Published in final edited form as:
Biol Blood Marrow Transplant. 2008 September ; 14(9): 993–1003. doi:10.1016/j.bbmt.2008.06.009.

OUTCOMES FOLLOWING HCT USING FLUDARABINE, BUSULFAN AND THYMOGLOBULIN: A MATCHED COMPARISON TO ALLOGENEIC TRANSPLANTS CONDITIONED WITH BUSULFAN AND CYCLOPHOSPHAMIDE

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Abstract

We have reported a lower incidence of acute graft versus host disease (aGVHD) with a novel conditioning regimen using low dose rabbit anti-thymocyte globulin (TG, Thymoglobulin) with fludarabine and intravenous busulfan (FluBuTG). To assess further this single center experience, we performed a retrospective matched pair analysis comparing outcomes of adult patients transplanted using the FluBuTG conditioning regimen with matched controls from patients reported to the CIBMTR receiving a first allogeneic hematopoietic stem cell transplant (HCT) after standard oral busulfan and cyclophosphamide (BuCy). 120 cases and 215 matched controls were available for comparison. Patients receiving FluBuTG had significantly less treatment related mortality (12% vs 34%, $p<0.001$) and grades II–IV aGVHD (15% vs 34% $p<0.001$) compared to BuCy patients. The risk of relapse was higher in the FluBuTG patients (42% vs 20%, $p<0.001$). The risks of chronic GVHD (cGVHD) and disease free survival (DFS) were similar in the cases and controls. These results suggest that the novel regimen FluBuTG decreases the risk of aGVHD and transplant mortality after HLA-identical sibling HCT, but is associated with an increased risk of relapse, resulting in similar DFS. Whether these conditioning regimens may be more suitable for specific patient populations based on relapse risk requires testing in prospective randomized trials.

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Key words or phrase

anti-thymocyte globulin; allogeneic transplantation; Busulfan

INTRODUCTION

Allogeneic HCT following standard myeloablative conditioning is associated with significant risks of regimen related morbidity, graft versus host disease (GVHD) and mortality. Attempts to reduce the intensity of the conditioning regimen have had mixed results. Although early morbidity and mortality has generally been lower, GVHD and non-relapse mortality remain a problem (1). In addition, reduced intensity conditioning approaches have been more successful in patients with indolent disease, as the conditioning regimen provides limited antitumor activity (1–6). Disease control relies instead on the graft versus malignancy effect which may take months to develop.

A combination of fludarabine + IV Bu (Flu + IV Bu) was developed to try and address the toxicity limitations of traditional regimens while providing disease control not seen with the very low intensity regimens (7, 8). The use of IV Bu results in more predictable Bu levels and the long half life allows for the convenience of once daily administration (7, 9, 10). Fludarabine, a strongly immunosuppressive purine analogue was selected to replace cyclophosphamide. Cyclophosphamide, long known to have dose limiting cardiac toxicity and risk of hemorrhagic cystitis is increasingly recognized as contributing to the morbidity and mortality of traditional regimens through hepatic toxicity (11, 12).

Thymoglobulin (TG), a rabbit derived anti-thymocyte globulin has been used for many years as prophylaxis for GVHD, the main cause of transplant-related mortality (TRM). Results in the alternative donor setting and subsequently in the related donor setting have been mixed (13–20); while GVHD and regimen related mortality have generally been decreased, infectious complications and relapse have been variably reported as increased. The effect on overall survival is also unclear.

There is evidence that myeloablative regimens based on Flu and IV Bu may provide effective control of hematologic malignancy with perhaps less toxicity than BuCy (7, 21). A report from the Alberta Blood and Marrow Transplant Program (ABMTP) in Calgary showed that the addition of a relatively low dose of TG to myeloablative regimens, largely Flu with oral or IV Bu resulted in decreased cGVHD and TRM but a trend to more relapse after HCT from matched siblings (22). This single center matched pair analyses was constrained by the heterogeneity of the patient population and the limited number from which to draw controls. To overcome these limitations we conducted a matched pair analysis of FluBuTG cases from the ABMTP using controls who received traditional oral Bu and Cy (BuCy) conditioning from the Center for International Blood and Marrow Transplant Research (CIBMTR) database. Outcomes studied were TRM, relapse, aGVHD, cGVHD and overall survival (OS).

PATIENTS AND METHODS

Data Sources

Data for this study were obtained from two data sources: the CIBMTR controls (n=215) and the ABMTP cases (n=120). The details of some of the cases from ABMTP have been previously reported (20, 22). The CIBMTR is a research affiliation of the International Bone Marrow Transplant Registry (IBMTR), Autologous Blood and Marrow Transplant Registry (ABMTR) and the National Marrow Donor Program (NMDP) that comprises a voluntary

working group of more than 500 transplant centers worldwide. Participating centers contribute detailed data on consecutive allogeneic and autologous hematopoietic stem cell transplants (HCT) to a Statistical Center at the Medical College of Wisconsin. Demographic and clinical data are collected on a representative sample of patients in the registry using a weighted randomization scheme.

Participating centers are required to report all consecutive transplant data; compliance is monitored by on-site audits. Patients are followed longitudinally, with yearly follow-up. The CIBMTR collects data at two levels: Registration and Research. Registration data include disease type, age, sex, pretransplant disease stage and chemotherapy-responsiveness, date of diagnosis, graft type (bone marrow, peripheral blood and cord blood derived hematopoietic stem cells), preparative regimen, post transplant disease progression and survival, development of secondary malignancies and cause of death. Requests for data on progression or death for registered patients are at six-month intervals. All CIBMTR teams contribute registration data. Research data are collected on subsets of registered patients and include comprehensive pre and post transplant clinical information. Computerized checks for errors, physician reviews of submitted data and on-site audits of participating centers ensure the quality of data.

Patients

Eligible subjects for the study were recipients of a first allogeneic bone marrow or peripheral blood cell transplantation from an HLA-identical sibling donor between 1999 and 2003 for ALL, AML, CML, MDS, NHL, HL, MM or CLL. Patients were aged 18 to 65 years inclusive.

Cases

The cases in this study were all from the ABMTP in Calgary, Canada and registered with the CIBMTR. As an additional eligibility criterion for the cases, patients had to have received FluBuTG for their pretransplant conditioning therapy as previously reported (20, 22) to be considered for the study. One hundred and thirty-three patients who met this condition were selected from CIBMTR database. A data set containing detailed pre and post transplant clinical information was provided by data managers at the ABMTP. For their GVHD prophylaxis the Calgary cases also received traditional cyclosporine (CSA) and short course methotrexate (MTX).

Selection of Matched Controls

Potential matched controls for the FluBuTG cases were selected from the CIBMTR database. Adult patients (≥18 years ≤ 65 years) who received a first allogeneic bone marrow or peripheral blood cell transplant from an HLA-identical sibling donor between 1999 and 2003 for the above named diseases were considered. Matched controls were selected from a pool of 573 patients who met the eligibility criteria. Patients in the control group received traditional ablative oral BU plus CY pretransplant conditioning therapy and CSA with short course MTX as GVHD prophylaxis.

Matching

Cases and controls were matched on disease and disease status prior to transplant (CR1, CP1 vs. CR2, CP2, AP vs. PIF, Relapse, and BP). For each case, a matched control was selected with the smallest age difference among potentially matched controls. The matching procedure was repeated twice for a maximum of one case to two controls matching. Of the 133 cases and 573 potential controls identified using the eligibility criteria, we were able to match 120 cases to 215 controls; 2 controls were identified for 95 cases, 1 for 25 cases and

for 13 cases, no suitable controls were identified. These 13 cases were excluded from the analysis.

Treatment Regimen

The conditioning regimen for the cases was fludarabine 50mg/m2/day for 5 days (days -6 to -2), IV BU (Busulfex, Orphan Medical, Minnetonka, MN) 3.2 mg/kg actual or adjusted ideal body weight ((ideal + 0.4(actual-ideal)) once daily for 4 days (day-5 to -2) as a 3 hour continuous infusion and TG (Thymoglobulin, Genzyme, Boston MA) 4.5 mg/kg in divided doses of 0.5, 2 and 2 mg/kg on days -2, -1 and 0, respectively (7). Controls received traditional oral Bu 16mg/kg in 16 divided doses and Cy 120mg/kg to 180mg/kg in 2 or 3 divided doses, respectively. Data on whether patients had targeted doses based on busulfan levels was not available in the CIBMTR database but based on the years from which controls were selected it is likely that the majority of patients did not have busulfan levels. Five controls received a cyclophosphamide dose of ≥ 180mg/kg. Both cases and controls received traditional CSA and short course MTX (days +1, +3, +6, and +11) graft versus host disease prophylaxis. Calgary cases also received folinic acid 5mg starting 24hrs after each methotrexate dose and continued every 6 hours until 12 hours before the next methotrexate dose (23). The CIBMTR database does not collect data on the tapering schedule of CSA, whether all 4 doses of methotrexate were given or whether patients received folinic acid. None of the cases or controls in the study population had T-cell depleted grafts.

Endpoints

The primary endpoints were TRM, hematologic relapse/disease progression, acute and chronic GVHD, OS and cause of death (COD). Acute GVHD was defined and graded based on the pattern and severity of organ involvement using established criteria (24). Chronic GVHD was defined as the development of any cGVHD based on clinical criteria. We defined relapse/progression as the time from transplant until relapse for those in continuous remission (CR) or disease progression for those who did not achieve CR with transplantation. Non-CR patients were primarily multiple myeloma (MM) patients. Transplant-related mortality was defined as death within 28 days of transplant, death from any cause in CR and death in the absence of disease progression for patients not in CR at transplant. Treatment failure was defined as death from any cause or disease progression / relapse. For analysis of OS, failure was death from any cause; surviving patients were censored at the date of last contact. Cause of death was reported by the individual teams involved in the care of the patient. Cause of death may or may not have been confirmed by autopsy. There was no central review of attribution of COD.

Statistical Analysis

Patient-, disease-, and transplant-related variables for patients in the case and control groups were compared using conditional logistic regression test to adjust for matched pair comparison. Univariate probabilities of TRM, relapse/progression, aGVHD and cGVHD were calculated using cumulative incidence curves to accommodate competing risks (25). Probability of OS was calculated using the Kaplan Meier estimator and the log rank test was used for univariate comparison. Estimates of standard error for the survival function were calculated by the Greenwood's formula and 95% CI, using log-transformed intervals. The univariate analyses are solely descriptive and were carried out on the individual groups (cases and controls) with no adjustments for differences between the groups.

Matched Pair Analysis

Multivariate analysis was performed by fitting a stratified Cox model on matched pairs. Controls were identified from the CIBMTR database to match the cases from the ABMTP.

To compare outcomes of TRM, aGVHD and cGVHD, relapse/progression, treatment failure and OS, a Cox proportional hazards model stratified on the matched pairs was used to adjust for potential imbalance in baseline characteristics between cohorts (FluBuTG vs. BuCy). A stepwise backward selection multivariate model was built to identify other covariates (other than those matched for) which influenced outcomes. The following variables were considered in multivariate analysis: the type of conditioning regimen: FluBuTG (cases) vs. conventional BuCy (controls), (main effect), age at transplant (continuous), Karnofsky performance score at transplant (<90 vs. ≥90) and graft type (BM vs. PB). Variables used to match are by definition not separately included in the Cox model. Year of transplant was not tested in the model as the window of time for cases in the study was only 5 years. The variable for the main effect was retained in all steps of model building. Any covariate with a p-value of 0.05 or less was considered to indicate statistical significance. The proportionality assumption for Cox regression was tested by adding a time-dependent covariate for each risk factor and each outcome. The proportionality assumption was met in all cases. Potential interactions between the main effect (FluBuTG vs. BuCy) and all significant risk factors were tested. No interactions were detected. Final results were expressed as relative risks (RR) of the event and its 95% confidence interval. All the analyses were performed using SAS software, version 9.1 (SAS Institute).

RESULTS

Matching

The total study cohort after matching was 120 cases and 215 controls. Among the 215 controls; 183 (85%) matched age difference within 5 years; 23 (11%) between 6 and 15 years; and 9 (4 %) between 16 and 37 years.

Patient Disease and Transplant Characteristics

Patient-, disease-, and transplant-related characteristics of the study population are described in Table 1. Compared to the controls, the matched cases had a lower percent of patients with a Karnofsky score (KPS) of ≥90 (30% vs. 83%, $p<0.001$) and were more likely to receive a PB graft (83% vs. 60%, $p<0.001$). Median follow up among surviving cases (n=69) was 59 (16–95) months and controls (n=115) was 51 (3–93) months. Eighty nine percent of the surviving controls and 99% of the surviving cases had at least 2 years follow up. Other variables were not different between the groups after matching.

Univariate Outcomes

Univariate outcomes for the cases and controls are shown in Table 2. These comparisons do not adjust for residual differences (KPS, graft source, year of transplant) between the cases and the controls and are not matched-pair analyzed. Comparisons between the groups are limited to the multivariate analyses. The cumulative incidence of TRM among the cases and controls at 1 year was 9% (95% CI 5–15%) and 24% (95% CI 18–30%), respectively.

The cumulative incidence of grade II–IV aGVHD at day 100 was 15% (95% CI 9–22%) and 34% (95% CI 28–41%) in the cases and the controls.

The cumulative incidence of relapse/progression at 1 year was 29% (95% CI 21–38%) and 12% (95% CI 8–17%) for the cases and the controls, respectively.

Multivariate Analysis

The results of the multivariate analysis for this study are shown in Table 3.

Acute GVHD

The cumulative incidence of aGVHD for each group is shown in Table 2. In the multivariate analysis, the risk for this study of aGVHD was significantly less in the cases than in the controls (RR = 0.36, p= 0.0003). No other covariates were significant in the multivariate analysis of aGVHD.

Chronic GVHD

The risk of cGVHD was not different between the cases and the controls (RR=1.28, p=0.26). No other covariates were significant in the multivariate analysis of cGVHD.

Treatment-Related Mortality

The risk of TRM was significantly lower in the cases compared to the controls (RR= 0.32, p=0.0013). No other covariates were significant in the multivariate analysis of treatment related mortality.

Relapse/Progression

The risk of relapse/progression was significantly higher in the cases than the controls (RR= 1.91, p=0.014). No other covariates were significant in the multivariate analysis of relapse.

Treatment Failure

The risk of treatment failure, the inverse of progression free survival, was not different between the cases and the controls (RR=0.90, p=0.59). No other covariates were significant in the multivariate analysis of treatment failure.

Survival

The risk of death was significantly less in the cases than the controls (RR=0.64, p=0.0298). No other covariates were significant in the multivariate analysis of survival.

Causes of Death

As is seen in Table 4, the major single cause of death was from primary disease. In addition to the overall risk of death being lower in the cases than the controls, the causes of death differed between the 2 groups, $p<0.01$. Mortality from the primary disease was observed to be higher among the cases (64% of deaths were due to relapse) compared to the controls (22% of deaths due to relapse). It is difficult to specifically determine deaths due to GVHD as some cases of infection are in patients being treated with immune suppression for GVHD. Considered together, GVHD and infection was the cause of death in 14% of the cases and 33% of the controls.

DISCUSSION

Oral BuCy is a commonly used traditional ablative conditioning regimen used for HLA-matched sibling donor transplants. In our case-matched study using multivariable analyses, we found that the FluBuTG regimen was associated with a decreased incidence of treatment related mortality and aGVHD compared to oral BuCy but no difference in the risk of cGVHD. The FluBuTG regimen was associated with an increased risk of relapse but overall survival remained higher. While the overall risk of death was decreased with FluBuTG the causes of death were different between the two groups. A higher proportion of deaths observed in the FluBuTG patients were from their primary disease. This raises the question of whether this regimen compromises the graft versus malignancy effect and which patient population should be transplanted with this regimen.

FluBuTG was developed to incorporate newer agents that decrease potential regimen related toxicity and simplify care delivery. Fludarabine, was introduced to replace Cy both for ease of administration and in an attempt to limit toxicities associated with Cy metabolites (7, 8). Intravenous Bu has been substituted for the oral drug because of an improved pharmacokinetic profile, ease of administration, and the ability to dose once daily. The later addition of Thymoglobulin was based on reports of decreased GVHD and early mortality in unrelated donor transplant recipients receiving ATG (13, 16, 18, 19, 26).

The use of TG as part of the pretransplant conditioning regimen has been associated with mixed results with respect to GVHD and is summarized in Table 5. These studies are primarily retrospective case series, case-control studies or small trials in heterogeneous patient populations. The major conclusions of these studies have been that TG decreases acute and/or chronic GVHD and may result in decreased TRM however; higher doses resulted in increased infections and relapse with no long-term improvement in overall survival.

Exemplary of these mixed results is a report of 2 small prospective randomized trials comparing conditioning with CYTBI +/− TG for unrelated marrow transplants (14). The first study comparing aGVHD using 7.5mg/kg TG was closed for futility after 54 patients. Subsequently, patients randomized to 15mg/kg TG were found to have significantly less grade III–IV aGVHD compared to patients receiving CYTBI alone. These results were complicated by a higher rate of infectious deaths in the high dose TG group. The multivariate analysis demonstrated that TG was associated with decreased cGHVD with a dose effect but not a decrease in TRM because of more infectious deaths in the high dose TG group. Long-term follow up of these studies demonstrated that TG was associated with decreased extensive cGVHD, decreased bronchiolitis obliterans and improved KPS (27). Mohty et al demonstrated a similar dose effect of TG on aGVHD in the HLA-identical sibling setting (15). In addition, one year PFS was higher in patients experiencing any type of GVHD suggesting that a GVT effect was maintained despite the addition of TG.

Russell et al recently reported a single center case control analysis of 54 patients who were treated with TG as part of various conditioning regimens and matched on disease and disease stage with patients who did not receive TG (22). Approximately 30 patients from this publication are included in our analysis. While the results are not necessarily the same as in our study, there are significant differences between the two studies. The sample size in ours is larger, including an additional 90 FluBuTG patients. In our study, the cases and controls each received a single conditioning regimen and were contemporaneous. Nonetheless, both studies identified lower TRM and higher relapse with TG.

Timing of the TG administration in relation to the graft infusion has also been raised as a critical factor (17) as administration of TG close to the time of graft infusion removes immunologically active cells in the graft. The final TG dose in the FluBuTG regimen is given very close to the infusion of the hematopoietic cell graft to try and ensure that donor lymphocytes infused with the graft will be removed by the circulating antibody.

The causes of death may depend on conditioning regimen. While the FluBuTG regimen in our study was associated with a lower risk of death, a greater proportion of those deaths were due to primary disease. Several possibilities exist to explain this observation. Misclassification is unlikely. It may reflect that due to higher early mortality, fewer BuCy patients were alive to later succumb to their underlying disease. Alternatively, it is possible the addition of TG results in a decreased GVT potential or that Cy is more cytotoxic (i.e. a better drug to kill cancer cells) than Flu. There is, however, recent evidence that a FluBu regimen has at least equivalent antileukemic activity to BuCy in AML (28).

The better tolerability of the FluBuTG regimen may allow for other strategies to be added in order to improve disease control. Russell et al have shown that the addition of 400Gy of total body irradiation to FluBUTG significantly reduces relapse in AML without increased TRM (29). Monitoring Bu levels allows dose adjustment not only to avoid toxic levels but also to target to higher levels in those diseases for which dose intensity may be important (20, 30, 31). Potentially, donor lymphocyte infusion can be added for those patients initially spared significant GVHD.

Another approach to decreasing toxicity has been to replace oral with intravenous Bu based on its consistent and predictable pharmacokinetics (7, 9, 21). The CIBMTR has reported decreased incidence of hepatic VOD and decreased 100 day mortality with IV Bu vs. oral Bu (32). It is unknown whether IV BuCy will result in similar long-term results as FluBuTG although the recent study in AML by Andersson et al suggests that the FluBu combination is superior to oral at least in that disease. At the time of our study, insufficient numbers of patients receiving IV BU had been reported to the CIBMTR to allow for this comparison to be conducted.

Other factors that may influence transplant outcome and have varied between reports are conditioning regimen, graft type, relapse risk based on diagnosis and disease status, GVHD prophylaxis, age, KPS and year of transplant. Our study was limited to adult cases with HLA-identical sibling donors undergoing their first transplant over a 5 year period. All the controls also received the same GVHD prophylaxis and were matched one age and disease except for the leukemia patients who were also matched on disease status. Multivariate analysis adjusted for age, KPS and graft type. As with all registry or observational studies, there are limitations related to collecting data from multiple centers and we acknowledge the caveats of our observational data. While a center effect was not identified for the controls, it is impossible to know the details of care such as the patterns of CSA tapering, the grading of GVHD, whether Bu levels and targeting were done or whether all 4 doses of methotrexate were administered, etc., at individual centers. As the CIBMTR is an observational database, we do not prescribe therapy; we collect only intended therapies and not data on individual variability of practice such as the items mentioned above. In practice, however, this is not really different from a prospective trial where clinical decisions take precedence over protocol therapy and is not anticipated to be systematically biasing the study in one direction. Similarly, the variability of individual patient selection for transplant is impossible to replicate between centers and although matched controls were randomly identified based on the selection criteria, unknown differences potentially remain between individual cases and controls. Again, it is unlikely to have resulted in systematic bias in favor of one group. Nonetheless, although not as robust as a randomized trial, our case-control analysis is a very good approach to using registry data in a single center comparative study that has a population-based patient group.

CONCLUSION

Many factors contribute to post transplantation outcomes with GVHD and relapse being the two main barriers to improved results. Other than patient selection, the choice of conditioning regimen and GVHD prophylaxis are the main variables that the transplant team can alter in an attempt to improve outcomes. In this study, the FluBuTG regimen resulted in less TRM and aGVHD but at the expense of increased relapse. This regimen may provide a platform upon which modifications can be based depending on the disease being treated. Thus, in acute leukemia, the regimen can be intensified to compensate for the trend to more relapse without an increase in TRM (29). In other conditions where dose intensity might be less critical, modifications can be based more on attempts to harness the GVT effect in patients spared the effects of early GVHD.

Acknowledgments

Supported by Public Health Service Grant U24-CA76518 from the National Cancer Institute, the National Institute of Allergy and Infectious Diseases, and the National Heart, Lung and Blood Institute; Office of Naval Research; Health Resources and Services Administration (DHHS); and grants from AABB; Aetna; American International Group, Inc.; American Society for Blood and Marrow Transplantation; Amgen, Inc.; Anonymous donation to the Medical College of Wisconsin; Astellas Pharma US, Inc.; Baxter International, Inc.; Bayer HealthCare Pharmaceuticals; BioOne Corporation; BloodCenter of Wisconsin; Blue Cross and Blue Shield Association; Bone Marrow Foundation; Bristol-Myers Squibb Company; Cangene Corporation; Celgene Corporation; CellGenix, GmbH; Cerus Corporation; Cubist Pharmaceuticals; Cylex Inc.; CytoTherm; DOR BioPharma, Inc.; Dynal Biotech, an Invitrogen Company; EKR Therapeutics; Enzon Pharmaceuticals, Inc.; Gambro BCT, Inc.; Gamida Cell, Ltd.; Genzyme Corporation; Gift of Life Bone Marrow Foundation; GlaxoSmithKline, Inc.; Histogenetics, Inc.; HKS Medical Information Systems; Hospira, Inc.; Infectious Diseases Society of America; Kiadis Pharma; Kirin Brewery Co., Ltd.; Merck & Company; The Medical College of Wisconsin; MGI Pharma, Inc.; Millennium Pharmaceuticals, Inc.; Miller Pharmacal Group; Milliman USA, Inc.; Miltenyi Biotec, Inc.; MultiPlan, Inc.; National Marrow Donor Program; Nature Publishing Group; Oncology Nursing Society; Osiris Therapeutics, Inc.; Pall Life Sciences; PDL BioPharma, Inc; Pfizer Inc; Pharmion Corporation; Roche Laboratories; Schering Plough Corporation; Society for Healthcare Epidemiology of America; StemCyte, Inc.; StemSoft Software, Inc.; SuperGen, Inc.; Sysmex; Teva Pharmaceutical Industries; The Marrow Foundation; THERAKOS, Inc.; University of Colorado Cord Blood Bank; ViaCell, Inc.; Vidacare Corporation; ViraCor Laboratories; ViroPharma, Inc.; and Wellpoint, Inc. The views expressed in this article do not reflect the official policy or position of the National Institute of Health, the Department of the Navy, the Department of Defense, or any other agency of the U.S. Government.

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Table 1

Characteristics of patients aged 18–65 years who received a first HLA-identical sibling PBSC or BM transplants for hematologic malignancies from 1999–2003. Patients received Fludarabine + Busulfan + Thymoglobulin (cases) or Busulfan + Cyclophosphamide (controls).

	CASES FluBuTG		CONTROLS BuCy		
Characteristics of patients	**N eval**	**N (%)**	**N eval**	**N (%)**	**P-value[a]**
Number of patients		120		215	
Number of centers		1		62	
Age at transplant, median (range), years	120	46 (18–65)	215	44 (18–63)	
18–45		57 (48)		120 (56)	0.01
46–65		63 (52)		95 (44)	
Male sex	120	72 (60)	215	127 (59)	0.87
Matching Groups	120		215		--**
AML, ALL, CML early(CR1, CP1)[b]		52 (44)		104 (48)	
AML, ALL, CML intermediate (CR2+, CP2+, AP)[b]		4 (3)		8 (4)	
AML, ALL, CML advanced (PIF, REL, BP)[b]		13 (11)		26 (12)	
MDS Treated		4 (3)		8 (4)	
MDS Untreated		18 (15)		36 (17)	
Multiple Myeloma		8 (8)		8 (4)	
NHL/HL PIF or REL		14 (12)		14 (6)	
NHL/HL CR		4 (3)		8 (4)	
CLL[c]		3 (2)		3 (1)	
Karnofsky score prior to transplant	120		208		<0.001
< 90		84 (70)		36 (17)	
≥ 90		36 (30)		172 (83)	
Graft type	120		215		
BM		21 (17)		87 (40)	<0.001
PBSC		99 (83)		128 (60)	
Time from diagnosis to transplant, median (range), months	118	5 (<1–154)	215	6 (<1–209)	
≤ 6 months		66 (56)		103 (48)	0.03
> 6 months		52 (44)		112 (52)	

Characteristics of patients	CASES FluBuTG N eval	CASES FluBuTG N (%)	CONTROLS BuCy N eval	CONTROLS BuCy N (%)	P-value[a]
Donor-recipient CMV status	114		204		0.25
Negative/Negative		22 (19)		51 (25)	
At least one positive		92 (81)		153 (75)	
Donor-recipient sex match	120		214		0.89
F-M		34 (28)		63 (29)	
Other[d]		86 (72)		151 (71)	
GVHD prophylaxis	120		215		
MTX + CSA only		120 (100)		215 (100)	
Year of transplant	120		215		<0.001
1999–2000		44 (37)		146 (68)	
2001–2003		76 (63)		69 (32)	
Year of diagnosis	118		215		0.07
1982–1996		4 (3)		14 (7)	
1997–2003		114 (97)		201 (93)	
Median follow-up of survivors, months	69	60 (16–95)	115	54 (3–93)	

Abbreviations: AML=acute myelogenous leukemia; ALL=acute lymphoblastic leukemia; CLL=chronic lymphocytic leukemia; CML=chronic myelogenous leukemia; NHL=non-Hodgkin lymphoma; HL= Hodgkin lymphoma; MDS/MPS=myelodysplastic/myeloproliferative disorders; CR= complete remission; REL= relapse; PIF= primary induction failure; CP= chronic phase; AP= accelerated phase; BP=blast phase; CSA= cyclosporine; MTX= methotrexate; BuCy= oral busulfan + cyclophosphamide; FluBuTG= fludarabine + IV busulfan + thymoglobulin; BM= bone marrow; PBSC= peripheral blood stem cells; RIC= reduced intensity conditioning; EVAL=evaluable; CMV= cytomegalovirus virus; F=female; M=male; HLA=human leukocyte antigen.

[a]The conditional logistic regression test was used for discrete covariates to detect the significant difference to for matched pair.

[b]The number of CML, AML and ALL patients and disease status among the cases and controls are given below.

Disease	Cases	Controls
CML	21	76
1st Chronic phase	19	69
2nd Chronic phase	-	2
≥ 2nd Chronic phase	2	2
Blast phase	-	3

Disease	Cases	Controls
AML	46	55
Primary induction failure	6	10
CR1	30	31
CR2	2	2
REL1	7	9
≥ REL2	1	3
ALL	2	6
CR1	2	3
CR2	-	1
CR3	-	1
REL1	-	1

[c] The two other CLL disease statuses are RAI stage 0 and RAI stage III.

[d] Other Donor-recipient sex match included: Male-Male (n=101); Male-Female (n=53); Female-Female (n=83).

** Matched variables.

Table 2

Univariate probabilities[a] of transplant outcomes among patients receiving Fludarabine + Busulfan + Thymoglobulin (Cases) or Busulfan + Cyclophosphamide (Controls).

	CASES FluBuTG		CONTROLS BuCy		
Outcome event	**N eval**	**Prob (95% CI)**	**N eval**	**Prob (95% CI)**	**P-value**
Grade II–IV AGVHD	120		215		
@ 100 days		15 (9–22) %		34 (28–41)%	<0.001++
Chronic GVHD	120		213[b]		
@ 1 year		39 (30–48) %		32 (25–39) %	0.20++
@ 2 years		39 (30–48) %		34 (27–41) %	0.357++
Treatment related mortality	120		215		
@ 1 year		9 (5–15) %		24 (18–30) %	<0.001++
@ 3 years		11 (6–17) %		30 (24–37) %	<0.001++
@ 5 years		12 (7–19) %		34 (27–41) %	<0.001++
Relapse/progression	120		215		
@ 1 year		29 (21–38) %		12 (8–17) %	<0.001++
@ 3 years		36 (27–45) %		20 (14–25) %	<0.001++
@ 5 years		42 (32–52) %		20 (15–26) %	<0.001++
Overall survival	120		215		0.157+
@ 100 days		91 (85–95) %		82 (76–87) %	0.01++
@ 1 year		76 (68–83) %		66 (60–73) %	0.06++
@ 3 years		65 (56–73) %		55 (48–62) %	0.07++
@ 5 years		58 (49–67) %		51 (43–58) %	0.22++

Abbreviations: CI= confidence interval; GVHD= graft-versus-host disease; BuCy= oral busulfan + cyclophosphamide; FluBuTG= fludarabine + IV busulfan + thymoglobulin; CY=Cyclophosphamide; AGVHD =acute graft-versus-host-disease.

[a]Probabilities of overall survival were calculated using the Kaplan-Meier product limit estimate. Probabilities of relapse, treatment-related mortality, acute GVHD and chronic GVHD were calculated using the cumulative incidence estimate.

[b] There are 2 patients missing CGVHD outcome data. These patients are missing the date of CGVHD onset.

[+] Log-rank test p-value.

[++] Pointwise p-value

Table 3

Multivariate analysis comparing transplant outcomes between HLA-identical patients who received Fludarabine + Busulfan + Thymoglobulin as conditioning (Cases) with HLA-identical patients who received Busulfan + Cyclophosphamide for their conditioning (Controls), after transplantation.

Outcome of interest	N eval	Relative Risk (95% Confidence Interval)	P - value
Acute GVHD[b]			
Main effect:			
Controls	215	1.00[a]	
Cases	120	0.36 (0.21–0.63)	0.0003
Chronic GVHD[b]			
Main effect:			
Controls	213	1.00[a]	
Cases	120	1.28 (0.83–1.98)	0.2607
Treatment related mortality[b]			
Main effect:			
Controls	215	1.00[a]	
Cases	120	0.322 (0.16 – 0.64)	0.0013
Relapse/progression[b]			
Main effect:			
Controls	215	1.00[a]	
Cases	120	1.91 (1.14–3.19)	0.0138
Treatment failure[b]			
Main effect:			
Controls	215	1.00[a]	
Cases	120	0.90 (0.62 – 1.31)	0.5901
Overall survival[b]			
Main effect:			
Controls	215	1.00[a]	
Cases	120	0.644 (0.43–0.96)	0.0298

Abbreviations: GVHD= graft-versus-host disease.

[a] Reference group.

[b] No other covariates were significant.

Table 4

Causes of death.

	CASES FluBuTG		CONTROLS BuCy	
	N eval	**N (%)**	**N eval**	**N (%)**
Number of patients	120		215	
Number of deaths	52		100	
Primary disease		33 (64)		22 (22)
New malignancy		1 (2)		0
Graft versus host disease		4 (8)		16 (16)
Infection		3 (6)		17 (17)
Organ failure		2 (4)		14 (14)
Interstitial pneumonia		2 (4)		9 (9)
Other cause		7 (14)		22 (22)

Table 5

Outcomes of Allogeneic Transplant Studies Using Anti-Thymocyte Globulin for GVHD Prophylaxis

Ref	n	Donor	ATG Dose	aGVHD	cGVHD	Toxicity	Rel/Prog
Bacigalupo (Ref 14)	No rATG 25 rATG 29	Unrel	7.5mg/kg rATG Sangstat	NS	NS	TRM p=NS	Survival p=NS
Bacigalupo (Ref 14)	No rATG 28 rATG 25	Unrel	15mg/kg rATG Sangstat	Grade III–IV 50% no rATG vs. 11% with rATG 11 (p=0.001)	In MVA, decreased risk of cGVHD with rATG	Infectious Deaths No rATG 7% With rATG 30% (p=0.02) In MVA, no decrease risk of TRM with rATG because of infectious deaths in high dose group	Survival p=NS
Mohty (Ref 15)	Low dose rATG 55 High dose rATG 46	HLA Id-sib	Low (2.5mg/kg) vs. High (7.5 or10mg/kg) rATG Sangstat	Gr II–IV 46% low vs. 24% high rATG (p=0.001)	Any cGVHD 76% low vs. 48% high rATG (p=0.02) rATG dose NS in MVA	TRM p=NS	Rel/Prog 29% with GVHD vs. 52% no GVHD p=0.02
Russell (Ref 22)	No rATG 54 rATG 54	Rel	4.5mg/kg rATG Sangstat	NS	96% no rATG vs. 55% with rATG p=0.002	100 day NRM 17% no rATG vs. 4% with rATG 1 year NRM 34% no rATG vs. 9% with rATG	4-yr Rel/Prog 22% no rATG vs. 43% with rATG p=0.05
Basara (Ref 16)	No ATG 68 ATG 87	Unrel	rATG Sangstat (5 to 15mg/kg) ATG Fresenius (45 or 60mg/kg)	NS	76% no rATG vs. 36% rATG p=0.0001	TRM no rATG vs. rATG p=NS	Rel/Prog p=NS LFS p=NS
Kröger (ref 18)	No ATG 57 ATG 45	Rel	ATG Fresenius 30, 60 or 90 mg/kg	47% no ATG vs. 20% with ATG (p=0.004)	67% no ATG vs. 36% with ATG	TRM p=NS	Rel p=NS 5-yr DFS p=NS
Remberger (Ref 26)	No rATG 52 rATG 52	Unrel	10mg/kg rATG Sangstat	5% no rATG vs. 12% with rATG	NS	NRM RR 0.30 with rATG p=0.005 CI 100 day NRM 21% no rATG vs. 6% with rATG	Rel/Prog p=NS MVA RR death 0.5 with rATG p=0.03
Remberger (Ref 19)	rATG 4mg/kg 51 6mg/kg 37 8mg/kg 19 10mg/kg 55	Unrel	4mg/kg, 6mg/kg, 8mg/kg, or 10mg,kg rATG Sangstat	GrII OR 2.67 for 4mg/kg vs. other doses rATG in MVA (p=0.01)	NS	TRM OR 0.35 6–8mg/kg rATG (p=0.03) Death OR 0.45 for 6–8mg/kg rATG (p=0.03)	Rel/Prog p=NS in MVA

Abbreviations: ATG=anti-thymocyte globulin; cGVHD=chronic graft versus host disease; CI=cumulative incidence; DFS=disease free survival; GrII-IV=grade II–IV acute graft versus disease; HLA Id-sib=HLA identical sibling donor; LFS=leukemia free survival; MVA=multivariate analysis; NRM=non-relapse mortality; NS=not significant; OR=odds ratio; rATG=rabbit antithymocyte globulin; Rel=HLA matched related donor; Rel/Prog=relapse or progression; RR=relative risk; TRM=treatment related mortality; Unrel=HLA matched unrelated donor.