Title

Comparative Analysis of Melphalan versus Busulphan T-Cell Deplete Conditioning using Alemtuzumab in Unrelated Donor Stem Cell Transplantation for Acute Myeloid Leukaemia

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Summary

There is limited data to guide the choice of reduced-toxicity T-deplete conditioning regimen in Acute Myeloid Leukaemia (AML). We conducted a multi-centre, retrospective analysis of two reduced toxicity T-deplete transplant protocols employing alemtuzumab and fludarabine in combination with either busulphan (FBC) or melphalan (FMC) in patients with AML in complete remission and without a matched sibling donor (n=117 FBC=47, FMC=70, median age 55). There were no differences in 5-year overall survival (OS), (52% v 46%,p=0.2) or relapse-free survival (45% v 45%,P=0.6) nor were there differences in non-relapse mortality (NRM) (17% v 15%,p=0.09) or cumulative incidence of relapse (CIR) (40% v 26%,p=0.26). Univariate and multivariate analyses showed age ≥60 years and HLA-mismatch to be the only features significantly associated with adverse OS. Higher NRM was seen in older patients (≥60) (42% v 15%,p=0.001), extensive v limited graft-versus-host disease (GVHD) (43% v 5%,p=0.0006), and HLA mismatch (36% v 18%,p=0.03). Higher CIR was seen in male recipients (5-year CIR 38% v 28%, p=0.02), and patients with *FLT3*-ITD (52% v 25%, p=0.02). Lower CIR was seen in those with chronic GVHD or strict full donor chimerism. Both regimens show equivalent outcomes with low incidence of graft failure and acute/chronic GVHD.

Key Words: Acute Myeloid Leukaemia, T-deplete conditioning, Allogeneic stem cell transplants, Alemtuzumab

Introduction

For the majority of patients with acute myeloid leukaemia (AML) who have intermediate or high-risk disease, allogeneic stem cell transplantation (allo-HSCT) offers the best chance of long-term cure(Döhner *et al*, 2017). Traditional allo-HSCT approaches have focused on myeloablative conditioning with high doses of cyclophosphamide, combined with total body irradiation (TBI) or busulphan. Reduced intensity conditioning (RIC) protocols, with a greater reliance on a graft-versus-leukaemia effect, allow the application of allo-HSCT to older patients and those with co-morbidities(Sengsayadeth *et al*, 2015). The morbidity and mortality associated with acute and chronic graft versus host disease (GVHD) has been reduced by in vivo T-cell depletion (TCD) most commonly using either anti-thymocyte globulin or alemtuzumab(Soiffer *et al*, 2011; Walker *et al*, 2016).

There is limited evidence to guide decision making regarding optimal allo-HSCT protocols in AML and as a result a variety of regimens exist(Bacigalupo *et al*, 2009; Jethava *et al*, 2017). We conducted a multi-centre retrospective analysis of two reduced toxicity T-deplete protocols in common use in the United Kingdom for unrelated donor allo-HSCT in patients with AML in complete remission (CR). Alemtuzumab was used for *in vivo* TCD with comparison made between fludarabine-melphalan-alemtuzumab (FMC) and fludarabine-busulphan-alemtuzumab (FBC) regimens.

Materials and Methods

Patients

Consecutive patients receiving a reduced intensity alemtuzumab-containing TCD HSCT from a matched unrelated donor for AML in CR at one of three hospitals, University College London Hospitals NHS Foundation Trust (UCLH), Royal Free London Hospital NHS Foundation Trust (RFH) and Kings College Hospital (KCH) between January 2005 and December 2014, were identified from local transplant databases. All patients received peripheral blood stem cells from an unrelated donor. CR was defined as per standard criteria(Cheson *et al*, 2003). Pre-transplant demographic data included recipient age at time of transplant, gender, transplantation in CR1 or CR2, cytogenetics at diagnosis according to Medical research Council (MRC) cytogenetic risk groups(Grimwade *et al*, 2010), fms-like tyrosine kinase 3- internal tandem duplication (*FLT3*-ITD) status in patients with normal karyotype, *de novo* or secondary/treatment-related disease, donor age, stem cell source, degree of HLA-mismatch (0, 1, 2 HLA antigens at major loci), and CMV serostatus of donor and recipient. All patients gave informed written consent for the data-collection.

Conditioning

Details of the conditioning regimens are shown in figure 1. The choice of conditioning regimen was based on centre dependent protocols; FBC(KCH) & FMC(UCLH/RFH). Additional GVHD prophylaxis at all sites consisted of cyclosporin-A administered from day -1. In the absence of GVHD, cyclosporin-A was tapered from day 56 post HSCT (FBC) or 3 months (FMC). Mycophenolate or methotrexate was not part of either protocol. Patients who had not attained full donor chimerism (FDC; see below) after cessation of immunosuppression were eligible to receive donor lymphocyte infusion (DLI) in the absence of active GVHD,

aiming for >95% donor T cell chimerism (KCH) or FDC (UCLH/RFH). DLI was also administered therapeutically as part of treatment for relapsed disease.

Chimerism

Chimerism was assessed using XY FISH (fluorescent in-situ hybridization) where a donor-recipient sex mismatch existed, and/or by PCR and fluorescent analysis of short tandem repeat/variable number tandem repeats sequences on bone marrow, whole blood; and peripheral CD3 and CD15 cell fractions as previously described(Bader *et al*, 2005; Mohamedbhai *et al*, 2012; Potter *et al*, 2014). Routine chimerism assessment began at three months (UCLH, RFH) or 28 days (KCH) and repeated 3-monthly unless otherwise indicated. Chimerism categories were defined as: 1) full donor (FDC): lack of detection of a previously determined recipient-specific peak and >95% donor T cell chimerism, 2) mixed T cell chimerism/predominant donor (MD): detection of both donor and recipient-specific peaks but with a predominant (50-95%) donor component, 3) very-mixed/predominant recipient (VM): >50% recipient chimerism; 4) Full recipient(R): no detectable donor cells.

GVHD

Acute and chronic GVHD (aGVHD, cGVHD) was assessed clinically according to established criteria(Przepiorka *et al*, 1995; Shulman *et al*, 1980) and confirmed histologically where possible.

Graft failure

Primary graft failure was defined as no evidence of engraftment or haematological

recovery of donor cells >28 days post HSCT without full donor chimerism. Secondary graft failure was defined as loss of a previously functioning graft, evident by cytopenia of at least two blood cell lineages with evidence of loss of donor cells (<95% donor chimerism) on the basis of cytogenetics, XY FISH or chimerism studies and in the absence of evidence of disease relapse.

Survival

The primary endpoint was overall survival (OS) measured from day 0 to death from any cause. Secondary end points included relapse-free survival (RFS), measured from day 0 to first relapse or death; cumulative incidence of relapse (CIR); and non-relapse mortality (NRM) measured from day 0 to death without evidence of relapse.

Statistics

Comparisons of baseline characteristics used Mann-Whitney U test, Fisher's exact test, or Chi-squared test for trend as appropriate. RFS and OS were calculated using the method of Kaplan and Meier & log-rank test (SPSS version 24.0). CIR and NRM were calculated using the Fine-Gray competing risks methods(Scrucca *et al*, 2010; Fine & Gray, 1999) using R version 3.4.1 (R Foundation for Statistical Computing, Vienna, Austria). Relapse and deaths without GVHD were considered competing risks for acute/chronic GVHD. Relapse was a competing risk for NRM. Multivariate analysis was conducted accounting for conditioning and pre-transplant variables using Cox proportional hazard regression method(Cox, 1972). The impact of GVHD on HSCT outcomes was studied as categorical variable

in absence of time specific data. P values were 2-tailed with values <0·05 considered statistically significant.

Results

Patients

One hundred and seventeen patients with AML in CR were identified, with patient and transplant characteristics detailed in Table 1. Median age of patients was 55 years (range 19-68). The FBC patient cohort were younger (p=0.04), more likely to have a fully matched (10/10) donor (p=0.03) and to receive a lower dose of alemtuzumab (p<0.0001). No other significant differences between the two groups were observed.

OS and EFS: univariate analyses

Median follow-up for all patients alive at the end of the study period (n=60) was 38.2 months (range: 4.6-106.1). The 5-year OS for the whole cohort (n=117) was 48% (Figure 2a) with no significant difference between FMC (46% 5-year OS) and FBC (52% 5-year OS, p=0.2) (Figure 2b). For those patients alive one-year post allo-HSCT (n=53), 5-year OS and RFS were 74% and 69% respectively. For patients alive *and* without any event at 1 year, the subsequent 5-year OS and RFS were 78% and 74% respectively.

On univariate analysis [Table S1 (supplementary)], age ≥60 years and HLA-mismatch were the only variables found to be significantly associated with worse OS. Patients aged ≥60 years had a poorer 5-year OS of 38% vs 55% (<60 years) (p=0.03) (Figure 2c). Patients with a mismatched unrelated donor (MMUD) had 5-

year OS of 29% compared with 60% for those with 10/10 HLA matched unrelated donor (MUD) (p=0.009) (Figure 2d). This difference was maintained for both 1 antigen (Ag) or 2 Ag mismatches. Patients <60 years with a 10/10 MUD had 73% 5-year OS, with no difference noted between the two regimens (p=0.14). There was no significant difference in OS/RFS for other variables measured including cytogenetic risk group and *FLT3*-ITD (Figures 2e-d, Table S1).

Non-relapse mortality (NRM): univariate analysis

Overall NRM at 1 and 5 years was 20% and 25% respectively and not different between the two conditioning regimens; (FMC, 21%,29% v FBC 15%,17%, p=0.09) (Figure 3a). Five-year NRM was higher for older patients (≥60yrs, 42% v <60yrs 15%, p=0.001) (Figure 3b) and in those with extensive vs limited cGVHD (43% vs 5%, p=0.0006) (Figure 3c). Higher NRM was noted for MMUD compared with the 10/10 MUD (5yr NRM 36% vs 18% respectively, p=0.03) (Table S2, supplementary).

Cumulative incidence of relapse (CIR): univariate analysis

The 5-year RFS was 45% for the whole cohort, the FMC cohort and the FBC cohort (p=0.68; Figure 3d). CIR was 16% and 30% at 1 and 5 years respectively, with no significant difference observed between the two conditioning cohorts (5yr CIR 26% FMC v 40% FBC, p=0.26) (Figure 3e). CIR was increased in male recipients (5-year CIR 38% male vs 18% female p=0.02) and patients with normal karyotype *FLT3*-ITD mutated (*FLT3*^{ITD}) versus wild type (*FLT3*^{WT}), with 5yr CIR of 52% vs 25% respectively (p=0.02). CIR was significantly reduced in patients with cGVHD (all grades) (p=0.02) with lower CIR in a sub-analysis of patients with extensive

cGHVD (Figure 3f, p-0.006). Similarly, CIR was significantly lower in sub-analysis of strict full donor T-cell chimerism (defined as >97% CD3 FDC) compared to non-full donor chimerism (<97%) patients (p=0.03). No similar observations were made with standard FDC (>95%) versus other chimerism groups. No other CIR differences were seen according to other patient features (Table S2, supplementary).

GVHD: univariate analysis

The FMC cohort was more likely to develop acute and cGVHD with differences observed in rates of grade 1 aGVHD and limited cGVHD, compared to FBC cohort (p<0.001) (Table 2). OS at 5-years in patients with grade 2-4 aGVHD was 50% v 38% in those with grade 0-1 (p=0.39; HR 0.73, 95% CI 0.35-1.51; Table S1). Grade 2-4 aGVHD did not significantly impact on either CIR or NRM. Incidence of severe (grade 3-4) aGVHD was low and did not differ between the FMC (n=4) and FBC cohorts (n=4). Grade 3-4 aGVHD was associated with higher 5-year NRM of 47% v 20% in those with grade 0-2; but did not reach significance due to small numbers of patients with severe aGVHD (p=0.20); while a trend towards lower CIR rates was observed in this group (31% vs 25%; p=0.06; Table S2)

Rates of extensive cGVHD were similar, with 17.5% and 19% recipients were affected (those alive >100days post HSCT) in the FMC and FBC cohorts respectively. For any grade of cGVHD vs no cGVHD there was an increased NRM (19% vs 6% p=0.04) and decreased CIR (22% vs 48% p=0.02) at 5-years post HSCT (Table S2). Extensive cGVHD was also associated with increased NRM (43% vs 5%; p=0.0006) and decreased CIR (42% vs 6%; p=0.006; Figure 3f) when

compared to patients with no or limited cGVHD; but this did not translate into differences in OS (5yr OS 38% vs 61% for extensive vs no or limited cGVHD, p=0.39).

Chimerism and DLI

Graft failure occurred in 4 patients in the FMC cohort and 1 patient in the FBC cohort (Table 2). Available lineage specific chimerism was compared at three months allowing analysis before pre-emptive DLI. For patients alive with a chimerism result at 3 months, a greater proportion of patients in the FMC cohort (24 of 33, 72%) had full donor T-cell chimerism (defined as >95% donor chimerism) than in the FBC cohort (18 of 42, 42%) while a higher proportion of FBC cohort had mixed donor chimerism (p=0.01). However, achievement of >95% donor chimerism at six months vs <95% mixed donor chimerism did not impact on subsequent 5-year OS (66% v 60% p=0.92) or EFS (64% vs 54%; p=0.54). Despite a decreased CIR in a sub-analysis of patients with strict FDC (>97% donor chimersim; n=26) vs non-FDC (n=91) there was no difference in OS or EFS.

Only 10 patients of the entire cohort (n=117) received DLI for mixed T-cell chimerism or predominant recipient chimerism in the absence of relapse (FMC, n=8; FBC n=2). The median time to first DLI post-transplant was 154 days (range 118-385), median number of DLI doses administered 2 (range 1-3), and median dose of $5x10^5$/kg CD3. Of the 10 patients who received DLI, 2 converted to FDC defined as >95% donor CD3 chimerism. All five patients with very mixed chimerism (>50% recipient), converted to predominant donor chimerism. Nine of 10 patients receiving DLI remain alive and in remission at the end of the study,

with only one patient developing cGVHD (skin and ophthalmic), and one dying of relapse.

Multivariate analysis

Age ≥60 years and HLA mismatch, retained significance on multivariate analysis. Extensive cGHVD (p=0.0004) and age ≥60 years (p=0.003) were being associated with higher NRM. Limited or no cGVHD was associated with increased CIR (p=0.048) (Table 3).

Outcome after relapse

At the completion of the study 33 patients had relapsed (FMC=17, FBC=16). The approach to relapse differed between the cohorts.

In the FMC cohort, one patient received further intensive chemotherapy, two received azacytidine and one received a *FLT3* inhibitor. All other patients received supportive/palliative care. There were no documented CRs and no patients received DLI.

In the FBC cohort (n=16), three patients received palliative therapy while all others were treated. Of the 8 patients who received DLI following chemotherapy (4 with fludarabine & high dose cytarabine +/- idarubicin (FLAG/FLAG-Ida), 2 with azacytidine, and 2 with low dose cyatarabine), five achieved CR, three had transient stable disease and 1 died from complications of DLI-related GVHD. Five others received chemotherapy alone (4 intensive protocols and 1 azacitidine) but

did not receive DLI due to inadequate response to chemotherapy. Of the 4 patients who were alive at the end of the study period following relapse, the median time to relapse was 470 days (range 350 – 887 days), the median follow-up from relapse was 276 days (range 155-967 days), and all had received chemotherapy (3 intensive, 1 azacytidine) followed by DLI (median 3 doses, range 1-3).

Median survival from relapse was longer in the FBC group than the FMC group 240 vs 52 days (p=0.0002) corresponding to a 2-year post relapse OS of 10 vs 0%, likely related to the intensity of treatment post-relapse rather than conditioning protocol.

Discussion

This retrospective study presents long-term outcome for UK patients treated with two different T-cell depleting allo-HSCT protocols for AML in CR. and demonstrated equivalent outcomes following treatment with FMC or FBC protocols. Of note, this patient population is different to the retrospective registry-based EBMT study comparing fludarabine-busulfan (FB) vs fludarabine-melphalan (FM), in which patients who had received T-cell depleting agents were *excluded* from that analysis(Baron *et al*, 2015). Interestingly, in the absence of TCD, other studies(Kawamura *et al*, 2017; Shimoni *et al*, 2007; Raida *et al*, 2014) also demonstrated a reduced incidence of relapse in the FM cohort, but similar overall survival with FB and FM.

The 2 and 5-year OS rates observed in our study compare favorably to other published RIC and myeloablative protocols(Luger *et al*, 2012; Shimoni *et al*, 2006).

Outcomes were particularly good for patients <60 years and those with 10/10 HLA matched donors due to a lower NRM, with no differences observed between the two regimens (p-0.14) despite relatively more younger patients and more proportion of MUDs in FBC cohort. These findings replicate that of other studies and are likely to be particularly important in the era of increasingly well-matched HLA donors(Rubio *et al*, 2017). Despite the worse outcomes observed in patients ≥60 years age, the overall survival at 5 years was 36% in this selected group of older patients (i.e. fit for transplant and with an appropriate donor) and a randomized controlled study comparing outcome for patients ≥60 years in CR1 or CR2 following chemotherapy alone or allo-HSCT using a TCD conditioning regimen(Pollyea *et al*, 2011; Sperr *et al*, 2016) will be beneficial.

As expected, rates of both acute and chronic GVHD were low. A lower dose (<100mg) of alemtuzumab was delivered in a proportion of patients (n=19) in the FBC group. This reduced dose did not appear to reveal any significant differences in OS, EFS or impact on NRM/CIR in this study, although numbers were too small to make any clear conclusions. A separate analysis is currently in progress to analyse the impact of alemtuzumab dose reduction on relapse in patients with AML and MDS at KCH. A previous study has demonstrated alemtuzumab can safely be reduced to 30mg in sibling donor setting in the context of FM conditioning without an adverse effect on clinical outcomes(Chakraverty *et al*, 2010). On multivariate analyses the presence of extensive cGVHD was associated with decreased relapse risk (CIR) suggestive of an achievable graft versus leukaemia effect even following *in vivo* TCD, however, as expected, the concomitant increase in NRM resulted in no improvement in OS.

The utility of DLI in patients with AML remains unclear with the current retrospective literature giving potentially biased results(Krishnamurthy *et al*, 2013; Yun & Waller, 2013; Liga *et al*, 2013; Jedlickova *et al*, 2016). Although some studies demonstrate increased relapse in the context of mixed chimerism no prospective randomized data exists to guide the appropriate indication, dosing or schedule in the setting of AML(Tsirigotis *et al*, 2016; Lee *et al*, 2015). Two prospective randomized trials in the UK designed to examine the effect of prophylactic DLI are currently recruiting (PRO-T4 and PRO-DLI); results are awaited (NCT01240525, NCT02856464). This current study had insufficient numbers of patients receiving DLI to draw any firm conclusions.

Other potential adverse factors were not shown to influence OS. While adverse karyotype has been shown to confer a worse prognosis in the post-transplant setting(Deeg *et al*, 2012), this was not demonstrated in our study, likely due to the small numbers in this group. Although increased CIR was evident in the *FLT3*^{ITD} group this did not translate into worse OS. The relapse potential of *FLT3*^{ITD} mutant status is influenced by other mutations(Ivey *et al*, 2016; Papaemmanuil *et al*, 2016), and we had sufficient additional information to assess this. Increasingly the importance of minimal residual disease(MRD) is also recognized as an important predictor of adverse outcome(Araki *et al*, 2016; Walter *et al*, 2011), a factor that was not available for this analysis.

Patients who relapsed post-transplant had poor outcomes. Salvage attempts with subsequent DLI in the FBC group did result in some patients achieving remission, which were durable with medium term follow up (median of 9 months). A previous published analysis of patients with MDS and AML who received a similar DLI strategy led to superior outcomes in those with minimal disease prior to DLI(Krishnamurthy *et al*, 2013). Diligent MRD monitoring may thus optimize the chances for an immunologically mediated intervention in reducing frank relapse risk. This is further supported by our findings of significantly lower CIR in patients who achieved strict FDC (>97%). This raises the possibility of adopting a lower threshold for maintaining strict FDC with early weaning of immunosuppression +/- DLI , which may decrease rates of relapse in high-risk patients.

In conclusion, this study represents first direct comparison of two different TCD regimens in unrelated donor allo-HSCT for AML in CR. While limitations of this analysis include the retrospective nature of the study, the differing approaches to chimerism monitoring and DLI and statistical limitations without time-dependent variable input may impact on the study findings; we believe that the observation of equivalent outcomes for both regimens is valid. Most relevant prognostic factors appear to be advanced patient age and degree of donor mismatch. Strategies to improve outcomes in these cohorts require a focus on decreasing NRM in the older age-group, donor selection and relapse prevention.

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1. **Dr Rob S. Sellar**[1,2§] conceived and designed the study, performed the research, analysed the data, wrote the manuscript, approved the final version and agreed to be accountable for all aspects of the work. This author can confirm that he has had full access to the data in the study and final responsibility for decision to submit for publication.
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17. **Prof Asim Khwaja**[1,2] contributed to interpreting results, revising the manuscript, approved the final version and agreed to be accountable for all aspects of the work.
18. **Dr Karl S. Peggs**[1,2] contributed to interpreting results, revising the manuscript, approved the final version and agreed to be accountable for all aspects of the work.
19. **Dr Kirsty J. Thomson**[2] reviewed clinical data, contributed to revising the manuscript, approved the final version and agreed to be accountable for all aspects of the work
20. **Prof Emma C. Morris**[2,4,6*] conceived and designed the study, reviewed clinical data, contributed to interpreting results, revising the manuscript, approved the final version, agreed to be accountable for all aspects of the work and has final responsibility for decision to submit for publication.

21. **Dr Victoria T. Potter[3]*** conceived and designed the project, contributed to writing and revising the manuscript, approved the final version, agreed to be accountable for all aspects of the work and has final responsibility for decision to submit for publication.

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TABLES

Table 1: Patient and transplant characteristics: All patients, FMC cohort, and FBC cohort.

	Total (n=117)	**FMC (n=70)**	**FBC (n=47)**	**p-value**
Median Age, years (range)	55 (19-68)	57 (27-68)	51 (19-67)	0.04*
Age (n)				
>60 years	41	25	16	0.85^
<60years	76	45	31	
Sex (n)				
-Male	43	28	15	NS^
-Female	74	42	32	
HLA match				
-10/10 HLA	71 (61%)	37 (53%)	34 (72%)	0.03^
-1 or 2 antigen mismatch	56 (38%)	33 (47%)	13 (18%)	
Remission status				
-CR1	76 (65%)	43 (61%)	33 (70%)	NS^
-CR2	41 (35%)	27 (39%)	14 (30%)	
AML type				
-De-novo	90 (77%)	54 (77%)	36 (77%)	NS^
-Secondary	37 (23%)	16 (23%)	11 (23%)	
Cytogenetics				
-Favourable	9 (8%)	5 (8%)	4 (9%)	NS^
-Intermediate	82 (70%)	47 (72%)	35 (76%)	
-Adverse	21 (18%)	14 (22%)	7 (15%)	
-Unknown	6 (5%)	5	1	
***FLT3* status**				
-ITD	20 (30%)	11 (27%)	9 (34%)	NS^
-No ITD (wild type)	25 (37%)	15 (37%)	10 (38%)	
-Unknown	22 (19%)	15 (37%)	7 (27%)	
Alemtuzumab dose				
<100mg	22 (19%)	3 (4%)	19 (40%)	<0.0001^
100mg	95 (81%)	67 (96%)	28 (60%)	
CMV IgG serostatus (recipient/donor)				
- +/+	53 (45%)	37 (53%)	16 (34%)	NS^
- +/-	14 (12%)	9 (13%)	5 (11%)	
- -/+	6 (5%)	1 (1%)	5 (11%)	
- -/-	44 (8%)	23 (33%)	21 (44%)	

*One way Anova; ^ chi square

AML- Acute Myeloid Leukaemia; CR- Complete remission; CMV- cytomegalovirus infection; FLT3- fms-like tyrosine kinase 3; ITD- internal tandem duplication; HLA- Human leukocyte antigen; NS- not significant

Table 2: GVHD, Graft failure and Peripheral blood chimerism

	FMC (n=70)	**FBC (n=47)**	p-value*
Acute GVHD			
All Grades	60%	25.5%	0.002
1	30%	6%	0.0001
2-4	30%	19%	NS
3-4	7.1%	8.5%	NS
Chronic GVHD grade	**FMC (n=57)**	**FBC (n=41)**	**p- value***
(alive>100d)	36.8%	78%	<0.0001
None	45.1%	2.4%	<0.0001
Limited	17.5%	19.5%	0.80
Extensive			
Chimerism (n) at 3 months:			
-FDC (>95)	24	18	
-Mixed	9	24	0.01
-Unknown or died prior	37	5	
Chimerism (n) at 6 months:			
-FDC (>95%)	23	16	NS
-Mixed	5	23	
-Unknown or died prior	41	8	
Graft failure (n)			
-Primary	1	1	NS
-Secondary	3	0	

* using chi-square pairwise comparison

NS=not significant; FDC=full donor chimerism; GVHD= graft-versus-host disease

Table 3: Multivariate analysis of Overall Survival (OS), relapse frees survival (RFS), cumulative incidence of relapse (CIR) and non-relapse mortality (NRM).

Overall Survival		HR	Lower CI	Upper CI	P value^
Patient Age (years)	**≥60 <60**	2.02	1.170	3.512	**0.012**
Degree of HLA match	**MUD**	1.0			**0.009**
	1Ag Mismatch	1.867	1.081	3.234	**0.025**
	2 Ag Mismatch	3.773	1.400	10.170	**0.009**

Relapse Free Survival (RFS)		HR	Lower CI	Upper CI	P value^
Degree of HLA match	**MUD**	1.0			**0.022**
	1Ag Mismatch	1.694	1.003	2.861	**0.049**
	2 Ag Mismatch	3.252	1.221	8.663	**0.018**
Patient Age (years)	**≥60 < 60**	1.891	1.119	3.196	**0.017**

Cumulative Incidence of Relapse (CIR)		HR	Lower CI	Upper CI	P value*
cGVHD	**Limited**	4.574	1.481	20.65	**0.048**
	Extensive				
Non-Relapse Mortality (NRM)		HR	Lower CI	Upper CI	P value*
Patient Age (years)	**≥ 60**	5.10	1.723	15.10	**0.003**
	<60				
cGVHD	**Limited**	0.11	0.03	0.39	**0.0004**
	Extensive				

^Cox regression (Gray method); *Fine Gray method

HR- Hazard ratio; CI- confidence interval; MUD- Matched unrelated donor; HLA- Human Leukocyte antigen; cGVHD- chronic Graft versus Host Disease.

Figure Legends

Figure 1: Schematic representation of FB and FM conditioning regimen with alemtuzumab (Campath®) based T cell depletion. In the FBC protocol, busalphan was delivered as a split dose (1.6mg IV twice daily) and from 2013, a total of 60mg alemtuzumab was given (20mg daily on days -4 to -2). CsA = cyclosporin A.

Figure 2: Overall survival (OS) of **a)** entire cohort of patients, **b**) between FBC and FMC regimens, **c)** according to patient age, **d)** between HLA matched unrelated (MUD) and HLA mismatched unrelated donors (MMUD), **e)** according to cytogenetic risk group, **f)** according to FLT3 mutation status (with normal karyotype).

Figure 3: Non-relapse mortality (NRM) according to **a)** conditioning regimen, **b)** patient age, **c)** type of chronic graft-versus-host disease (GVHD) grade; AND **d)** Cumulative incidence (CIR) & **e)** Relapse free survival (RFS) according to conditioning regimen (FMC vs FBC); **f)** CIR according to chronic GVHD grade.