Durable graft-versus-leukaemia effects without donor lymphocyte infusions- results of a Phase II study of sequential T-replete allogeneic transplantation for high-risk acute myeloid leukaemia and myelodysplasia

Jeff K. Davies,[1,3*] Sandra Hassan,[1*] Shah-Jalal Sarker,[2] Caroline Besley[1], Heather Oakervee,[3] Matthew Smith,[3] David Taussig,[1,3**] John G. Gribben[1,3] and Jamie D. Cavenagh.[1,3]

[1] Centre for Haemato-oncology, Barts Cancer Institute, Queen Mary University of London, UK

[2] Centre for Experimental Cancer Medicine, Barts Cancer Institute, Queen Mary University of London, UK, and [3]Department of Haemato-Oncology, Barts Cancer Centre, St Bartholomew's Hospital, Barts Health NHS Trust, London, UK

*JKD and SH contributed equally to this study

Running Title: T-replete sequential allotransplantation for AML/MDS

Corresponding author: Dr Jeff Davies, Centre for Haemato-Oncology, Barts Cancer Institute, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ UK.
Tel: 020 7882 3815, Fax: 020 7882 3891 E-mail: j.k.davies@qmul.ac.uk

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Summary

Allogeneic haematopoietic stem-cell transplantation remains the only curative treatment for relapsed/refractory acute myeloid leukaemia (AML) and high-risk myelodysplasia but has previously been limited to patients who achieve remission before transplant. New sequential approaches employing T-cell-depleted transplantation directly after chemotherapy show promise but are burdened by viral infection and require donor lymphocyte infusions (DLI) to augment donor chimerism and graft-versus-leukaemia effects. T-replete transplantation in sequential approaches could reduce both viral infection and DLI usage. We therefore performed a single-arm prospective Phase II clinical trial of sequential chemotherapy and T-replete transplantation using reduced-intensity conditioning without planned DLI. The primary endpoint was overall survival. Forty-seven patients with relapsed/refractory AML or high-risk myelodysplasia were enrolled with 43 proceeding to transplantation. High levels of donor chimerism were achieved spontaneously with no DLI. Overall survival of transplanted patients was 45% and 33% at 1 and 3 years. Only one patient developed CMV disease. Cumulative incidences of treatment-related mortality and relapse were 35% and 20% at 1 year. Patients with relapsed AML and myelodysplasia had the most favourable outcomes. Late-onset graft-versus-host disease protected against relapse. In conclusion, a T-replete sequential transplantation using reduced-intensity conditioning is feasible for relapsed/refractory AML and myelodysplasia and can deliver graft-versus-leukaemia effects without DLI.

(Trial registered at ISRCTN 32336114/Eudract 2007-000806-64)

Introduction

The outlook for patients with relapsed or refractory acute myeloid leukaemia (AML) and high-risk myelodysplasia (MDS) is very poor. As targeted cellular immunotherapies such as chimeric antigen receptor T-cells have not yet been developed for these diseases, allogeneic haematopoietic stem-cell transplantation (AHST) remains the only potentially curative therapy, harnessing donor immune-cell mediated graft-versus-leukaemia (GvL) effects to prevent relapse. However, the established strategy of salvage chemotherapy to achieve remission followed by AHST results in 3-year survival rates of only 8-30% for relapsed/refractory AML (Tavernier et al. 2003; Mato, Morgans, and Luger 2008; Thomas et al. 2003; Breems et al. 2005; Fung et al. 2003; Cook et al. 2006). Importantly, these studies report outcomes only for patients who achieved remission prior to AHST, but many patients with relapsed/refractory AML cannot achieve remission and few studies are reported on full intent-to-treat basis enrolling patients at onset of salvage therapy rather than at AHST.

Similarly, AHST remains the only curative therapy for high-risk myelodysplasia (MDS) (Gangat, Patnaik, and Tefferi 2016). Achieving remission of MDS prior to AHST results in better outcomes (Castro-Malaspina et al. 2008) but this approach is associated with delayed haematological recovery and toxicity that may make successful AHST difficult to deliver.

To address these significant challenges, delivering AHST earlier after induction chemotherapy in AML patients with high genetic risk factors or induction failure have been explored The German Study Alliance Leukemia (SAL) group reported the feasibility of this approach in such patients in an initial pilot study (Platzbecker et al. 2006), followed by a larger retrospective analysis of AHSCT in patients in aplasia after first induction chemotherapy (Stolzel et al. 2013), and this strategy was incorporated in the larger prospective SAL AML 2003 study (Schetelig et al. 2015). In parallel, an approach of delivering chemotherapy to induce remission followed directly by reduced intensity-conditioned (RIC) AHST was first reported in 2005 (Schmid et al. 2005). This *sequential* strategy, employing a T-cell depleted transplant platform to reduce

toxicity and planned donor lymphocyte infusion (DLI) to strengthen donor immune graft-versus-leukaemia (GvL) effects, resulted in improved outcomes for patients with relapsed/refractory AML and high-risk MDS. Subsequently, several other groups have reported successful application of sequential chemotherapy and AHST using a variety of RIC regimens, confirming the utility of this approach (Liu et al. 2009; Cluzeau et al. 2011; Chemnitz et al. 2012; Saure et al. 2012; Buchholz et al. 2012; Krejci et al. 2013; Schneidawind et al. 2013; Pfrepper et al. 2016). However, all used T-cell depletion with anti-thymocyte globulin which increases viral infection after AHST (Hamadani et al. 2009) and may increase relapse after RIC regimens (Soiffer et al. 2011). Most also employed planned DLI, which can be difficult to obtain especially from unrelated donors.

We have previously reported use of a non-T-depleted ('T-replete') RIC-AHST platform in patients with AML in first remission which results in low toxicity, very infrequent viral infection and a low requirement for DLI (Taussig et al. 2003; Davies et al. 2006; Davies et al. 2013). We therefore reasoned that incorporation of this transplant approach in a sequential strategy might be feasible and could potentially reduce infectious problems and the need for DLI. We now report the results of a prospective Phase II clinical trial of sequential chemotherapy and T-replete AHST using our established RIC platform for patients with relapsed/refractory AML and high-risk MDS.

Methods

Study Design and Eligibility

We performed a prospective single-centre Phase II clinical trial of sequential chemotherapy and T-replete RIC-AHST. Patients with relapsed or refractory AML, high-risk MDS (IPSS Int. 2/high (Greenberg et al. 1997)) or other high-risk myeloid malignancy were eligible for the study. The study enrolled 53 patients, 47 of whom had AML/MDS. To facilitate comparison with published sequential studies, only AML/MDS patients are included in this report. Patients with functionally refractory AML (those not entering complete remission (CR, <5% bone marrow blasts) after one cycle of induction therapy or remitting but relapsing with a CR1 shorter than 6 months) were grouped together as outcomes for these two groups are very similar (Breems et al. 2005). The protocol was approved by the local ethics committee with written informed consent obtained in accordance with the Declaration of Helsinki. The primary study endpoint was overall survival (OS). Secondary endpoints included engraftment, chimerism, treatment-related mortality (TRM), acute and chronic GvHD, disease response/relapse and use of DLI.

Sequential chemotherapy and reduced-intensity T-replete conditioning protocol

Patients received daunorubicin 45mg/m^2 (D-15 to D-13) and cytarabine 1500mg/m^2 twice daily (D-15-D-9) followed by fludarabine 25mg/m^2 (D-6-D-2) and cyclophosphamide 1g/m^2 (D-3-D-2, as previously reported (Davies et al. 2013). Donors were fully matched by high resolution typing at HLA-A, -B, -C, -DRB1, and DQB1 loci. All patients received granulocyte colony-stimulating factor-mobilized peripheral blood stem-cells except one who received unmanipulated bone marrow. GvHD prophylaxis was methotrexate (5 mg/m^2 on D+1,+3 and +6), and the calcineurin inhibitor (CNI) ciclosporin (CSA) adjusted to maintain therapeutic trough blood levels between 150–300µg/L. Tapering of CSA commenced at D+90 in the absence of GvHD and earlier in patients with <80% donor chimerism at D+60. Supportive care is detailed in **Supplemental Methods.**

Engraftment and Chimerism and DLI

Neutrophil recovery was defined as time to a count of 0.5×10^9/L for 2 consecutive days. Chimerism was performed on whole peripheral blood and purified $CD3^+$ T-cells by PCR analysis of variable number tandem repeat polymorphisms monthly to D+90 and thereafter as clinically appropriate. Full donor chimerism was defined as greater than 95% donor. DLI was not planned but could be used at investigators discretion for mixed chimerism or disease relapse.

Definitions, outcomes and statistical analyses

TRM was defined as death from any cause in the absence of disease persistence/relapse. Acute and chronic GvHD were diagnosed/graded using consensus/NIH criteria and managed by institutional protocol (Przepiorka et al. 1995; Filipovich et al. 2005). Differences in pre-transplant variables were assessed using the two-sided Fisher's exact test (FET). Initial disease response was assessed by bone marrow biopsy at D+30. Patients with persistent disease were censored at this day for relapse. OS curves were constructed using the method of Kaplan and Meier (Kaplan 1958) and differences between groups were assessed using the log-rank statistic (Peto et al. 1977). Patients were censored at last follow-up by January 2015. For donor chimerism, TRM, GvHD and disease persistence/relapse, cumulative incidence with competing risks was calculated using the method of Fine and Gray (Fine 1999). Data were analyzed using SPSS v22.0 (IBM, New York) and Stata 12 (StataCorp, College Station, Texas). Additional statistical considerations are found in the **Supplemental Methods.**

Results

Patient, donor and graft characteristics

Forty-seven patients with AML/MDS were enrolled between 2007 and 2014. Four patients did not undergo transplantation; 2 had donors deemed unfit to donate by donor registries and no alternate donors could be identified, and 2 died prior to AHST, one from infection and one from AML). Consequently, 43/47 (92%) AML/MDS patients proceeded to AHST as per protocol. Details of transplanted patients, donor and graft characteristics are summarized in **Table 1**. AML patients received primary induction therapy mostly as directed by the UK MRC AML study current at the time of their induction (standard ADE/DA or FLAG-Ida on AML15 or DA with daunorubicin 60mg/m^2 or 90mg/m^2 on AML17). All patients had bone marrow blasts >5% at the time of study entry. All patients with relapsed AML had a Breems' score of 7 of higher. Patients were transplanted using HLA-matched related (n=21) or unrelated donors (n=22).

Engraftment and Chimerism

Five patients died of infection or multi-organ failure by D+15, prior to engraftment. Of the remaining 38 patients, all engrafted. The median time of neutrophil engraftment was D+21 (range D+11-D+29). At the earliest time point where donor chimerism was evaluated (D+30), the median unsorted donor chimerism level was 96%, with 71% of patients achieving 90% or higher and less than 25% of patients achieving less than 80% donor chimerism. After tapering CSA, most patients who had mixed chimerism at earlier time points converted to full donor chimerism without need for DLI, with a median unsorted donor chimerism level of 98% and only 12% of patients with less than 80% donor chimerism in unsorted peripheral blood at D+180 (**Figure 1A**). Similarly, T-cell lineage-specific donor chimerism levels were high even at early time points (median 94% with only 12% of patients achieving less than 80% donor T-cell chimerism at D+30) with most patients with lower levels converting to full donor T-cell chimerism (FTTC) upon tapering CSA, with a median level of 96% and only 10% of patients

with less than 80% donor T-cell chimerism in peripheral blood at D+180 (**Figure 1B**). Graft failure (in the absence of disease persistence or relapse) occurred in only two patients; one patient had autologous reconstitution and successfully underwent a second RIC AHST, and one patient died with aplasia induced after tapering CSA.

Overall Survival

At the time of data analysis, 15 of 43 (35%) transplanted patients were alive with a median follow-up of surviving patients of 4 years**.** The actuarial overall survival (OS) and 95% CIs for transplanted patients was 45% (29-61%), 39% (24-54%) and 33% (18-48%) at 1, 2 and 3 years respectively (**Figure 2A**). One surviving patient relapsed and underwent a second RIC AHST and is currently in remission. No surviving patients had relapsed disease at the point of data analysis so current disease-free survival was equal to OS. OS was most favourable in patients with MDS or with relapsed AML with 2-year OS of 71% and 52% respectively, (**Figure 2B-C**). For AML patients, there was a trend toward better OS in patients with intermediate-risk cytogenetics at diagnosis compared to those with high-risk cytogenetics (2-year OS of 44% vs 20% respectively, $p = 0.09$). No other pre-transplant patient or donor factors listed in **Table 1** significantly impacted on OS. To capture the true outcome of the whole patient group, we performed survival analysis on an intention to treat basis on all AML/MDS patients enrolled in the study not just those who proceeded to RIC AHST. The OS of all patients (from the date of study entry) was 43%, 36% and 30% at 1, 2 and 3 years respectively (**Figure 1D)**.

Toxicity and GvHD

Of transplanted patients, 17 died from treatment-related mortality (TRM) and the cumulative incidence (CI) of TRM with relapse as a competing risk was 35% and 40% at 1 and 3 years respectively (**Figure 3A**). CI of TRM was significantly higher in older patients (those above the median age of 53 years, **Figure 3B**) but was not significantly impacted upon by HCT-CI or other pre-transplant patient or donor factors. The CI of classical acute GVHD was 24% (grade 2-4) and 7% (grade 3-4) occurring at a median D+47. CI of late onset acute/chronic GvHD

was 52% of assessable patients, occurring at a median D+159 (**Figures 3C-D**). The majority of these patients had extensive chronic GvHD requiring systemic immunosuppression. A total of 7 deaths were attributable to either acute or chronic GvHD. Ten patients died from regimen-related toxicity without GvHD (8 of bacterial infection and 2 of multi-organ failure).

Viral reactivation, viral disease

Only 10 of 43 transplanted patients (23%) had one or more episodes of CMV reactivation representing 33% of patients at higher risk of CMV reactivation. Only one patient developed CMV disease (colitis), in the setting of systemic immunosuppression for GvHD but responded to antiviral treatment and is a long term survivor. No other clinically significant viral infections occurred.

DLI

No patients received DLI.

Disease Response and relapse

Of transplanted patients, 38/43 were alive at D+30 and evaluable for disease response, of which 32 had a bone marrow evaluation at this time point. Three of 32 had persistent disease (and were censored as disease relapse at this time point) and 29/32 (91%) were in complete remission (CR). The 6 patients not assessed at D+30 had normal count recovery with predominantly donor cell chimerism and were therefore presumed to be in CR. A further 9 patients relapsed and the CI of relapse was 30% at 3 years. The median time to relapse was 95 days. At the time of analysis no patient has relapsed after 2 years (**Figure 4A**). The CI of relapse was significantly higher in patients with functionally refractory AML than in relapsed AML or MDS, and in patients receiving below the median dose of $CD34^+$ cells compared to those receiving above the median dose (**Figure 4B-C**). In contrast the CI of relapse was not significantly different in AML patients grouped by cytogenetic risk-group (at diagnosis) or for all patients grouped by infused $CD3^+$ T-cell dose (**Supplementary Figure S1A-B**). Only one

of 13 patients transplanted with relapsed AML went on to relapse post-sequential transplant and 6 of these patients achieved an inversion of remission length, with a longer CR2 than CR1 (**Figure 4D**).

Impact of post-transplant factors influencing GvL effects on outcome

Finally we assessed the impact on disease relapse of three post-transplant factors that might influence the development of donor immune cell mediated GvL effects; post-transplant T-cell chimerism levels, CNI exposure and clinical alloreactivity in the form of *de novo* chronic GvHD. Individual T-cell chimerism patterns are shown in **Figure 5A** grouped according to outcome (ongoing CR, relapse or TRM). There was no significant difference in the level of TCC (one way ANOVA) or in the proportion of patients achieving FTCC (FET) at any single individual time point between these groups However, all patients in ongoing CR (14/14) achieved FTCC vs only 6/9 patients who achieved CR post-transplant but subsequently relapsed (p=0.047 FET). These data are consistent with the attainment of FTCC post-transplant being necessary but not sufficient to provide a sustained GvL effect capable of protecting against relapse.

In view of this we examined the impact of immunosuppression early post-transplant which could modulate development of T-cell mediated GvL effects. We used an area under the curve analysis to quantify CNI exposure over the first 21 days post-transplant in patients who survived to this time point. The degree of CNI exposure (AUC_{21}) over this period did not impact the achievement of FTCC or the development of early acute GvHD whilst on CNI, (**Supplementary Figure S2A-B**). However, early CNI exposure was significantly lower in patients who went on to develop clinical alloreactivity after tapering CNI compared to those who did not, demonstrating that T-cell alloresponses mediating delayed GvHD/GvL effects are impacted by immune modulation early post-transplant in this setting (**Figure 5B**). We finally assessed the impact of the development of clinically apparent alloreactivity after CNI withdrawal on subsequent relapse. A landmark analysis of cumulative incidence of relapse (with TRM as a competing risk) of patients alive and in remission at D+100 is shown in **Figure**

5C. In such patients, late onset acute/de novo chronic GvHD after the attainment of FTCC was associated with a significant reduction in subsequent relapse (**Figure 5B**) demonstrating that late onset clinical alloreactivity was associated with provision of immune GvL effects. This effect was most prominent in patients with AML, resulting in significantly better OS (**Figure 5D**).

Discussion

In the last decade the application of sequential chemotherapy and AHST strategies for AML/MDS have led to better outcomes than historical approaches, but all have all used TCD mostly followed by planned DLI. The ability to deliver a sequential approach using a T-replete transplant platform without planned DLI has not previously been reported, and is of considerable interest as TCD is associated with more viral infections, and DLI can be difficult to obtain from unrelated donors and exposes donors and patients to additional medical procedure. In this study we demonstrate that sequential chemotherapy and T-replete RIC AHST results in high levels of donor chimerism without any use of DLI, very little morbidity from viral infection, and can result in durable remissions, particularly in patients with relapsed AML or MDS.

A major potential advantage for using a T-replete transplant platform is that high levels of donor chimerism, particularly T-cell chimerism, can be achieved spontaneously without DLI, whereas persistent split chimerism particularly in the T-cell lineage is common after T-cell depleted approaches and DLI is more frequently required to augment chimerism levels (Dey et al. 2003; Shaw et al. 2007). Previous early AHST or sequential AHST strategies for relapsed/refractory AML have used T-cell depletion either for all patients (Schmid et al. 2005; Cluzeau et al. 2011; Chemnitz et al. 2012; Buchholz et al. 2012; Krejci et al. 2013), (Pfrepper et al. 2016) or for patients receiving transplants from unrelated donors (Liu et al. 2009; Saure et al. 2012; Platzbecker et al. 2006; Stolzel et al. 2013; Schetelig et al. 2015) with the majority administering planned DLI. In contrast, almost all of our assessable patients achieved full donor chimerism spontaneously despite the non-myeloablative conditioning we employed. Furthermore we also observed the achievement of high levels of donor T-cell chimerism which has been reported to be key in prevention of relapse after RIC-AHST (Saito et al. 2008; van Besien et al. 2009) suggesting that a GvL effect may be established spontaneously without DLI after our approach.

Although direct comparison of outcomes from our study with those of other early AHST or sequential transplant approaches is not possible, the 2-year OS of transplanted patients (39% ± 15%) we report is comparable to outcomes reported by the SAL study group of early AHST (Stolzel et al. 2013; Schetelig et al. 2015) and to both individual reports of different individual sequential strategies using either FLAMSA or clofarabine-based chemotherapy followed by cyclophosphamide and low dose TBI (Schmid et al. 2005) or busulphan-based RIC platforms (Schneidawind et al. 2013; Mohty et al. 2017), and to the pooled outcome data from a recently published registry-based survey by the EBMT of patients with AML treated with sequential chemotherapy and AHST (Ringden et al. 2017). In the latter series, the authors report that the 3-year OS of patients treated with T-cell depleted approaches was superior to that of those who received T-cell replete transplants (31% versus 20%). However it should be noted that only a small minority of patients in this series received T-replete transplants reflecting current sequential practice, and our current study provides a much larger patient group to assess the impact of T-replete transplantation on sequential strategies for AML/MDS.

Importantly, published data for sequential AHST for AML/MDS is restricted to outcome of patients who proceed to transplant rather than the outcome of the patient group for whom the treatment is intended. Furthermore, the SAL AML 2003 study utilizing early AHST in patients with refractory AML demonstrated a survival benefit for early AHST, but low proportions of eligible patients with available donors proceeded to early AHST (Schetelig et al. 2015). In contrast, over 90% of AML patients in our study proceeded to AHST as per protocol. Additionally, we provide important new data demonstrating that the 3-year OS for all AML/MDS patients enrolled on our study on an intention to transplant basis is 30%.

Although we had a strikingly low frequency of CMV disease, our approach was however associated with somewhat higher overall TRM (40%) than that reported in sequential strategies utilizing T-cell depletion (20-33%) and in the recent EBMT series of pooled registry data (26%). In order to develop strategies to address this we performed an analysis of risk

factors for TRM. Although we did not see a clear association of TRM with HCT-CI (which is validated across most AHST platforms, but not yet in the context of sequential approaches) we did see significantly higher TRM in older patients who tended to have higher HCT-CI scores. This suggests that a stratified approach to patient selection may help reduce TRM to better apply our T-replete sequential approach in the future.

Interestingly, we observed the most favourable outcomes for AML patients in those with relapsed disease, and our approach was less effective in patients with functionally refractory AML, which contrasts with reports of FLAMSA-based strategies which can be particularly effective in the latter patient group (Schmid et al. 2006). This difference is likely to reflect the lower level of direct anti-leukemic activity in our induction chemotherapy and RIC-platform (which is truly non-myeloablative) and thus our approach may offer less effective protection from disease persistence/relapse in the early post-transplant period. However, our strategy was more effective in patients with relapsed AML and MDS, with very low incidences of relapse and favourable overall survival. Notably patients with relapsed AML included in our report all had high Breems' scores (Breems et al. 2005) predicting a 5-year OS of 4-18%. Although the number of patients with relapsed AML in our study is small, the 5-year OS observed in these patients (43%) suggests our approach is superior to historical approaches to treatment for this patient group.

The demonstration of a clear association with spontaneous GvHD after tapering immunosuppression and protection from relapse demonstrates the potential for our strategy to deliver a potent GvL effect and is further evidence that the strategy might be more effective in relapsed rather than primary refractory AML. Our analysis of chimerism patterns and outcome demonstrate that a high level of T-lineage-specific chimerism is necessary but not sufficient for a clinically significant GvL effect. Importantly, although early exposure to CNI in the first 3 weeks post-transplant did not impact on T-lineage chimerism, increased early CNI was associated with a reduced frequency of subsequent GvHD suggesting that the GvL effect

may be tractable in this context by modulation of immunosuppression. Although there is undoubtedly a burden of morbidity that comes with chronic GvHD, in our previous experience using a T-replete RIC approach for AML most patients with chronic GvHD required systemic immunosuppression for less than a year (Davies et al. 2013), and studies have shown quality of life is comparable after either T-cell depleted or T-replete AHST (Lee et al. 2002).

In summary we report a strategy of sequential chemotherapy and T-replete RIC-AHST for relapsed/refractory AML/MDS that demonstrates comparable outcomes to published series of T-cell depleted approaches without requirement for DLI which is a major practical advantage particularly in the unrelated donor setting. This approach should be considered for incorporation into new Phase III comparative studies to define the best strategy of sequential chemotherapy and AHST for management of relapsed/refractory AML.

Authors' Contributions: The study was conceived and overseen by JDC. SJS provided statistical support in study design and analysis. HO, MS, DT, JGG and JDC provided patients for the study. Data collection and analysis was performed by JKD and SH with assistance from CB and SJS. JKD, SH, JGG and JDC wrote the manuscript. All authors finally approved the manuscript

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Table 1. Patient, donor and graft characteristics

Characteristic	Median (range)	Subgroup	Number of patients (%)	Cytogenetic risk group[1]
Transplanted patients			43 (100%)	
Patient age (years)	53 (23-68)	50 years or older	24 (56%)	
		60 years or older	8 (19%)	
Gender		Male	28 (65%)	
		Female	15 (35%)	
HCT-CI	2(0-6)	Low (0)	17 (40%)	
		Intermediate (1-2)	12 (28%)	
		High (3 or more)	14 (33%)	
Diagnosis		AML (Refractory)	21 (49%)	14 IR, 7 HR
		AML (Relapsed)	13 (30%)	11 IR, 2 HR
		AML (Therapy-related)[2]	2 (5%)	1 IR, 1 HR
		MDS (Untreated)	7 (16%)	
Previous AHST		No	36 (84%)	
		Yes	7[3] (16%)	
Disease status at study entry		Not in CR	43 (100%)	
Stem cell donor		Matched related	21 (49%)	
		Matched unrelated	22 (51%)	
Stem cell source		PBSC	42 (98%)	
		BM	1 (2%)	
ABO mismatch		Present	12 (28%)	
		Not present	31 (72%)	
Sex mismatch[4]		Present	9 (21%)	
		Not present	34 (79%)	
CMV risk[5]		Higher	30 (70%)	
		Lower	13 (30%)	
$CD34^+$ cell dose[6]	5.6 (1.8-26)			
$CD3^+$ cell dose[6]	321 (36-1337)[7]			

IR, intermediate risk; HR, high-risk; HCT-CI, Haematopoietic stem cell transplantation Co-morbidity Index; CR, complete remission; IPSS, international prognostic scoring system; PBSC, G-CSF-mobilised peripheral blood stem cells; BM, Bone Marrow.

[1] Cytogenetic risk for AML patients at initial diagnosis by standard UK MRC AML17 study criteria

[2] Untreated AML with prior history of cytotoxic chemotherapy exposure; [3] 6 patients with AML had undergone prior RIC-AHST and 1 patient as Bu-Cy autograft [4] sex mismatch defined as female donor, male recipient; [5]Higher risk = donor and/or recipient CMV seropositive pre-transplant; [6] x 10^6 cells/kg; [7] includes the single patient who received HLA-matched bone marrow from a related donor containing 10 x 10^6 $CD3^+$ T-cells/kg

Figure 1

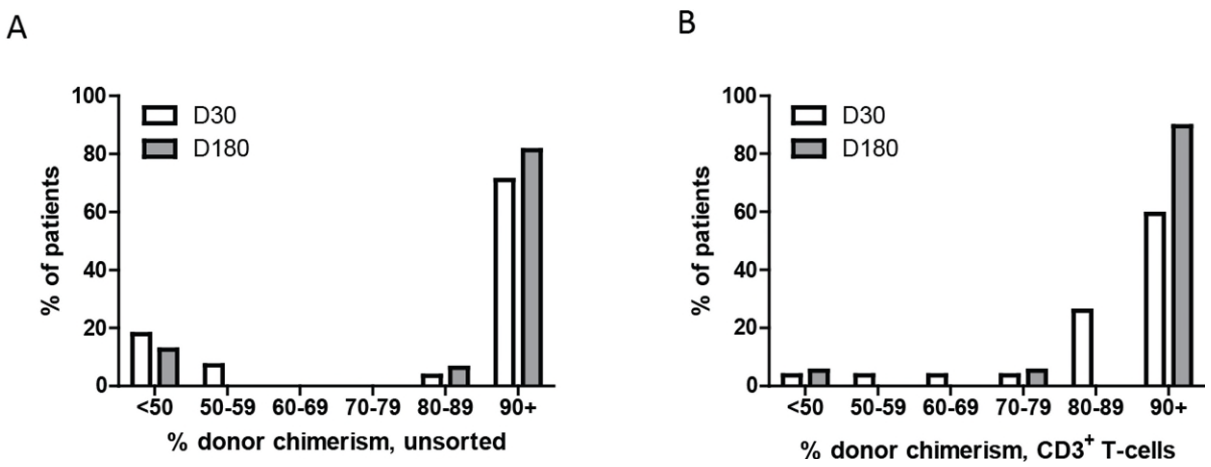

Figure 1 Donor Chimerism

(A) Proportion of patients with different levels of donor chimerism in unsorted peripheral blood at D+30 and D+180 after transplant

(B) Proportion of patients with different levels of donor chimerism in purified $CD3^+$ T-cells from peripheral blood at D+30 and D+180 after transplant.

Figure 2

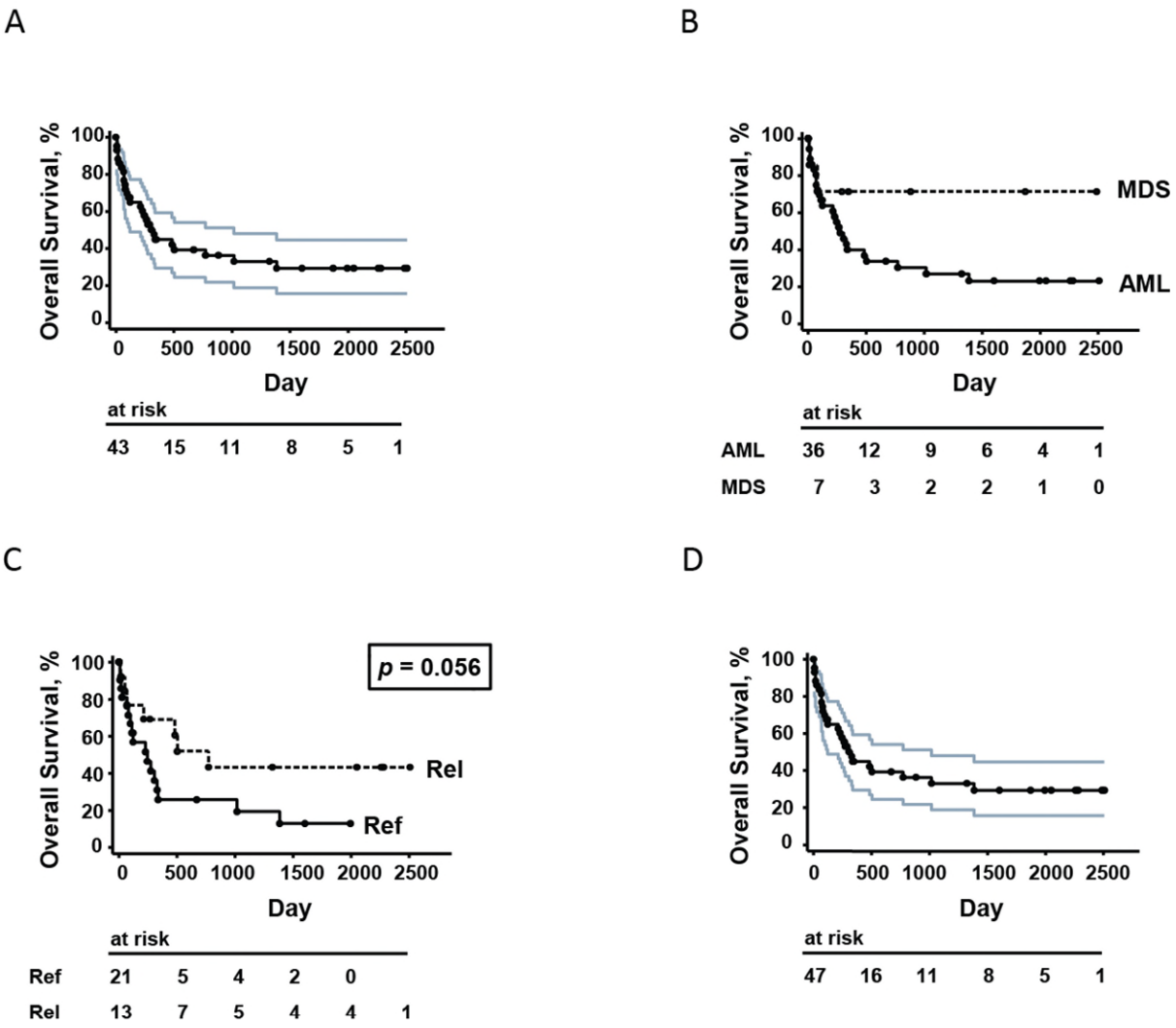

Figure 2 Overall Survival

(A) Actuarial overall survival (OS) of transplanted patients. Grey lines show 95% confidence intervals.

(B) Comparison of OS of transplanted patients with AML and MDS

(C) Comparison of OS of transplanted patients with primary refractory AML (REF) and relapsed (REL) AML.

(D) OS of all AML/MDS patients enrolled on study. Grey lines show 95% confidence intervals.

Figure 3

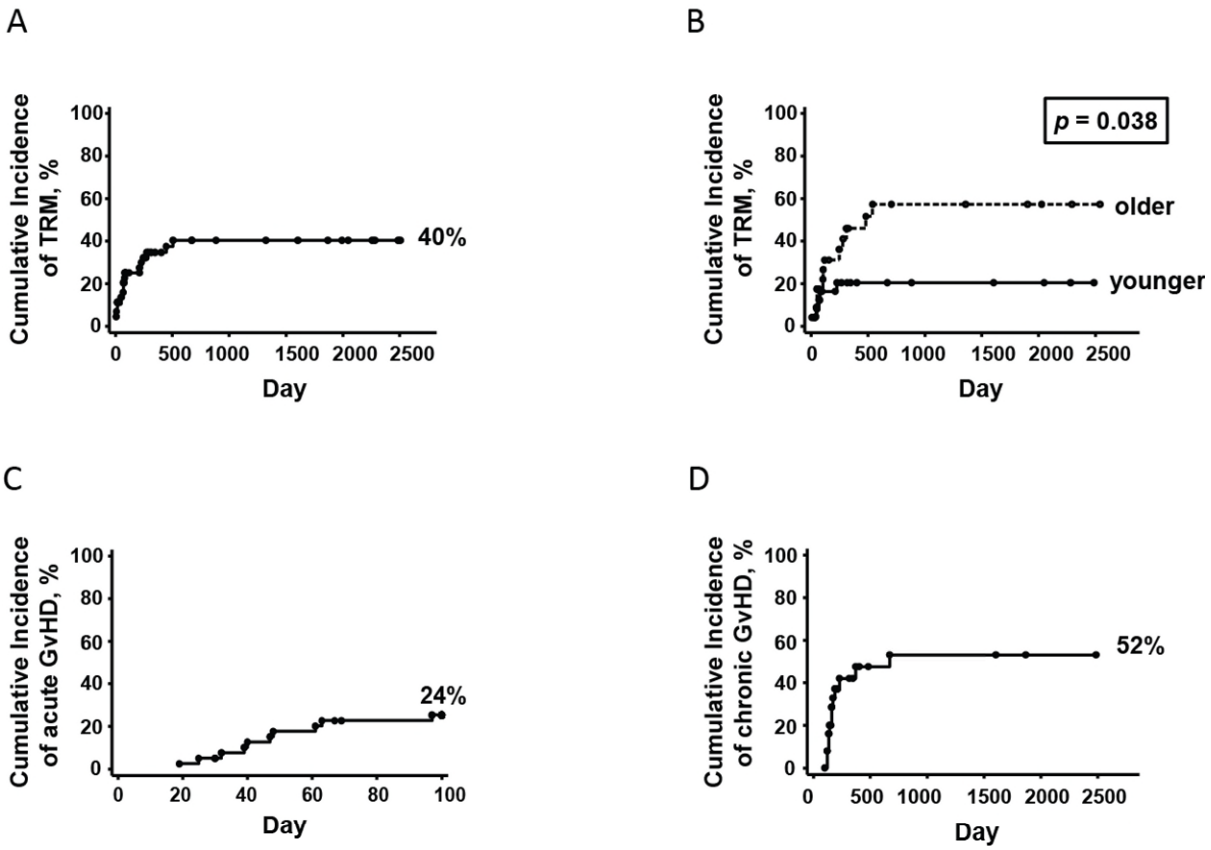

Figure 3 Treatment-related mortality and Graft-versus-Host Disease

(A) Cumulative incidence (CI) of treatment-related mortality (TRM) in transplanted patients

(B) Comparison of CI of TRM in transplanted patients grouped according to age (older, above median age (53 years), younger, on or below median age).

(C) CI of acute Graft-versus-Host Disease (GvHD, grades II-IV) in transplanted patients

(D) CI of chronic GvHD in transplanted patients

Figure 4

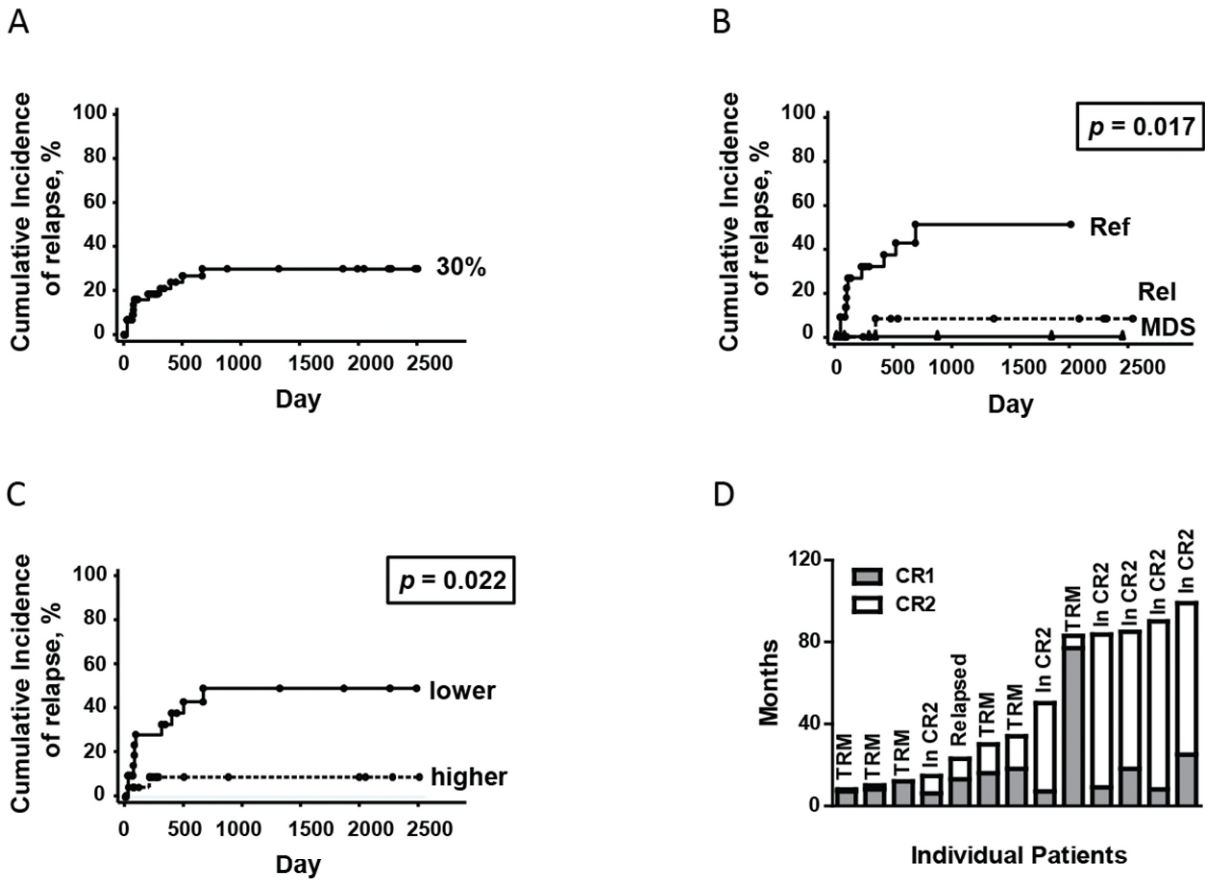

Figure 4 Relapse

(A) Cumulative incidence (CI) of disease persistence/relapse in transplanted patients

(B) Comparison of CI of disease persistence/relapse in transplanted patients with primary refractory (REF) or relapsed (REL) AML or MDS

(C) CI of disease persistence/relapse in transplanted patients according to transplanted $CD34^+$ cell dose (lower, on/below median dose; higher, above median dose)

(D) Length of CR1 and CR2 and individual outcomes of AML patients transplanted in first relapse.

Figure 5

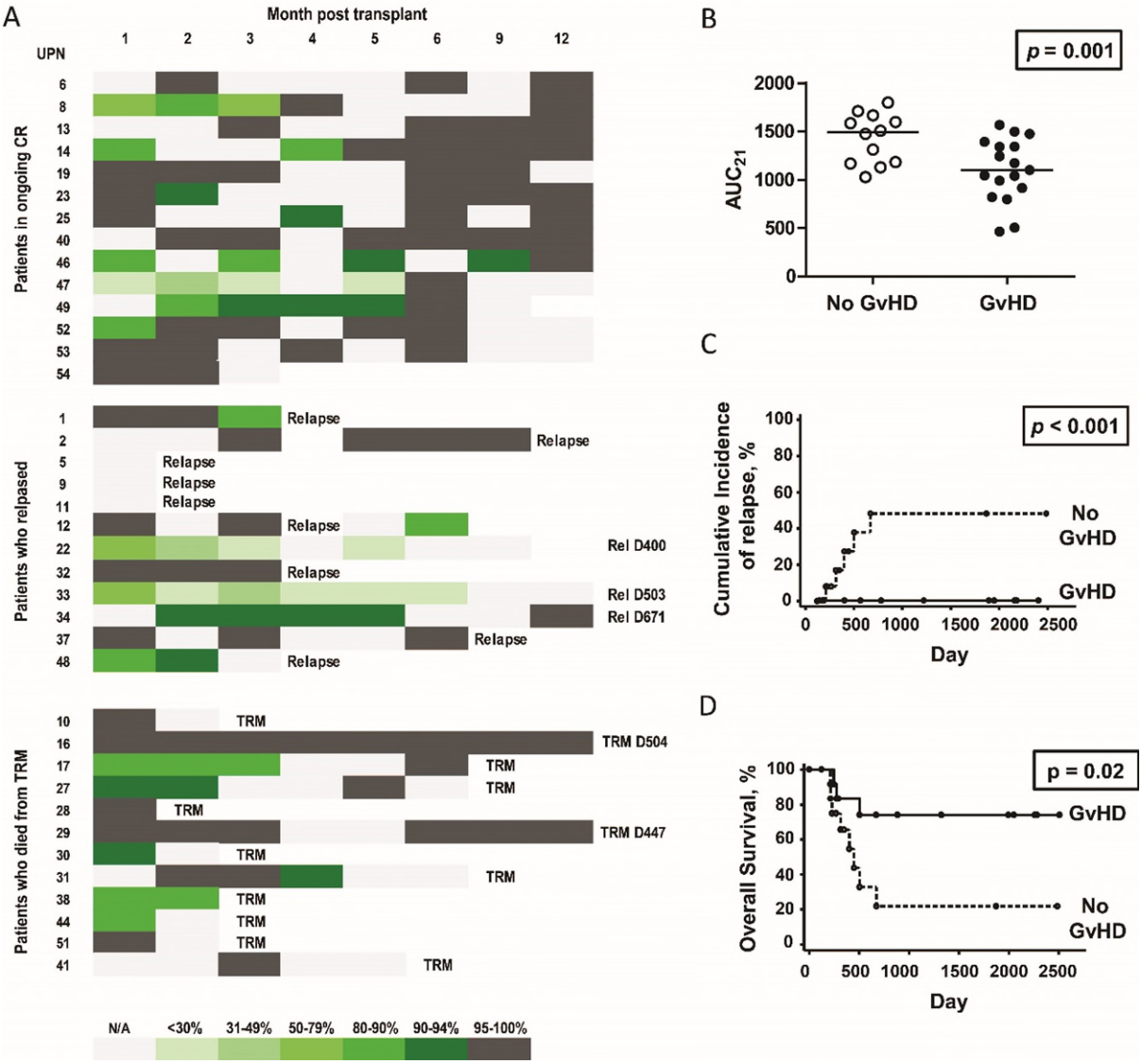

Figure 5 Post-transplant factors influencing graft-versus-leukaemia effects

(A) Donor T-cell chimerism levels in all patients who were transplanted and survived to D+30, grouped according to outcome. N/A, not available; CR, complete remission; TRM, treatment-related mortality

(B) Degree of exposure to CNI in the early post-transplant period in patients who did or did not develop GvHD after tapering CNI. Horizontal lines are medians and *p* value is for a two-tailed Mann Whitney test. Analysis is restricted to transplanted patients who were alive and in remission at D+100. Horizontal lines are medians and *p* value is for a two-tailed Mann Whitney test.

(C) Comparative CI of relapse in transplanted patients who did and did not develop *de novo* GvHD after tapering CNI immunosuppression

(D) Comparative OS of transplanted AML patients who did and did not develop *de novo* GvHD after tapering CNI immunosuppression