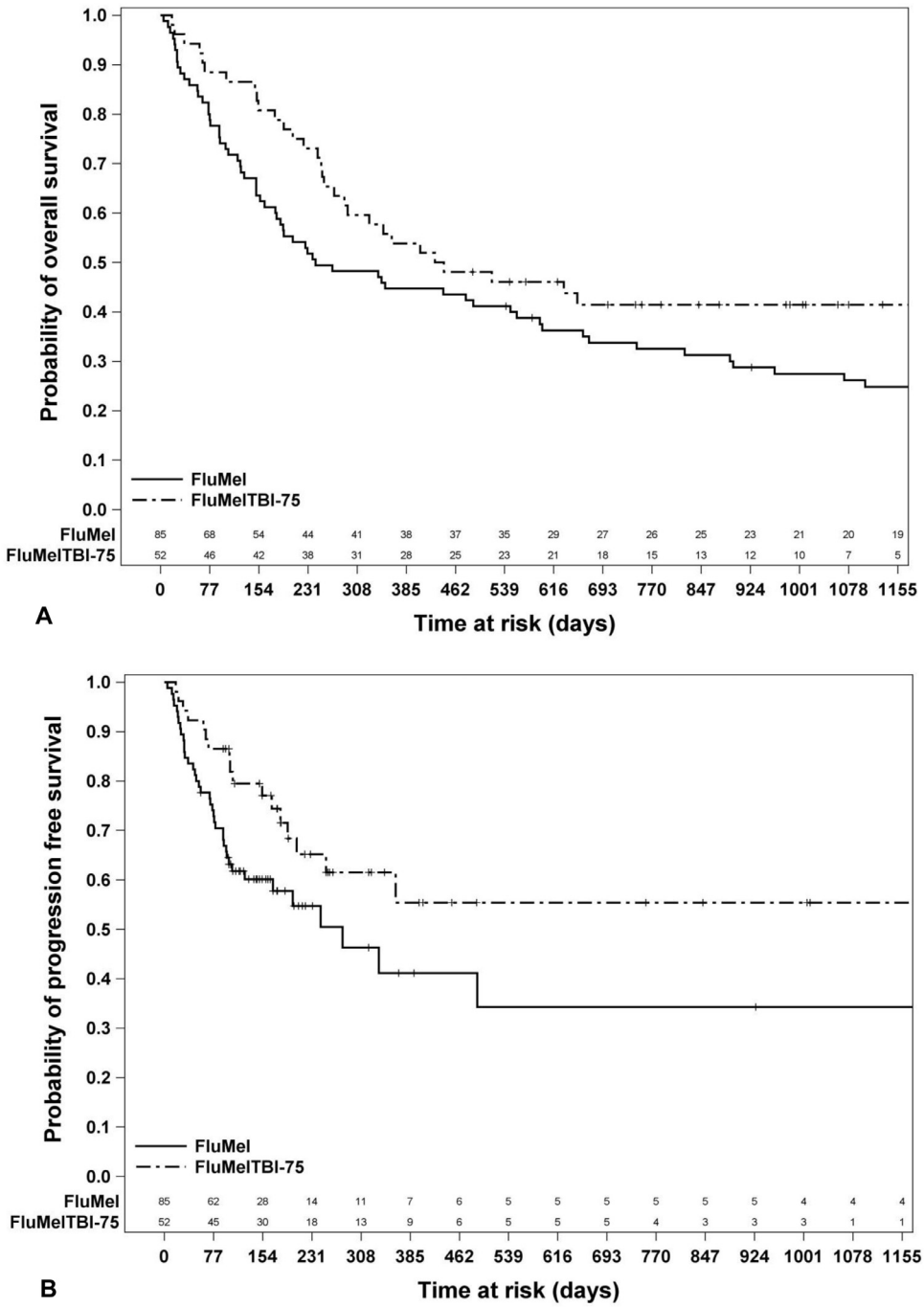


**Figure 4.** Survival for patients not in complete remission on allogeneic hematopoietic cell transplantation. (A) Overall survival in patients not in CR on alloHCT. There is a trend ($P = .10$) toward longer OS in patients who received FluMelTBI-75. (B) Progression-free survival in patients not in CR on alloHCT. PFS was significantly longer in patients who received FluMelTBI-75 compared with FluMel ($P = .03$). The cumulative incidence of PFS was calculated with GvHD and death as competing risks.

biased the clinical outcomes against FluMelTBI-75, yet in our univariate analyses, FluMelTBI-75 was superior. The differences in underlying disease among the 3 groups are more difficult to interpret. FluMelTBI-75 had more myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) than FluMel or FluMelTBI-50, which may have affected PFS and OS. However, when we examined the specific effect of MDS/MPN on outcome in univariate analyses (data not shown), no significant interaction was observed.

In conclusion, we demonstrate the efficacy of FluMelTBI-50 and FluMelTBI-75 for enabling safe allogeneic hematopoietic cell transplantation in 2 phase II clinical trials with a contemporaneous comparator. Phase III randomized clinical trials may not be practical in rare diseases for which resources and numbers of patients available for clinical trials are limited, and outcome data from well-designed and -conducted phase II trials may be the best available. Thus, in alloHCT, for which approximately 8500 procedures are performed annually in the United States for a variety of patient/disease factors and conditioning regimens, clinical application of data from well-designed and -conducted phase 2 trials has become common [31]. The clinical outcomes from this trial can be cautiously applied to

alloHCT practice in the continued absence of phase III randomized clinical trial data, especially in patients with residual disease at the time of alloHCT.

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*Conflict of interest statement*: There are no conflicts of interest to report.

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