ARTICLE

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Long-term survival with mixed chimerism in patients with AML and MDS transplanted after conditioning with targeted busulfan, fludarabine, and thymoglobulin

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We evaluated long-term outcome in 40 patients with MDS or AML, transplanted from related or unrelated donors following conditioning with targeted busulfan (Bu, over 4 days), fludarabine (Flu, 120 [$n = 23$] or 250 [$n = 17$] mg/m^2) and thymoglobulin (THY). Compared to 95 patients conditioned with Bu/Cyclophosphamide (Cy) without THY, BuFluTHY-conditioned patients had lower rates of chronic graft-vs.-host disease (GVHD). Adjusted hazard ratios (HR) for BuFlu(120)THY and BuFlu(250)THY-conditioned patients were 1.60 (95% confidence interval (CI) 0.66–3.86) and 1.87 (0.68–5.11), respectively, for relapse; 0.77 (0.30–1.99) and 1.32 (0.54–3.23) for non-relapse mortality; 0.81 (0.42–1.57) and 1.38 (0.72–2.57) for overall mortality; and 0.78 (0.30–2.05) and 1.62 (0.63–4.41) for relapse or death (failure for relapse-free survival). At one year, 45% of BuFlu(120 or 250)THY-conditioned patients had mixed CD3+ chimerism compared to 0% with BuCy ($p < 0.0001$). None of 7 patients with long-term mixed chimerism had chronic GVHD; two relapsed, five remained stable mixed chimeras. THY is effective in reducing chronic GVHD, and long-term mixed T-cell chimerism can be compatible with relapse-free survival. However, Thy may also be associated with an increased risk of relapse and, dose-dependent, with non-relapse mortality.

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INTRODUCTION

Disease relapse and graft-vs-host disease (GVHD) remain the two major causes of failure after allogeneic hematopoietic cell transplantation (HCT). An essential factor in preventing relapse is the immunological reaction of donor cells against malignant cells in the patient, the graft-vs-tumor or graft-vs-leukemia (GVL) effect, ideally without the occurrence of GVHD [1]. Efforts to achieve this goal have involved a shift away from high dose radiation-based regimens to the use of cytotoxic and immunosuppressive drugs [2], and from high intensity (myeloablative) to reduced intensity (RIC) or so-called non-myeloablative conditioning regimens [3–5]. Incorporation of thymoglobulin (THY) into the conditioning regimen [6–10] and the administration of cyclophosphamide (Cy) after donor cell infusion [11, 12] have been two stratregies aimed at GVHD prevention. One concern with the administration of THY has been incomplete donor cell engraftment (mixed chimerism) and increased relapse risk [1, 13]. Here, we report late results of two sequential phase 2 trials in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) who were transplanted after conditioning with busulfan (Bu), fludarabine (Flu), and THY and followed for more than a decade.

MATERIALS AND METHODS

Patients

Patients were enrolled from 2004 through 2006. All had given informed consent to participate in clinical trials at the Fred Hutchinson Cancer Research Center (FHCRC) as approved by the Institutional Review Board of the FHCRC. Patient and disease characteristics are shown in Table 1, and conditioning regimens are summarized in Supplementary Table S1.

Protocol 1913: sequential BuFlu(120)THY—NCT01056614. Twenty-three patients (12 male and 11 female, median age 47 [range 19–60] years) with AML, MDS, or MDS/MPN overlap were enrolled in this single-arm trial between October 2004 and February 2006. Stopping rules included excess graft failure and relapse. Conditioning consisted of once daily IV Flu (30 mg/m^2/d on days −9 to −6 for a total dose of 120 mg/m^2) followed on days −5 to −2 by once daily IV Bu (Busulfex, Otsuka Pharma US). The starting dose of Bu was 3.2 mg/kg/ day (actual weight, unless >100% ideal body weight, when the dose was based on adjusted ideal body weight). Blood for Bu pharmacokinetics was collected at the end of the infusion and at 3.25, 4.5, 6, 8, 11, and 24 h following the start of the infusion after the first, second, and third doses [14]. Phenytoin as seizure prophylaxis was administered during Bu administration [14]. Bu doses were adjusted to target concentrations at steady-state (Css) of 900 ± 100 ng/mL as previously described [15] (expressed in harmonized units: for a 24-h dosing interval and a Css of 900 ng/ml = 21.6 mg × h/L [per dose]; for 4

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Table 1. Patient and Disease Characteristics.

	Protocol 1913 BuFlu (120)THY	Protocol 2041 BuFlu (250)THY	BuCy
No. patients	23	17	95
Median age, yr (range)	47 (19–60)	51 (34–66)	50 (20–67)
Male (%)	12 (52%)	7 (41%)	51 (54%)
Diagnosis (No. [%])			
Good risk	9 (39%)	5 (29%)	61 (64%)
AML (CR)	5	3	37
MDS-RA, RARS, MLD	4	2	24
Poor risk	14 (61%)	12 (71%)	34 (36%)
MDS/AML or tAML	3	4	6
MDS-RAEB	5	2	18
Secondary MDS	1	0	0
Hypoplastic MDS	0	0	1
MDS/MPN Overlap	3	4	8
CMML	2	2	1
Transplant Status (CR1)	5 (22%)	7 (41%)	36 (38%)
HCT-CI (No. [%])			
Low (Score = 0)	7 (29.5%)	1 (6%)	27 (28%)
Intermediate (Score = 1–2)	9 (39%)	8 (47%)	38 (40%)
High (Score > 2)	7 (29.5%)	8 (47%)	30 (32%)

Baseline factors including median age, gender distribution, disease type stratified by good vs. poor risk, median time from diagnosis to transplantation, and HCT-comorbidity index (HCT-CI) scores are shown [52]. Disease risk was conventionally defined [53]. There were more patients with "Good risk" disease in the BuCy group ($p = 0.0064$).
THY thymoglobulin, *AML-CR1/CR2* acute myelogenous leukemia in first or second complete morphologic remission, *MDS* myelodysplastic syndrome, *RA* refractory anemia, *RARS* refractory anemia with ring sideroblasts, *MDS/AML* AML with antecedent MDS, Secondary MDS treatment-related MDS, *RAEB* refractory anemia with excess blasts, *CMML* chronic myelomonocytic leukemia, *MDS/MPN* myelodysplastic syndrome/myeloproliferative disease overlap.

doses = 86.4 mg x hr/L [cumulative AUC]). THY (Genzyme, Cambridge, MA) was given IV at doses of 0.5 mg/kg (actual weight) on day −3, 2.5 mg/kg on day −2, and 3 mg/kg on day −1 (total dose 6 mg/kg), infused over 4- 6 hours. Serum levels of THY were determined on day −3 prior to the first dose, on day −1 one hour after completion of the infusion, and on day +1. THY doses were fixed and levels were determined retrospectively [16]. Levels of total THY were determined by ELISA and levels of active THY (available for binding to human lympohcytes) were determined by flow cytometery, as described by Regan et al[17].

Donor and HCT characteristics are summarized in Table 2. Eleven patients were transplanted from HLA-identical siblings and 10 from unrelated donors matched for HLA-A, -B, -C, -DRB1, and -DQB1 by high-resolution typing. Two patients received transplants from HLA-nonidentical unrelated donors, one mismatched for HLA-C alleles bidirectionally, and one mismatched for an HLA-A allele in the host-vs.-graft direction. GVHD prophylaxis, in addition to the pre-transplant administration of THY, consisted of tacrolimus given as a continuous IV infusion (0.02 mg/kg/hr) beginning on day −1, and methotrexate given at doses of 10 mg/m^2 IV on days +1, +3, +6, and +11. Tacrolimus levels were monitored starting on day +1, and dosing was adjusted to achieve steady-state serum levels of 5-10 ng/ml. Tacrolimus was changed to the oral formulation at a dose conversion of 1:4 (IV:oral) given in divided doses twice daily as soon as tolerated by the patient. In the absence of GVHD, tacrolimus was tapered by 20% per month starting on day +56 and discontinued on day +180. Diagnoses of acute or chronic GVHD were based on established criteria [18, 19]. GVHD treatment was as previously described [20].

Table 2. Donor and transplant characteristics.

	Protocol 1913 BuFlu (120)THY	Protocol 2041 BuFlu (250)THY	BuCy
Total No. donors	23	17	95
Median donor age, yr (range)	37 (20–60)	48 (25–65)	37 (17–77)
Male (%)	14 (61%)	12 (71%)	59 (62%)
Donor graft source (*N*)			
PBSC	23	17	95
BM	0	0	0
Donor/Patient relationship (*N*, %)			44 (46%)
HLA-matched, related	11 (48%)	12 (71%)	44 (46%)
HLA-matched, unrelated	10 (43%)	5 (29%)	7 (7%)
HLA-mismatched, unrelated	2 (9%)	0	0
Donor/Patient CMV serology (*N*, %)			27 (28%)
D+/R+	4 (17%)	4 (24%)	36 (38%)
D−/R+	11 (48%)	4 (24%)	4 (4%)
D+/R−	2 (9%)	1 (4%)	28 (29%)
D−/R−	6 (26%)	8 (48%)	7.9 (3.7)
Mean (SD) CD34 cells × 10^6/kg	6.7 (2.0)	7.5 (2.7)	95

Donor age, gender, graft source, transplant type, CMV serology, and CD34 dose are shown. The median donor age was higher in the BuFlu(250)THY group ($p = 0.0084$).
BM bone marrow; *CMV* cytomegalovirus; *D* donor, *HLA* human leukocyte antigen; *PBSC* G-CSF mobilized peripheral blood cells; *R* recipient.

Protocol 2041: concomitant BuFlu(250)THY—NCT00346359. Because of two graft failures, protocol 1913 was stopped and a new single-arm pilot protocol was designed that increased the total Flu dose to 250 mg/m^2 (50 mg/m^2/day administered as a 30 min IV infusion on days −6 to −2). The starting dose of IV Bu was 4 mg/kg based on the average clearance observed in protocol 1913, and Bu was given as a 3-hour infusion immediate after Flu, on days −5 to −2, with the goal of achieving synergistic cytotoxicity [21]. Phenytoin administration, monitoring of Bu levels with dose adjustments, and supportive care were as in protocol 1913. Similar to protocol 1913, THY was given IV at doses of 0.5 mg/kg (actual weight) on day −3, 2.5 mg/kg on day −2, and 3 mg/kg on day −1 (total dose 6 mg/kg), infused over 4–6 h. Stopping rules included graft failure, relapse and non-relapse mortality (NRM). Seventeen patients (7 male and 10 female, median age 51 [range 34–66] years) with AML, MDS or MDS/MPN overlap were enrolled from April to November 2006. Twelve patients were transplanted from HLA-identical siblings and 5 from unrelated donors matched for HLA-A, -B, -C, -DRB1, and -DQB1 by high-resolution typing. HLA-mismatched donors were excluded. GVHD prophylaxis was as in protocol 1913.

Comparison regimen (controls). To put results into perspective, outcome in the two protocols was compared to patients with similar diseases undergoing allogeneic peripherbal blood stem cell (PBSC) transplant between January 2004 and December 2006 after conditioning with "standard" Bu (1 mg/kg every 4 h for 16 doses, administered orally on days −7 to −4) along with phenytoin, and Cy (60 mg/kg, administered IV on days −3 and −2 along with equivalent doses of mesna) [22]. In 44 patients (46%) the donors were HLA-identical siblings, in 44 (46%) matched unrelated donors, and in 7 (7%) HLA-nonidentical unrelated donors. GVHD prophylaxis included tacrolimus and methotrexate ($N = 66$, 69%), cyclosporin and methotrexate ($N = 25$, 25%), and tacrolimus, methotrexate, and either rapamycin ($N = 2$) or mycophenolate mofetil ($N = 2$).

Engraftment and chimerism

Time of neutrophil engraftment was defined as the first of 3 consecutive days on which the absolute neutrophil count (ANC) exceeded 0.5 × 10^9/L. Platelet engraftment was defined as the first of 7 consecutive days on which the platelet count exceeded 20 × 10^6/L, untransfused. Evidence of graft rejection was sought when the ANC failed to reach 0.5 × 10^9/L or declined below 0.5 × 10^9/L after engraftment, unassociated with drug toxicity or relapse. To determine engraftment of donor T cells and granulocytes (donor chimerism), DNA was isolated form T cells (CD3-positive) and granulocytes (CD33-positive), which were sorted from peripheral blood by flow cytometry. Donor-host chimerism for recipients of sex-mismatched transplants was determined by fluorescence in situ hybridization (FISH) using probes for X- and Y-chromosomes. As of July 2007 (in long-term follow-up) short tandem repeat technique was used instead [23]. For recipients of sex-matched transplants, chimerism was determined by PCR analysis of variable number tandem repeats or short tandem repeats unique to donors or recipients [24]. Chimerism status was determined at different time intervals as clinically indicated. Significant mixed chimerism was defined as <85% donor cells, a cut-off that has been previous reported [25].

Relapse

All patients had marrow samples examined morphologically and by cytogenetic and flow cytometric analysis on approximately day +28, day +80, and at one year. Later marrow examinations were carried out as clinically indicated. Relapse was defined morphologically by marrow dysplasia or the presence of >5% myeloblasts determined by microscopic exam and by flow cytometry, or by the re-appearance of cytogenetic abnormalities present before transplantation. No DNA mutation data were available.

Supportive care

Antimicrobials were administered per standard practice. Transfusion products were irradiated with 25 Gy before infusion. Cytomegalovirus (CMV)-seronegative patients were given transfusions from CMV-seronegative donors, or leuko-reduced blood products if CMV-negative products were unavailable. Patients were monitored sequentially for CMV as well as Epstein-Barr virus (EBV) reactivation and pre-emptive therapy with ganciclovir or rituximab, respectively was initiated upon documentation of rising copy numbers and administered as described [26, 27].

Statistical methods

The probabilities of overall survival (OS) and relapse-free survival (RFS) were estimated using the method of Kaplan and Meier. The probabilities of acute and chronic GVHD and NRM were expressed in terms of cumulative incidence [28], where death without GVHD and relapse was treated as competing risk for GVHD and NRM, respectively. Cox regression models were fit to compare the cause-specific hazards of failure between THY-containing cohorts and the BuCy controls for the appropriate endpoints, with adjustment for confounding factors as needed and as allowed for by the number of events relevant to each endpoint examined. The association of CD3 chimerism with the risk of relapse was examined by modeling chimerism as a time-dependent covariate. Failure of RFS was defined as the occurrence of relapse or death. Data were analyzed as of December 2, 2020.

RESULTS

Conditioning

The median Css (AUC) of Bu in protocols 1913, 2041, and controls were 874 (83.9), 954 (91.5) and 869 (83.4) ng/ml (mg × h/L), respectively, and >90% of patients had levels within the target range (Table S1). The level of *active* THY on day +1 was 0.9–3.5 µg/ml (median 1.9 µg/ml) in patients conditioned with BuFlu(120)THY and 1.7–7.6 µg/ml (median 3.9 µg/ml) for patients conditioned with BuFlu(250)THY. The half-life of active THY was 9.1 ± 3.4 days, similar to that reported previously by Waller et al. [29].

Engraftment and mixed chimerism

Protocol 1913: sequential BuFlu(120)THY. All patients achieved initial engraftment of neutrophils by 12–26 (median 15.5) days and platelets by 9–21 (median 14) days. The patient whose graft was mismatched at HLA-A in the direction of rejection became neutropenic after day +50 at which time donor T-cell (CD3)

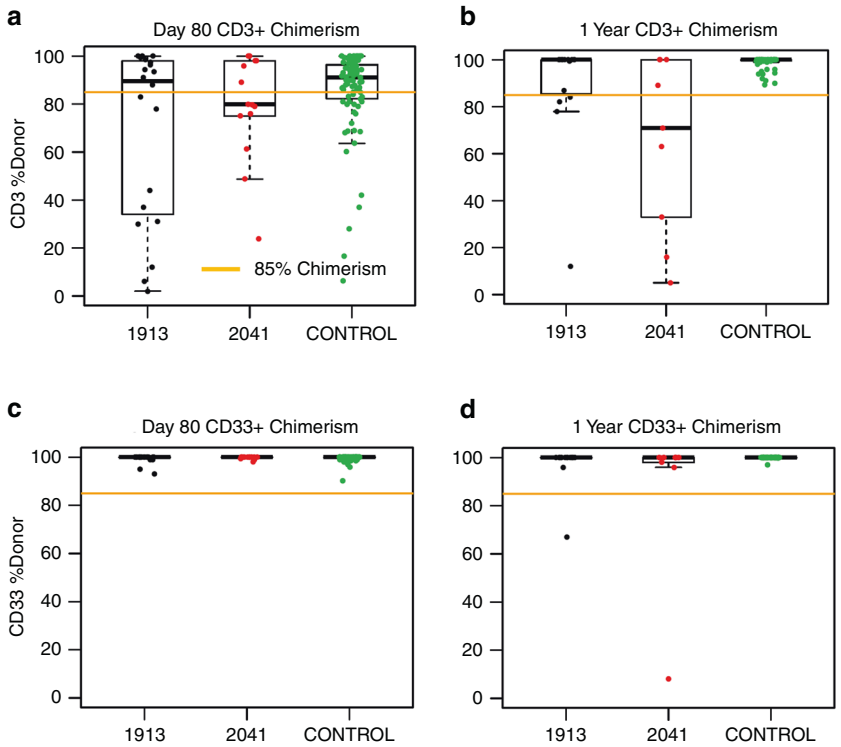

Fig. 1 CD3+ and CD33+ chimerism levels. Shown are the proportions of donor CD3+ and CD33+ cells at day +80 and at one year post-transplant. **a** Day +80 CD3+ chimerism. **b** 1 year CD3+ chimerism. **c** Day +80 CD33+ chimerism. **d** 1 year CD33+ chimerism. Conditioning regimens: Protocol 1913—BuFlu(120)THY; Protocol 2041—BuFlu(250)THY; Control—BuCy.

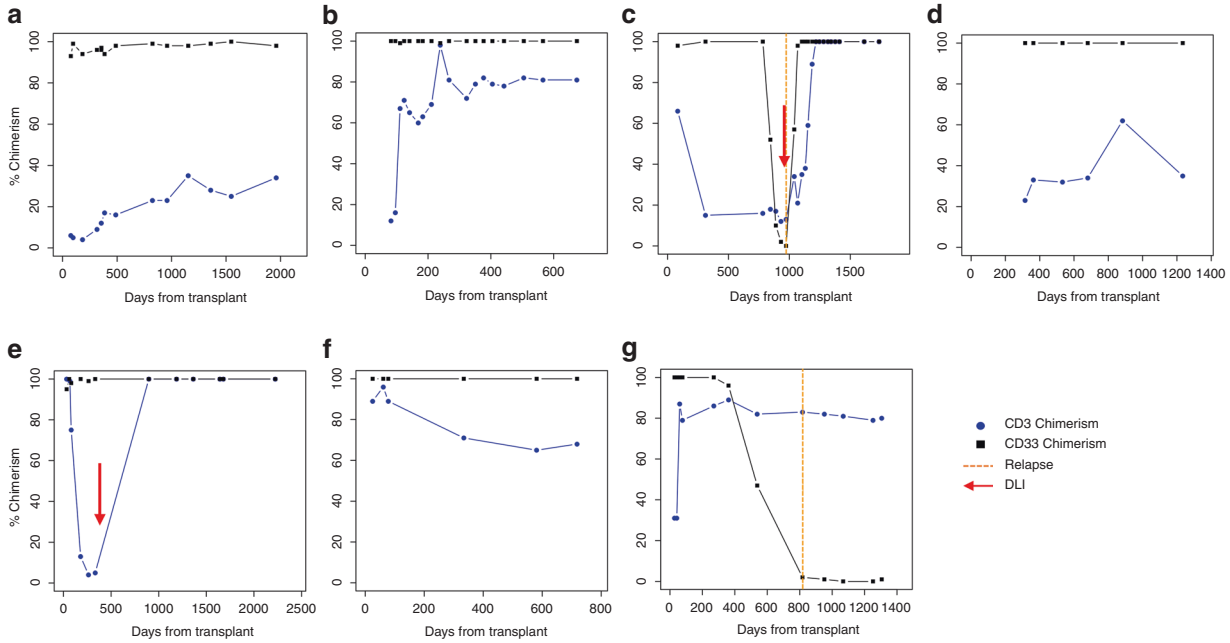

Fig. 2 Individual chimerism levels in seven patients over an extended time period. Percent donor CD3+ (blue) or CD33+ (black) cells as a function of time post-transplant following conditioning with either BuFlu(120)THY (**a**, **b**) or BuFlu(250)THY (**c**–**g**). **a** 50-year-old female with CMML transplanted from an unrelated donor. **b** 29-year-old female with AML-CR2 transplanted from an unrelated donor. **c** 49-year-old male with MDS/MPN overlap syndrome transplanted from a related donor. **d** 49-year-old female with AML-CR1 transplanted from a related donor. **e** 59-year-old female with AML-CR1 transplanted from a related donor. **f** 42-year-old female with MDS/MPN overlap transplanted from a related donor. **g** 61-year-old male with MDS/MPN overlap transplanted from a related donor. The red arrow shows timing of DLI. Vertical orange dashed line shows time of relapse.

chimerism was only 2%, although granulocyte (CD33) chimerism remained 100%. The patient died after a salvage transplant from an HLA haploidentical sibling. The patient with a graft bidirectionally mismatched at HLA-C experienced an ANC decline after starting ganciclovir therapy for CMV reactivation on day +47. While CD33 chimerism remained at 100%, donor CD3 chimerism was 5% on day +73 and the patient died of fungal pneumonia.

Day +80 chimerism studies in the initial 8 patients on study showed full (100%) donor chimerism in CD33+ granulocytes, but only one patient showed full donor CD3+ chimerism. In the remaining 7 patients donor CD3+ T-cell chimerism ranged from 5% to 94%, and accrual was suspended pending determination of day +80 chimerism in the remaining patients who had been enrolled. Four of these 14 patients on whom samples were available had mixed donor CD3+ T-cell chimerism at day +80. In total (excluding the two patients with graft rejection), there were 9 (of 21) patients (43%) with apparently stable mixed CD3 chimerism at day +80.

Protocol 2041: concomitant BuFlu(250)THY. Only patients with HLA-matched donors were enrolled. All 17 patients achieved neutrophil engraftment by 11–30 (median 16) days, and platelet engraftment by 7–13 (median 13) days, except for two patients who remained platelet transfusion-dependent until their deaths from non-relapse causes at days +36 and +76, respectively. Protocol 2041 was stopped after 17 patients as the NRM of 24% at day +100 met the stopping criterion. In total (excluding three patients who did not survive to d + 80), there were 8 (of 14) patients (57%) with mixed CD3 chimerism at day +80.

Chimerism analysis in protocols 1913 and 2041. Data on CD3+ T-cell and CD33+ myeloid chimerism by day +80 and 1 year post-HCT are summarized in Fig. 1. In the two BuFluTHY trials combined, day +80 CD3+ chimerism was available in 35 patients and ranged from 2% (in a rejecting patient, see above) to 100% (median 85%, Fig. 1a). In 17 of these 35 patients (49%), CD3+ chimerism was ≤85%. In contrast, CD33+ myeloid chimerism was >90% donor in all patients (Fig. 1c).

At one year post-HCT, 25 of 27 surviving patients had CD3+ T-cell chimerism determined. Nine patients (36%) had ≤85% donor T cells (Fig. 1b); two of these who had concurrent mixed CD33+ chimerism experienced morphological relapse (Fig. 1d). Donor T-cell chimerism was measured up to 6 years post-HCT in seven of these nine patients (78%) (Fig. 2). Two of the seven patients eventually relapsed (days +974 and +820, respectively, Fig. 2c, g) as indicated by a decrease in donor CD33 chimerism followed by clinical relapse. One patient achieved a second remission with recovery of CD33 chimerism to 100% donor cells by day +1068 (Fig. 2c), following donor lymphocyte infusion (DLI, 1×10^7 CD3+ cells/kg). In another patient (Fig. 2e), concern about persistent mixed donor chimerism of about 5% prompted administration of DLI (1×10^6 CD3+ cells/kg) on day +424, despite no evidence of relapse, and resulted in conversion to full donor CD3+ T-cell chimerism. The remaining five patients with persistent mixed CD3+ chimerism, representing 12% of the entire cohort, have remained in complete remission of their disease for more than 10 years post-HCT.

Comparison regimen, sequential BuCy (controls). Among 95 comparable BuCy-conditioned patients all but 1 patient achieved sustained neutrophil engraftment by 10–28 (median 16) days and platelet engraftment by 7–66 (median 13) days. At day +80 chimerism data were available in 82 patients, and 29% had mixed CD3+ chimerism (Fig. 1a). At 1 year, chimerism data were available in 53 patients in the BuCy group and none had mixed CD3+ chimerism ($p < 0.001$) compared to the BuFluTHY cohorts (Fig. 1b).

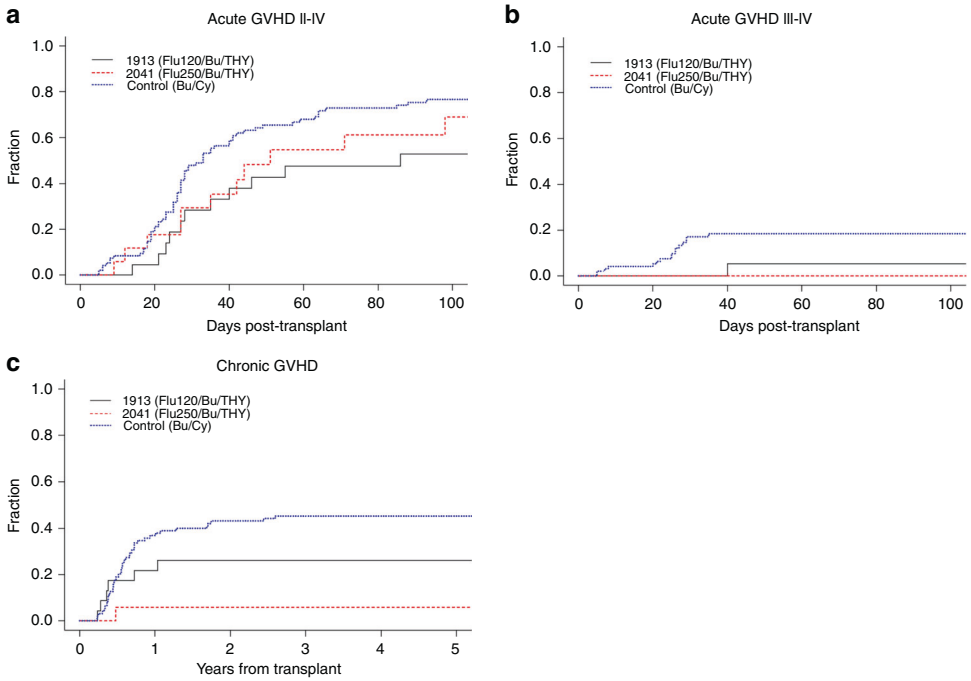

Fig. 3 Incidence of acute and chronic GVHD. a Cumulative incidence of grades II-IV acute GVHD. **b** Cumulative incidence of grades III-IV acute GVHD. **c** Cumulative incidence of extensive chronic GVHD. Conditioning regimens: BuFlu(120)THY (protocol 1913), solid black line; BuFlu(250)THY (protocol 2041), dashed red line, BuCy, dotted green line.

Table 3. Multivariable analysis of GVHD.

	HR	95% CI	*p* value
Acute GVHD grade ≥ 2			
Group (p1913 vs. Control)	0.50	[0.26–0.95]	**0.04**[a]
Group (p2041 vs. Control)	0.72	[0.37–1.39]	0.32
Chronic GVHD (extensive)			
Group (p1913 vs. Control)	0.38	[0.15–0.93]	**0.03**[b]
Group (p2041 vs. Control)	0.11	[0.01–0.82]	**0.03**[b]

GVHD graft-vs-host disease.
[a]Model adjusted for age, donor graft (matched related donor [MRD] vs. other), patient/donor CMV serostatus (Patient and/or donor + vs. both negative), severity of disease (poor risk vs. good risk), and disease status at transplant (remission vs. other).
[b]Model adjusted for age, donor graft (MRD vs. other), severity of disease (poor risk vs. good risk), and disease status at transplant (remission vs. other).
Bold values indicate statistical significance.

Graft-vs.-host disease

Grades II-IV acute GVHD was observed in 22 of 40, and extensive chronic GVHD in 7 BuFluTHY-conditioned patients. In 16 of the 22 patients (73%) with acute GVHD, manifestations were mild and restricted to the upper GI tract (grade IIa) [30]. Treatment consisted of oral beclomethasone and budesonide alone or combined with a short course of systemic prednisone [31]. Summarized in Fig. 3a, b are the observed rates of acute GVHD in patients given THY and BuCy-conditioned control patients. After adjusting for patient age, type of donor, risk of disease, and patient/donor CMV serostatus, the estimated hazard ratio (HR) between BuFlu(120)THY and controls was 0.50 (95% confidence interval (CI) 0.26–0.95, Table 3). Grade III acute GVHD was observed in one BuFluTHY-conditioned patient (Fig. 3b).

The estimated probability of extensive chronic GVHD by 2 years was 6% after BuFlu(250)THY conditioning, 26% with BuFlu(120)THY conditioning, and 43% in the BuCy group (unadjusted results, Fig. 3c). The adjusted HRs for chronic GVHD in patients who had received BuFluTHY at the lower and higher dose of Flu relative to BuCy-conditioned patients were 0.38 (95% CI, 0.15–0.93) and 0.11 (95% CI, 0.01–0.82), respectively (Table 3). None of the seven patients with persistent mixed CD3+ chimerism at 1 year developed chronic GVHD.

Relapse

Shown in Fig. 4a are the unadjusted probabilities of relapse as a function of time for all groups. The adjusted HRs for relapse are summarized in Table S2. Among all patients (FluBuTHY and BuCy regimens), there was very little observed correlation between percent CD3 chimerism and the risk of relapse ($p = 0.98$). Among patients who did relapse, relapse was heralded by a marked decrease in donor CD33+ cells, as shown in Fig. 2c.

Non-relapse mortality

Day 100 NRM in the BuFlu(250)THY cohort was 24% versus 9% in the BuFlu(120)THY cohort, contributing to an adjusted HR = 2.68 (95% CI, 0.81–8.92, $p = 0.11$) for NRM at any time. Compared to the BuCy cohort, the adjusted HR of NRM (at any time) in BuFlu(120)THY-conditioned patients was 0.55 (95% CI, 0.21–1.45) and the adjusted HR in the BuFlu(250)THY cohort was 1.48 (95% CI, 0.58–3.74, Table S2, unadjusted estimates as a function of time Fig. 4b).

Overall mortality (OM) and failure of relapse-free survival (RFS)

The adjusted HRs for mortality and RFS failure for BuFlu(120)THY-conditioned patients relative to BuCy were 1.14 (95% CI, 0.57–2.27) and 1.15 (95% CI, 0.59–2.22), respectively, while the adjusted HRs for BuFlu(250)THY-conditioned patients relative to BuCy were 1.93 (95% CI, 0.91–4.09) and 1.83 (95% CI, 0.87–7.84), respectively (Table S2, unadjusted estimates as function of time Fig. 4c, d).

Viral reactivation

Cytomegalovirus. When patient, donor, or both were CMV seropositive, reactivation occurred in 14 of 17 patients (82%)

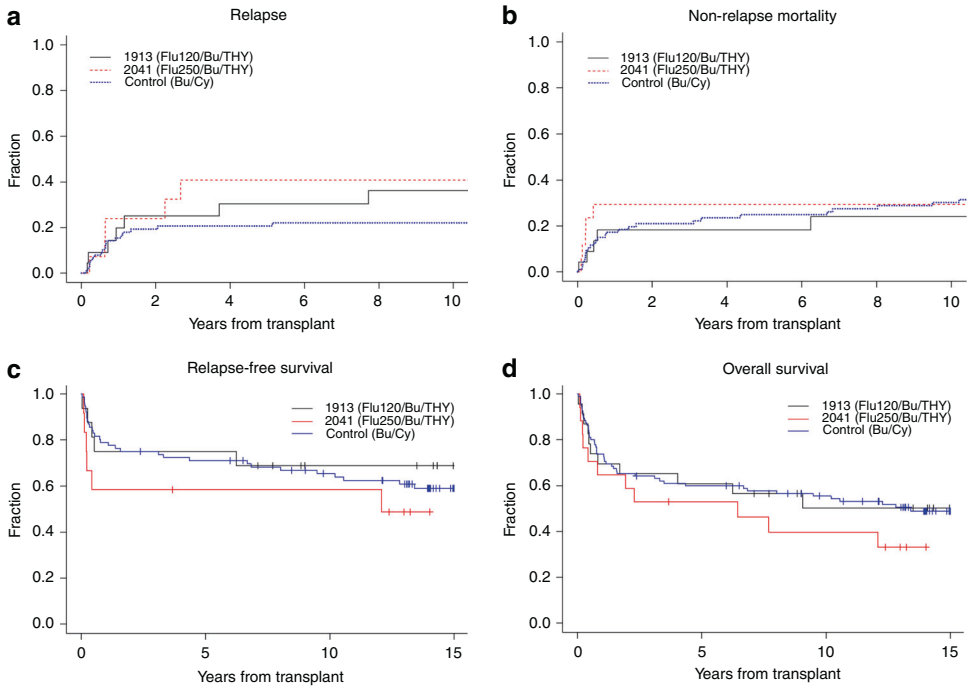

Fig. 4 Non-relapse mortality (NRM), relapse-free survival (RFS), and overall survival (OS). a Cumulative incidence of relapse. **b** Cumulative incidence of non-relapse mortality. **c** Kaplan-Meier plot of relapse-free survival (tick marks indicate censored data points). **d** Kaplan–Meier plot of overall survival. Conditioning regimens: BuFlu(120)THY, solid black line; BuFlu(250)THY, dashed/solid red line, BuCy, dotted/solid green line.

who received BuFlu(120)THY, in 8 of 9 patients (89%) who received BuFlu(250)THY, and 40 of 67 patients (60%) in the BuCy control group. The adjusted HRs for patients who received THY compared to BuCy were 2.04 (95% CI 1.10 to 3.80) for BuFlu(120) THY, and 3.18 (95% CI 1.42 to 7.15 for BuFlu(250)THY, Table S3). The median time to viral reactivation (time at which the probability of reactivation reached 50%) was 46 days for BuFlu (120)THY, 34 days for BuFlu(250)THY, and 76 days for BuCy-conditioned patients. All patients responded to pre-emptive therapy and none developed clinical CMV-associated disease.

Epstein-Barr virus. Reactivation of EBV occurred in 5 of 23 patients (22%) in the BuFlu(120)THY cohort and in 14 of 17 patients (82%) in the BuFlu(250)THY cohort, compared to 3 of 95 patients (3%) conditioned with BuCy. This led to adjusted HRs of 9.45 (95% CI, 2.19 to 40.75) for BuFlu(120)THY compared to BuCy and 93.5 (95% CI 23.28–375.54) for BuFlu(250)THY compared to BuCy (Table S3). Four patients in the BuFluTHY cohorts required therapy with rituximab. While there were no cases of post-transplant lymphoproliferative disease, the data indicated a higher rate of EBV reactivation in patients who received THY, particularly at the higher dose level of Flu.

DISCUSSION

Polyclonal anti-T-cell sera such as equine anti-thymocyte globulin (ATG) or THY generated in rabbits, first tested in the immunosuppressive treatment of aplastic anemia [32] and solid organ transplantation [33], have been used for several decades in HCT to prevent or treat GVHD [7, 34, 35]. However, with a half-life of at least 9 days, THY when given as part of conditioning, could also react with donor-derived T cells post-HCT, possibly interfering with engraftment or with the donor T-cell mediated GVL effect, thereby increasing the risk of relapse [35, 36].

The present results are in agreement with previous reports showing a decreased incidence of acute and chronic GvHD in patients given THY as part of conditioning with BuFlu [37] or BuCy [16]. Previous reports showed probabilities of 40-60% after conditioning with BuFlu without THY [37, 38] or BuCy [37, 39] as reflected in the present comparator group, closely resembling results reported by Russell, et al[40]. Furthermore, with THY-mediated in vivo T-cell depletion, acute GVHD was mild, restricted to the upper GI tract [30] in 80% of patients, and responsive to short courses of systemic steroids and oral beclomethasone [31]. Only one patient had grade III acute GVHD, and no grade IV GVHD was observed.

NRM among patients conditioned with BuFlu(120)THY was 9% at day 100 and 17% at one year, comparable to other studies using BuFluTHY conditioning [37–39]. However, with the use of higher doses of Flu given concomitently with Bu (BuFlu(250)THY), 4 of 17 patients (24%) died of transplant-related causes by day +100, meeting stopping criteria for the protocol. This regimen resulted in higher steady-state Bu levels than observed in patients who received the lower dose of Flu, either related to the dose of Flu or the fact that Flu and Bu were administered on the same day rather than sequentially. The high incidence of NRM with the higher Flu dose was in contrast to the incidence of 4%, reported by Russell et al. [40]. The reasons are not clear; however, disease risk (as defined by HCT-CI scores of ≥3) was higher in our two pilot trials, and the total THY dose of 6 mg was higher than the 4.5 mg in the Russell study.

A second concern with the use of THY has been graft failure, particularly after reduced-intensity conditioning [13]. We observed two cases in patients transplanted from HLA nonidentical unrelated donors, but none in patients transplanted from HLA-matched related or unrelated donors. Yet there was a high rate of long-lasting mixed donor/host chimerism, i.e., *incomplete* engraftment, of CD3+ donor cells, which, however, was not associated with significantly increased incidence of relapse. Further, none of the seven patients who remained mixed CD3+ chimeras by 1 year after HCT and were followed to beyond 10 years, developed chronic GVHD, supporting the concept of a tolerogenic effect of long-term donor-host cell interactions [33].

Persistent mixed donor/host chimerism has been reported previously [41]. One study found that early (d + 80 to d + 120)

mixed T-cell chimerism following BuFlu±THY conditioning was associated with an increased risk of relapse [42], and a second study showed that increasing chimerism was a risk factor for relapse [43]. In the current trials of "myeloablative" i.e. high-intensity conditioning with BuFluTHY, despite high levels of mixed T-cell chimerism in 45% of patients at one year, and one-third of patients surviving beyond 10 years, there was no definitive increase in relapse associated with mixed CD3 chimerism. However, the number of relapses in this analysis was quite low, rendering definitive conclusions difficult.

Retrospective data [44] and animal models [45] indicate that mixed donor–recipient chimerism is a reflection of the immunosuppressive potency of the conditioning regimen. The present findings suggest, therefore, that despite the immunosuppressive characteristics of THY, when given with BuFlu as part of conditioning, it is less effective in securing complete donor cell engraftment than observed with BuCy or BuFlu without THY. Conceivably, this is related to the fact that in addition to suppressing the host immune system THY also lessens the graft-enhancing activity of donor-derived T lymphocytes [46]. The persistence of mixed CD3 chimerism for years post-HCT following in vivo T-cell depletion by THY implies a state of mutual donor-recipient tolerance as also reflected by the prevention of chronic GVHD. It was encouraging that there was no clinically significant loss of the GVL effect and that long-term mixed T-cell chimerism was compatible with RFS [25, 44, 47, 48]. One potential disadvantage of the augmented immunosuppression with THY in BuFlu-containing regimens was the high rate of reactivation of CMV and EBV, as also reported by others [26, 49, 50].

An important weakness of the present studies is the small number of THY-conditioned patients. Also, these were prospective, but not randomized, single-arm pilot trials. A major strength, however, is the maturity of the data. Follow-up extends to 15 years for the lead patients, who survive in remission despite prolonged persistence of donor/recipient CD3+ mixed chimerism.

To summarize and conclude, the inclusion of THY in BuFlu conditioning regimens resulted in promising data on GVHD prevention. However, the higher dose of Flu (given on the same days as Bu) led to increased OM, decreased RFS, increased relapse, and increased NRM. However, there were only 17 patients who received this higher dose and statistical power was limited. The sustained presence of mixed donor/recipient CD3+ chimerism was associated with a lower incidence of chronic GVHD. These observations could be exploited to further improve HCT results. The balance between the risk of chronic GVHD and relapse would likely affect the selection of patient that would benefit most from this approach. A trial using an intermediate dose of Flu, maybe 150 mg/m^2, given sequentially with Bu as used here, and comparing a total THY dose of 4 mg (to reduce viral reactivation) to 6 mg for patients transplanted from HLA-matched donors would be of interest. The recent publication of three randomized trials using prophylactic ATG or THY underscores the continued interest in and the potent role of THY in HCT [9, 10, 51].

REFERENCES

1. Negrin RS. Graft-versus-host disease versus graft-versus-leukemia. Hematol Am Soc Hematol Educ Program. 2015;2015:225–30. https://doi.org/10.1182/asheducation-2015.1.225.
2. Ben-Barouch S, Cohen O, Vidal L, Avivi I, Ram R. Busulfan fludarabine vs busulfan cyclophosphamide as a preparative regimen before allogeneic hematopoietic cell transplantation: systematic review and meta-analysis. Bone Marrow Transplant. 2016;51:232–40. https://doi.org/10.1038/bmt.2015.238
3. Deeg HJ, Sandmaier BM. Who is fit for allogeneic transplantation? Blood. 2010;116:4762–70.
4. McDonald GB, Sandmaier BM, Mielcarek M, Sorror M, Pergam SA, Cheng GS, et al. Survival, non-relapse mortality, and relapse-related mortality after allogeneic hematopoietic cell transplantation: Comparing 2003-2007 versus 2013-2017 cohorts. Ann Intern Med. 2020;172:229–39. https://doi.org/10.7326/M19-2936.
5. Sorror ML, Storer BE, Fathi AT, Brunner A, Gerds AT, Sekeres MA, et al. Multisite 11-year experience of less-intensive vs intensive therapies in acute myeloid leukemia. Blood. 2021;138:387–400. https://doi.org/10.1182/blood.2020008812.
6. Bonifazi F, Rubio MT, Bacigalupo A, Boelens JJ, Finke J, Greinix H, et al. Rabbit ATG/ATLG in preventing graft-versus-host disease after allogeneic stem cell transplantation: consensus-based recommendations by an international expert panel. Bone Marrow Transplant. 2020;55:1093–102. https://doi.org/10.1038/s41409-020-0792-x.
7. Mohty M, Labopin M, Balere ML, Socie G, Milpied N, Tabrizi R, et al. Antithymocyte globulins and chronic graft-vs-host disease after myeloablative allogeneic stem cell transplantation from HLA-matched unrelated donors: a report from the Societe Francaise de Greffe de Moelle et de Therapie Cellulaire. Leukemia. 2010;24:1867–74.
8. Walker I, Panzarella T, Couban S, Couture F, Devins G, Elemary M, et al. Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. Lancet Oncol. 2016;17:164–73. https://doi.org/10.1016/S1470-2045(15)00462-3
9. Kroger N, Solano C, Wolschke C, Bandini G, Patriarca F, Pini M, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. N Engl J Med. 2016;374:43–53. https://doi.org/10.1056/NEJMoa1506002.
10. Finke J, Bethge WA, Schmoor C, Ottinger HD, Stelljes M, Zander AR, et al. Standard graft-versus-host disease prophylaxis with or without anti-T-cell globulin in haematopoietic cell transplantation from matched unrelated donors: a randomised, open-label, multicentre phase 3 trial. Lancet Oncol. 2009;10:855–64.
11. Luznik L, O'Donnell PV, Symons HJ, Chen AR, Leffell MS, Zahurak M, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008;14:641–50. https://doi.org/10.1016/j.bbmt.2008.03.005
12. McCurdy SR, Luznik L. Post-transplantation cyclophosphamide for chimerism-based tolerance. Bone Marrow Transplant. 2019;54:769–74. https://doi.org/10.1038/s41409-019-0615-0.
13. Soiffer RJ, Lerademacher J, Ho V, Kan F, Artz A, Champlin RE, et al. Impact of immune modulation with anti-T-cell antibodies on the outcome of reduced-intensity allogeneic hematopoietic stem cell transplantation for hematologic malignancies. Blood. 2011;117:6963–70.
14. Yeh RF, Pawlikowski MA, Blough DK, McDonald GB, O'Donnell PV, Rezvani A, et al. Accurate targeting of daily intravenous busulfan with 8-hour blood sampling in hospitalized adult hematopoietic cell transplant recipients. Biol Blood Marrow Transplant. 2012;18:265–72.
15. Slattery JT, Sanders JE, Buckner CD, Schaffer RL, Lambert KW, Langer FP, et al. Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics [Erratum appears in Bone Marow Transplantation 1996 Oct; 18(4):829]. Bone Marrow Transplant. 1995;16:31–42.
16. Deeg HJ, Storer BE, Boeckh M, Martin PJ, McCune JS, Myerson D, et al. Reduced incidence of acute and chronic graft-versus-host disease with the addition of thymoglobulin to a targeted busulfan/cyclophosphamide regimen. Biol Blood Marrow Transplant. 2006;12:573–84. https://doi.org/10.1016/j.bbmt.2006.12.036
17. Regan JF, Lyonnais C, Campbell K, Smith LV, Buelow R, U. S. Thymoglobulin Multi-Center Study Group. Total and active thymoglobulin levels: effects of dose and sensitization on serum concentrations. Transpl Immunol. 2001;9:29–36. https://doi.org/10.1016/s0966-3274(01)00048-x.
18. Przepiorka D, Weisdorf D, Martin P, Klingemann HG, Beatty P, Hows J, et al. 1994 consensus conference on acute GVHD grading. Bone Marrow Transplant. 1995;15:825–8.
19. Lee SJ, Vogelsang G, Flowers ME. Chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2003;9:215–33. https://doi.org/10.1053/bbmt.2003.50026.
20. Martin PJ, Rizzo JD, Wingard JR, Ballen K, Curtin PT, Cutler C, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2012;18:1150–63. https://doi.org/10.1016/j.bbmt.2012.04.005.
21. McCune JS, Woodahl EL, Furlong T, Storer B, Wang J, Heimfeld S, et al. A pilot pharmacologic biomarker study of busulfan and fludarabine in hematopoietic cell transplant recipients. Cancer Chemother Pharmacol. 2012;69:263–72. https://doi.org/10.1007/s00280-011-1736-3.
22. Haselberger MB, Schwinghammer TL. Efficacy of mesna for prevention of hemorrhagic cystitis after high-dose cyclophosphamide therapy [Review]. Ann Pharmacother. 1995;29:918–21. https://doi.org/10.1177/106002809502900914.
23. Crescenzi B, Fizzotti M, Piattoni S, La Starza R, Matteucci C, Carotti A, et al. Interphase FISH for Y chromosome, VNTR polymorphisms, and RT-PCR for BCR-ABL in the monitoring of HLA-matched and mismatched transplants. Cancer Genet Cytogenetics. 2000;120:25–29. https://doi.org/10.1016/s0165-4608(99)00245-9.

24. Deeg HJ, Salit RB, Monahan T, Schoch G, McFarland C, Scott BL, et al. Early mixed lymphoid donor/host chimerism is associated with improved transplant outcome in patients with primary or secondary myelofibrosis. Biol Blood Marrow Transplant. 2020;26:2197–203. https://doi.org/10.1016/j.bbmt.2020.07.013.
25. Mossallam GI, Kamel AM, Storer B, Martin PJ. Prognostic utility of routine chimerism testing at 2 − 6 months after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2009;15:352–9. https://doi.org/10.1016/j.bbmt.2008.12.496.
26. Boeckh M, Fries B, Nichols WG. Recent advances in the prevention of CMV infection and disease after hematopoietic stem cell transplantation (Review). Pediatr Transplant. 2004;8:19–27. https://doi.org/10.1111/j.1398-2265.2004.00183.x.
27. van Esser JW, van der Holt B, Meijer E, Niesters HG, Trenschel R, Thijsen SF, et al. Epstein-Barr virus (EBV) reactivation is a frequent event after allogeneic stem cell transplantation (SCT) and quantitatively predicts EBV-lymphoproliferative disease following T-cell-depleted SCT. Blood. 2001;98:972–8. https://doi.org/10.1182/blood.v98.4.972.
28. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695–706.
29. Waller EK, Langston AA, Lonial S, Cherry J, Somani J, Allen AJ, et al. Pharmacokinetics and pharmacodynamics of anti-thymocyte globulin in recipients of partially HLA-matched blood hematopoietic progenitor cell transplantation. Biol Blood Marrow Transplant. 2003;9:460–71. https://doi.org/10.1016/s1083-8791(03)00127-7.
30. Martin PJ, McDonald GB, Sanders JE, Anasetti C, Appelbaum FR, Deeg HJ, et al. Increasingly frequent diagnosis of acute gastrointestinal graft-versus-host disease after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transpl. 2004;10:320–7. https://doi.org/10.1016/j.bbmt.2003.12.304.
31. Hockenbery DM, Cruickshank S, Rodell TC, Gooley T, Schuening F, Rowley S, et al. A randomized, placebo-controlled trial of oral beclomethasone dipropionate as a prednisone-sparing therapy for gastrointestinal graft-versus-host disease. Blood. 2007;109:4557–63. https://doi.org/10.1182/blood-2006-05-021139.
32. Speck B, Gluckman E, Haak HL, Vanrood JJ. Treatment of aplastic anaemia by antilymphocyte globulin with and without allogeneic bone-marrow infusions. Lancet. 1977;2:1145–8.
33. Starzl TE, Demetris AJ, Murase N, Thomson AW, Trucco M, Ricordi C. Donor cell chimerism permitted by immunosuppressive drugs: a new view of organ transplantation (Review). Immunol Today. 1993;14:326–32. https://doi.org/10.1016/0167-5699(93)90054-o.
34. Bacigalupo A, Lamparelli T, Barisione G, Bruzzi P, Guidi S, Alessandrino PE, et al. Thymoglobulin prevents chronic graft-versus-host disease, chronic lung dysfunction, and late transplant-related mortality: long-term follow-up of a randomized trial in patients undergoing unrelated donor transplantation. Biol Blood Marrow Transplant. 2006;12:560–5. https://doi.org/10.1016/j.bbmt.2005.12.034
35. Soiffer RJ, Kim HT, McGuirk J, Horwitz ME, Johnston L, Patnaik MM, et al. Prospective, randomized, double-blind, phase III clinical trial of anti-T-lymphocyte globulin to assess Impact on chronic graft-versus-host disease-free survival in patients undergoing HLA-matched unrelated myeloablative hematopoietic cell transplantation. J Clin Oncol. 2017;35:4003–11. https://doi.org/10.1200/JCO.2017.75.8177.
36. Kumar A, Reljic T, Hamadani M, Mohty M, Kharfan-Dabaja MA. Antithymocyte globulin for graft-versus-host disease prophylaxis: an updated systematic review and meta-analysis. Bone Marrow Transplant. 2019;54:1094–106. https://doi.org/10.1038/s41409-018-0393-0.
37. Bredeson CN, Zhang MJ, Agovi MA, Bacigalupo A, Bahlis NJ, Ballen K, et al. Outcomes following HSCT using fludarabine, busulfan, and thymoglobulin: a matched comparison to allogeneic transplants conditioned with busulfan and cyclophosphamide. Biol Blood Marrow Transplant. 2008;14:993–1003. https://doi.org/10.1016/j.bbmt.2008.06.009.
38. Bornhauser M, Storer B, Slattery JT, Appelbaum FR, Deeg HJ, Hansen J, et al. Conditioning with fludarabine and targeted busulfan for transplantation of allogeneic hematopoietic stem cells. Blood. 2003;102:820–6. https://doi.org/10.1182/blood-2002-11-3567.
39. Bartelink IH, van Reij EM, Gerhardt CE, van Maarseveen EM, de Wildt A, Versluys B, et al. Fludarabine and exposure-targeted busulfan compares favorably with busulfan/cyclophosphamide-based regimens in pediatric hematopoietic cell transplantation: maintaining efficacy with less toxicity. Biol Blood Marrow Transplant. 2014;20:345–53. https://doi.org/10.1016/j.bbmt.2013.11.027.
40. Russell JA, Kangarloo SB, Williamson T, Chaudhry MA, Savoie ML, Turner AR, et al. Establishing a target exposure for once-daily intravenous busulfan given with fludarabine and thymoglobulin before allogeneic transplantation. Biol Blood Marrow Transplant. 2013;19:1381–6. https://doi.org/10.1016/j.bbmt.2013.07.002.
41. Levrat E, Roosnek E, Masouridi S, Mohty B, Ansari M, Villard J, et al. Very long term stability of mixed chimerism after allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies. Bone Marrow Res. 2015;2015:176526 https://doi.org/10.1155/2015/176526.
42. Lee HC, Saliba RM, Rondon G, Chen J, Charafeddine Y, Medeiros LJ, et al. Mixed T lymphocyte chimerism after allogeneic hematopoietic transplantation is predictive for relapse of acute myeloid leukemia and myelodysplastic syndromes. Biol Blood Marrow Transplant. 2015;21:1948–54. https://doi.org/10.1016/j.bbmt.2015.07.005.
43. Tang X, Alatrash G, Ning J, Jakher H, Stafford P, Zope M, et al. Increasing chimerism after allogeneic stem cell transplantation is associated with longer survival time. Biol Blood Marrow Transplant. 2014;20:1139–44. https://doi.org/10.1016/j.bbmt.2014.04.003.
44. van Leeuwen JE, van Tol MJ, Joosten AM, Wijnen JT, Verweij PJ, Khan PM, et al. Persistence of host-type hematopoiesis after allogeneic bone marrow transplantation for leukemia is significantly related to the recipient's age and/or the conditioning regimen, but it is not associated with an increased risk of relapse. Blood. 1994;83:3059–67.
45. Storb R, Yu C, Wagner JL, Deeg HJ, Nash RA, Kiem HP, et al. Stable mixed hematopoietic chimerism in DLA-identical littermate dogs given sublethal total body irradiation before and pharmacological immunosuppression after marrow transplantation. Blood. 1997;89:3048–54. e-pub ahead of print 1997/04/15
46. Mohty M. Mechanisms of action of antithymocyte globulin: T-cell depletion and beyond (Review). Leukemia. 2007;21:1387–94. https://doi.org/10.1038/sj.leu.2404683.
47. Boeck S, Hamann M, Pihusch V, Heller T, Diem H, Rolf B, et al. Kinetics of dendritic cell chimerism and T cell chimerism in allogeneic hematopoietic stem cell recipients. Bone Marrow Transplant. 2006;37:57–64. https://doi.org/10.1038/sj.bmt.1705217.
48. Doney KC, Loken MR, Bryant EM, Smith AG, Appelbaum FR. Lack of utility of chimerism studies obtained 2-3 months after myeloablative hematopoietic cell transplantation for ALL. Bone Marrow Transplant. 2008;42:271–4. https://doi.org/10.1038/bmt.2008.155.
49. Storek J, Mohty M, Boelens JJ. Rabbit anti-T cell globulin in allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2015;21:959–70. https://doi.org/10.1016/j.bbmt.2014.11.676.
50. Ocheni S, Kroeger N, Zabelina T, Sobottka I, Ayuk F, Wolschke C, et al. EBV reactivation and post transplant lymphoproliferative disorders following allogeneic SCT. Bone Marrow Transplant. 2008;42:181–6. https://doi.org/10.1038/bmt.2008.150.
51. Walker I, Panzarella T, Couban S, Couture F, Devins G, Elemary M, et al. Addition of anti-thymocyte globulin to standard graft-versus-host disease prophylaxis versus standard treatment alone in patients with haematological malignancies undergoing transplantation from unrelated donors: final analysis of a randomised, open-label, multicentre, phase 3 trial. Lancet Haematol. 2020;7:e100–e111. https://doi.org/10.1016/S2352-3026(19)30220-0.
52. Sorror ML, Storb RF, Sandmaier BM, Maziarz RT, Pulsipher MA, Maris MB, et al. Comorbidity-age index: a clinical measure of biologic age before allogeneic hematopoietic cell transplantation. J Clin Oncol. 2014;32:3249–56. https://doi.org/10.1200/JCO.2013.53.8157.
53. Stem Cell Trialists' Collaborative Group. Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. J Clin Oncol. 2005;23:5074–87.

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AUTHOR CONTRIBUTIONS

PVO'D, JMcC, and HJD designed the protocols; HJD, ACY, BES, TG and JPC analyzed the data. ACY and HJD wrote the manuscript. JSMcC was responsible for the busulfan pharmacokinetics; MEDF provided long-term follow-up data; MLS scored the comorbidities; GS maintained the database; CMcF carried out the chimerism determinations; PJM, KD, and FRA provided critical comments. All authors read and approved the manuscript.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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