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Alemtuzumab with fludarabine and cyclophosphamide reduces chronic graft versus host disease after allogeneic stem cell transplantation for acquired aplastic anemia

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ABSTRACT

We evaluated a novel alemtuzumab-based conditioning regimen in hematopoietic stem cell transplantation (HCT) for acquired severe aplastic anemia (SAA). In a multi-center retrospective study, 50 patients were transplanted from matched sibling donors (MSD) (n=21) and unrelated donors, UD (n=29), using fludarabine 30mg/m^2 for 4 days, cyclophosphamide 300mg/m^2 for 4 days and alemtuzumab median total dose of 60mg (range:40-100mg). Median age was 35 years (range 8-62). Overall survival at 2 years was 95% +/-5% for MSD and 83% for UD HCT (p 0.34). Cumulative incidence of graft failure was 9.5% for MSD and 14.5% for UD HCT. Full donor chimerism (FDC) in unfractionated peripheral blood was 42%; no patient achieved CD3 FDC. Acute GVHD was observed in only 13.5% patients (all grade I-II) and only two patients (4%) developed chronic GVHD. A low incidence of viral infections was seen. Factors influencing overall survival were HCT co-morbidity index (92% with score 0-1 vs 42% with score ≥ 2, p<0.001) and age (92% for age < 50 years vs 71% ≥ 50 years, p<0.001). Our data suggests that the use of an alemtuzumab-based HSCT regimen for SAA results in durable engraftment with a low incidence of chronic GVHD.

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) offers the chance of long term cure for patients with severe aplastic anemia (SAA)[1-5] but chronic graft versus host disease (GVHD) represents a current major challenge that impacts on quality of life as well as survival[6]. The ideal conditioning regimen for SAA is one that results in sustained engraftment, minimal regimen-related toxicity and absence of both acute and chronic GVHD. There is no advantage for any degree of chronic GVHD in AA, in contrast to the beneficial impact of graft versus leukemia effect on HCT for myeloid malignancies.

Over the last few decades, survival after HCT for acquired SAA has improved greatly. HLA matched sibling donor (MSD) HCT results in long term survival in at least 80% of patients[1-2, 4-5, 7-8]. The standard of care conditioning for young patients undergoing MSD BMT for AA is high dose cyclophosphamide (CY) 200mg/kg and ATG, with ciclosporin (CSA) and methotrexate (MTX) as post graft immunosuppression. Long term survival occurs in 80-90% of patients, although this is age-dependent, with only 50% survival above the age of 40-50 years[3, 9-10]. Graft rejection occurs in 5-10%, and acute GVHD in 12-30%. Notably, chronic GVHD remains a major problem for 30-40% of patients. In addition, incremental impact of increasing age is observed on the cumulative incidence of acute and chronic GVHD[3]. Unrelated donor (UD) HCT had previously been reserved for those patients who fail to respond to two courses of immunosuppressive therapy (IST), but outcomes after UD HSCT have improved significantly during the last decade[11-15] A prospective paediatric study from Japan comparing repeat course of IST with UD HCT showed benefit in favour of early UD HCT in AA[16]. UD HCT is now considered after failing one course of IST[17-18]. Improved outcomes are likely due to improved

HLA matching techniques, better supportive care and improved conditioning regimens. For example, the use of fludarabine, low dose CY and ATG (FCATG) conditioning regimen for young patients, and FCATG with low dose (2Gy) TBI for older patients, results in 75 and 79% overall survival at 5 years, respectively. Graft rejection occurs in 17% of patients. Although acute GVHD grade II-IV occurs in only 13% of patients, chronic GVHD is observed in 27% of young patients and 50% in the older age group[12].

We have previously evaluated the CD52 monoclonal antibodies Campath-1G and the humanised antibody Campath 1-H (alemtuzumab), in pilot studies for MSD and UD HCT for SAA[19-23]. The rationale was to develop a conditioning regimen that provided good anti-GVHD prophylaxis with the addition of Campath to CSA, without the necessity for methotrexate, thereby reducing hepatotoxicity and mucositis. At the same time, in order to achieve long term haematopoietic engraftment, additional immunosuppression would be provided by the addition of CSA. In the early studies, high dose CY was used with Campath and CSA for MSD HCT and demonstrated good survival, with a low risk of acute and chronic GVHD. A high incidence of graft failure was associated with the use of Campath given both pre and peri-transplant, and reduced when given only pre-transplant[20]. For UD HSCT, alemtuzumab was used with low dose CY (300mg/m^2 x 4) and fludarabine[21].

In this retrospective, multi-centre study from 5 centres, we have analysed outcomes of 50 patients with acquired SAA who have been transplanted with a minimal intensity conditioning regimen using alemtuzumab with fludarabine and CY 300mg/m^2 x 4 (FCC). We confirm that the use of alemtuzumab in this setting is the first reported manoeuvre to result in a much lower

incidence of chronic GVHD compared with previously reported conditioning regimens used in SAA. We also show that this regimen is associated with a low incidence of infections, low toxicity, sustained engraftment and excellent long term survival. Additionally, we show that survival after UD HCT is not significantly different from MSD HCT.

MATERIALS AND METHODS

1. PATIENTS

From 1999 to 2009, 50 adults and children with acquired SAA were transplanted at 5 centres (King's College Hospital, London; St George's Hospital, London; Bristol; Nottingham; and Princess Margaret Hospital, Toronto). The diagnosis of aplastic anaemia and assessment of disease severity were established according to published criteria[24-26]. Fanconi anaemia was excluded in all cases by chromosome breakage studies on cultured peripheral blood lymphocytes with diepoxybutane. Eight patients included in this study were previously reported[21, 23].

Patient characteristics are shown in Table 1. The median follow up of patients was 18.2 months (range: 2.3-118.2). Twenty-one patients were transplanted from either an HLA matched sibling donor (MSD), and 29 from an unrelated donor (UD). UD HCT was performed after failure to respond to at least one course of immunosuppressive therapy. All donor-recipient pairs were matched for HLA-A, -B, -C, -DRB1 and DQB1 using high resolution DNA typing, apart from two patients who received a 9/10 antigen matched unrelated donor. Each local institutional research ethics committee approved the study and informed consent was obtained from all participating patients in accordance with the Declaration of Helsinki.

2. STUDY PROTOCOL

The transplant conditioning regimen used (FCC) comprised fludarabine 30mg/m^2 intravenous (IV) daily for 4 days given from day - 7 to - 4, CY 300mg/m^2 IV daily for 4 days on day – 7 to – 4. Alemtuzumab (Genzyme Therapeutics) was administered IV/SC pre-HCT at a total dose of 40-100mg (median 60mg) between days -7 to -3: 100mg (n=9), 75mg (n=11), 60mg (n=21), 50mg (n=8) and 40mg (n=1). Each centre used their approved regimen for alemtuzumab (See Supplemental data for each institutional protocol). Stem cell source was bone marrow (BM) in 24 (49%), G-CSF primed BM in 7 (14%), PBSC in 14 (27%) and BM+PBSC in 5 (10%) of patients. Post-transplant GVHD prophylaxis was achieved with CSA (2.5mg/kg IV BD) from day-1 titrated to plasma trough levels of 200-300 ng/ml. Oral CSA was substituted when a good oral intake was achieved, and continued for 12 months with tapering at 9 months only in the presence of stable haematological parameters and without declining mixed donor chimerism.. Acute and chronic GVHD were graded using standard criteria[27].

3. SUPPORTIVE CARE

Supportive care was provided according to institution practices as previously described[20, 23, 28-29]. All patients were screened for CMV infections/reactivation at least weekly for the first 3-6 months using CMV antigenemia or PCR based assay. .

4. STUDY DEFINITIONS

Neutrophil engraftment was defined as the first of 3 consecutive days with an ANC $\geq 0.5 \times 10^9$/l unsupported by G-CSF. Platelet engraftment was defined as the first of 3 consecutive days with a platelet count $\geq 20 \times 10^9$/l without platelet transfusion support for 7 preceding days. Patients

were evaluable for engraftment if they survived > 21 days after transplantation. Primary graft failure was defined as the absence of neutrophil count ≥ 0.5 x 10^9/l on 3 consecutive days and late graft failure as recovery followed by recurrent pancytopenia with a hypocellular bone marrow in the absence of severe GVHD. Platelet refractoriness was defined as a platelet count increment of < 10 x 10^9/l at one hour following random donor platelet transfusion, in the absence of fever or bleeding. GVHD was diagnosed on clinical grounds with histopathological biopsy where possible and graded according to published criteria. The hematopoietic stem cell transplantation comorbidity index (HCT-CI) was used to assign a pre-transplant co-morbidity score to patients[30].

5. ANALYSIS OF CHIMERISM

Chimerism assessments were scheduled for days 30, 60, and 100; 6 months; and then yearly. Chimerism was assessed in unfractionated bone marrow samples as well as fractionated peripheral blood CD3+ T-cell and CD15+ granulocyte populations whenever possible[31]. Thirty-nine patients had serial unfractionated bone marrow chimerism samples available for interpretation, and 21 patients had additional peripheral blood CD3+ chimerism data.

Full donor chimerism (FDC) was defined as the presence of >95% donor hematopoietic cells, while mixed donor chimerism (MDC) was defined as 5-95% donor cells post-transplant[32]. Progressive mixed chimerism was defined as ≥ 15% increase in recipient cells over 3 months and stable mixed chimerism as < 5% fluctuation in the % recipient cells over time. Patients who showed mixed donor-recipient chimerism but who subsequently reverted to full donor chimerism

were classified as having transient mixed chimerism[33]. Declining donor chimerism was defined as a 10% or greater increase in recipient chimerism on two consecutive measurements.

6. STATISTICAL ANALYSIS

Overall survival (OS) was measured from day 0 to death from any cause or last known follow-up. Failure-free survival (FFS) was defined as survival with sustained engraftment, death and graft failure were categorised as treatment failure. FFS was measured from day 0 to the first indicator of graft failure, death of any cause, or last known follow-up. Univariate comparisons and multivariate analysis used the Cox proportional hazards regression model. Variables analysed included: recipient age (≥ or < 50 years), Severity of AA (non-severe vs severe vs very severe), donor status (MSD vs UD), stem cell source, HCT-CI (0-1 vs ≥2), and time from diagnosis to HCT (≥ or < 12 months). On multivariate analysis, independent variables with p>0.1 were sequentially excluded from the model. All data was censored as of the 5th March 2010.

RESULTS

1. PATIENT CHARACTERISTICS

Table 1 summarises the patient characteristics. Of the 50 patients, 29 were transplanted using an UD and 21 a MSD. The median age was 35 years (range 8-62), of whom 38 patients (76%) were aged <50 years and 12 (24%) ≥50 years at time of HCT. Eighty-eight percent (44/50) of transplants were performed after 2005. The median interval from diagnosis to HCT was 6 months (range 2-252) for MSD and 10 months (range 4-137) for UD (p=0.75). A PNH clone was detected by flow cytometry, with or without FLAER, in 9 (18%) patients at time of SCT. The median size (and range) of the clone was 0.07% (0-3.1%) for red cells, 0.4% (0-97.1%) for

granulocytes and 2% (0-52.8%) for monocytes. In two patients the size of the granulocytic clone was > 10%.

2. GRAFT FAILURE

The median time to neutrophil recovery was 17 days (range: 2-50) and platelet recovery was 19 days (range: 10-57). Graft failure occurred in 6 patients (12%), of whom 3 had early graft failure following UD HCT and 3 had late graft failure on days + 32, 86 and 126, two after MSD and one after UD HCT. All patients had been on immunosuppressive therapy at time of graft failure**.** Of these 6 patients, 4 had received BM, one G-BM and one PBSC. The median cell dose (and range) was 1.68 CD34 $x10^6$/kg (1.2-6.35) compared with 3.19 CD34 $x10^6$/kg (1.10-7.83) for patients who had sustained engraftment (p=0.24). Two patients died of complications related to graft failure, 2 patients had autologous reconstitution following graft failure with improvement in haematological parameters, and 2 patients proceeded to a second rescue allograft.

The cumulative incidence of graft failure was 9.5% for MSD and 14.5% for UD HCT. As all patients in the cohort had persistent mixed donor chimerism, it was unsurprising that preceeding donor chimerism had no correlation with subsequent graft failure of these 6 patients. There was no difference in engraftment according to HCT-CI score, with 92% engraftment for a score of 0-1 and 80% for ≥ 2 (p = 0.5). At last follow-up, 29 patients in the cohort were at least 1 year post-transplant, of which 22 patients had stopped their immunosuppression. There have been no episodes of last onset graft failure/rejection or acute GvHD in the cohort to date.

3. CHIMERISM DATA

39 patients (78%) had sequential data on unfractionated whole peripheral blood chimerism available, of whom 21 patients had additional sub-lineage CD3 (lymphocyte) chimerism data available for analysis. At day 100 post-transplant, 21 patients had attained full donor chimerism (FDC) in the unfractionated PB. However, at the same time-point, no patients had attained CD3 FDC, with a median CD3 chimerism level of 45% (range: 9-94%). Likewise, at 1 year post-transplant, none of the 16 evaluable patients had attained CD3 FDC (see Figure 2). Of note, no patients received donor lymphocyte infusions**,** and all patients were blood and platelet independent after 1 year post-transplant. Only one of the patients who had subsequently developed GVHD (acute) had available chimerism for assessment (CD3 chimerism was 29% at time of GVHD).

4. GVHD

Acute GVHD was observed in 7 (13.7%) patients, all grade I-II. Only two patients (4%) developed chronic GVHD, one limited and one extensive. The cumulative incidence of acute and chronic GVHD at 1 year was 16.5% +/- 8% and 7% +/- 4%, respectively. Chronic GVHD contributed to death of one patient (on day + 427) who also had CMV disease.

5. INFECTIONS

Epstein-Barr virus (EBV) viremia was seen in 4 of 49 patients (8.2%), of whom 2 patients had post transplant lymphoproliferative disease (PTLD). One patient was successfully treated with one course of R-CHOP, but another patient died of progressive PTLD. There were 9 cases of CMV reactivation but only one case of CMV disease, who unfortunately died from

complications associated with GVHD and CMV. Seven of 42 patients (16.7%) had adenovirus viremia.

6. FERTILITY

While the fertility status of patients was not uniformly assessed in all patients post-transplant, out of 14 female patients in the age group 16-40 years at HCT, one patient delivered a full term healthy baby at 31 months post HCT and another had a medical termination for unwanted pregnancy.

7. OVERALL SURVIVAL

The actuarial overall survival at 2 years for all patients is 88%+/-5%, with a 2-year FFS of 80%+/-6%. At time of last follow-up, a total of five patients had died, 4 following UD HCT. The individual causes of death were chronic GVHD with CMV disease (day + 427), presumed invasive pulmonary fungal infection (day +14), graft failure in 2 patients (days + 137 and + 199)) and PTLD (day + 180). There was no significant difference in 2-year OS between MSD with UD HCT, 95% and 83%, respectively (Figure 1a). The following factors were shown to significantly influence overall survival.

(i) **CO-MORBIDITY INDEX (Figure 1b):** For patients with a Sorror score of 0-1 ($n = 37$), the 2-year OS was 95% compared with only 42% with a score of ≥2 ($n = 6$), ($p < 0.001$). Likewise, 2-year FFS was 88%+/-7% for HCT-CI 0-1 vs 44%+/-22% for HCT-CI ≥2 ($p=0.02$)

(ii) **AGE (Figure 1c):** Advanced recipient age (≥50 years) adversely impacted on the OS of the cohort. HCT was performed in 11 patients aged ≥ 50 years, with an OS of 71% compared with 92% for patients < 50 years old (p = 0.02). Three patients were aged ≥ 60 years who received UD HCT, of whom two are alive; one had grade 1 acute GVHD, the second had fungal pneumonia at time of HCT, died on day +14 from progression of fungal infection. 2-year FFS was 86.5% +/- 6% (<50 years) vs 72.9% +/-13.5% (≥50years), p=0.50.

(iii) **STEM CELL SOURCE (Figure 1d):** While there was no significant difference on the 2 year overall survival between all stem cell sources, with PBSC (n=14) 73%+/-13%, BM (n=24) 93%+/-6%, GCSF-BM (n=7) 86%+/-13%, and BM+PBSC:100% (p=0.72), there was a significant difference on pair-wise log-rank analysis in OS between patients who received BM vs PBSC (p=0.03). Of note, there was no difference in 2 year FFS based on stem cell source, BM 88% +/- 7% vs PBSC 70% +/- 13% (p=0.36).

(iv) **TIME FROM DIAGNOSIS TO HCT:** The time from initial diagnosis to transplantation had no significant impact on overall survival in this study, with those transplanted within 12 months having a 2-year OS of 95.8% +/-4% vs those ≥12 months 75.7%+/- 11% (p=0.07). 2 year FFS for those receiving transplantation within 12 months of diagnosis was 90% +/-6% vs those ≥12 months: 65% +/- 13% (p=0.09).

8. IMPACT OF ALEMTUZUMAB DOSE

Due to the retrospective nature of this study, there was a degree of variability in the dosage of alemtuzumab used between centres. Twenty patients (40%) received a total dose of >60mg of alemtuzumab while 30 (60%) received a total dose of 60mg or less. There was no significant difference in donor type (MSD vs MUD), stem cell source, or stem cell dose, between patients who received > or </= 60mg alemtuzumab. In terms of transplant outcomes, there was no difference in OS (>60mg: 81%+/-9% vs </=60mg: 96%+/-4%, p=0.23) or FFS (≥60mg:74%+/-9% vs </=60mg: 88%+/-7%, p=0.41) between groups, likewise, there was no significant difference in CMV, EBV or GVHD incidence.

DISCUSSION

We have shown that the use of alemtuzumab in combination with fludarabine and low dose CY (FCC) in MSD and UD HCT for acquired AA is associated with a very low risk of chronic GVHD (4%), a low risk and low grade of acute GVHD (14%, and all cases grade I or II) and excellent overall survival. The incidence of graft failure is comparable to conditioning regimens using ATG instead of alemtuzumab, and infection rates were low.

We first explored an alternative approach to reduce GVHD initially using the monoclonal antibody Campath-1G during the 1980s, and later the humanised monoclonal antibody Campath-1H (alemtuzumab) when it became available in 1999[19-21]. The aim was to develop a conditioning regimen for HCT in SAA that produced sustained engraftment, with minimal toxicity and GVHD. Pharmocokinetic studies have shown that alemtuzumab is detectable in the plasma for

several weeks after administration, resulting in depletion of recipient autoreactive lymphocytes and prevention of GVHD by depletion of donor alloreactive T-cells[34-35]. For MSD HCT, Campath-1G or alemtuzumab was used instead of ATG, with CY 200mg/kg and CSA[21]. Avoidance of MTX as GVHD prophylaxis helps reduce regimen related toxicity. Excellent 5 year survival of 81% was associated with a very low incidence of GVHD (14% grade II-IV acute GVHD and 4% chronic GVHD), limited in all cases, but graft rejection was high at 24%. The high graft rejection was associated with the initial use of Campath-1G given both pre- and peri-transplant in the first 21 patients from day -8 to +5, in an attempt to achieve maximum GVHD prophylaxis. Subsequent patients received Campath monoclonal antibody pre-transplant only, resulting in a reduction in graft rejection to 11%, while maintaining a low incidence of GVHD. The high survival of early patients with early graft failure was in part due to a high incidence of autologous recovery, in around 50% of patients[19, 22]. We previously used alemtuzumab with fludarabine based conditioning for UD HCT and reported excellent engraftment in a small series of patients with both acquired and inherited SAA[21].

Risk factors for chronic GVHD after HCT for SAA include acute GVHD, older age and donor chimerism. The use of peripheral blood stem cells (PBSC) in MSD HCT using ATG-containing regimens, results in a high incidence of chronic GVHD among younger patients and worse survival for both young and older patients[36]. A low incidence of chronic GVHD using alemtuzumab instead of ATG in HCT for SAA was also observed separately from one of the centres participating in this study[23]. In addition to deleting alloreactive donor T-cells, alemtuzumab targets host antigen presenting dendritic cells which trigger the alloreactive response[37]. In our study the use of alemtuzumab was associated with a very low incidence of

GVHD. Acute GVHD occurred in 8 patients, 4 who received BM and 4 who received PBSC. Of the two cases of chronic GVHD, one with extensive chronic GVHD received BM, the other with limited disease received PBSC.

Mixed donor chimerism occurs frequently after HCT for SAA[33, 38-40]. Progressive mixed chimerism carries a high risk of graft rejection but stable mixed chimerism is associated with absence of chronic GVHD and excellent survival. Analysis of chimersim showed that while the majority of patients attain FDC in the unfractionated bone marrow by day 100, none of the patients achieve CD3 FDC even at 1 year post-HCT. Persistent mixed CD3 donor chimerism, particularly in the backdrop of T-cell depletion with alemtuzumab has been shown to be associated with a low incidence of GVHD. The data presented here suggests that using an FCC protocol, patients are able to maintain sustained mixed donor chimerism even after the cessation of immunosuppression, with maintenance of clinical remission and haematological parameters. Similar observations have been reported using alemtuzumab with TBI (3Gy) and sirolimus in HCT for sickle cell disease, with complete absence of both acute and chronic GVHD in association with a high incidence of stable mixed chimerism[41]. The proposed advantage of using sirolimus instead of CSA in that study was that sirolimus promotes differentiation of T regulatory cells and T helper cells which may have contributed to the absence of GVHD. However, sirolimus is associated with toxicity from hyperlipidaemia, arthralgia, pneumonitis, hyperglycaemia and myelosuppression.

A low stem cell dose is associated with an increased risk of graft failure in HCT for AA[42-43]. We have recently shown that infusion of $< 2.0 \times 10^6$ CD34 cells/kg is associated with increased graft

failure, increased incidence of bacterial infections and delay in neutrophil engraftment, although neutrophil engraftment is more dependent on stem cell source[28]. One study reported more chronic GVHD when >3.7 x 10^8 nucleated cells/kg were infused, but data on CD34 cells was lacking[44]. We have shown in this study that the use of PBSC with alemtuzumab-based conditioning permits collection of an adequate cell dose without causing significant chronic GVHD.

Concerns have been raised that the use of alemtuzumab may increase the risk of viral infections post HCT. As in our previously reported studies in HCT for SAA[19-20], and for treatment of refractory autoimmune cytopenias[45], we confirmed a low incidence of viral infections and we observed only two cases of EBV PTLD. We have previously shown that there is a strong association of EBV PTLD post HCT for SAA when ATG is used as IST for AA before HCT[22].

We observed no expansion of PNH clones post HCT. Lymphocytes deficient in the expression of GPI-anchored proteins may emerge after treatment with alemtuzumab, mimicking a PNH clone, as a result of immune selection by the anti-CD52 antibody[46]. A PNH clone was present in 9/34 patients before HCT, but only detectable post-HCT in 2 patients, which disappeared at 6 months in one patient and detectable in only 0.1% granulocytes and monocytes at 4 months in the other patient. In the remaining 7 patients the PNH clones disappeared post HCT. However, we did not examine expression of GPI-anchored proteins on peripheral blood lymphocytes.

There is much debate concerning the upper age limit for HCT in SAA. Using standard CY 200mg/kg with ATG, overall survival of 65 % with median follow up of 9.1 years, was recently

reported among 23 patients aged 43-68 years from Seattle[47]. However there are concerns about cardiac toxicity of CY at this dose in older patients; fluid overload was reported in 6 patients in that series, which contributed to cause of death in 4 patients[47]. A recent study from the Centre for International Blood and Marrow Transplantation Research (CIBMTR) showed worse survival among patients > 40 years of age transplanted for SAA from MSD. Older patients were more likely to (i) have a worse performance status (ii) receive IST prior to HCT (iii) have an interval from diagnosis to HCT of > 3 months and (iv) have received a PBSC graft. With the use of fludarabine-based conditioning regimens, improved outcomes have been reported in older patients[9]. Previously, first line MSD HCT was recommended for patients < 40 years of age, because of worse outcome after SCT in patients > 40 years old. Our institution at King's College Hospital has pioneered the use of alemtuzumab with reduced intensity conditioning for allogeneic HCT in myelodysplastic syndrome (MDS)[29, 48]. Furthermore, in a retrospective study from the EBMT, patients older than 60 years transplanted for MDS show similar non-relapse mortality to patients aged 50-60 years[49]. Another CIMBTR study in MDS showed that older age has no impact on overall survival, disease free survival in addition to non-relapse mortality[50]. In our study we show excellent outcomes in a small sub-group of patients > 50 years of age (range 50-62) with 71% survival. The low toxicity of FCC, as well as low risk of GVHD and infections, makes this an especially attractive regimen for older patients, and further studies in older patients are now indicated.

An important prognostic factor for survival in our study was the co-morbidity index, as assessed by the Sorror score, and this emphasises the importance of careful selection of patients prior to planned HCT. A score of > 2 in SAA indicated a worse outcome with overall survival of only

42% after FCC HCT, and this may be especially important in decision planning in older patients. Patients receiving bone marrow as the stem cell source had an improved OS when compared with those receiving peripheral blood stem cells (93% vs 73%, p=0.03). Given the possible confounding factors influencing the outcomes in our study, larger studies will be required to truly establish the influence of stem cell source on outcomes of patients receiving the FCC regimen.

The excellent outcome of UD HCT using FCC adds further support to the current recommendation of early HCT after failure of only one course of IST, instead of waiting until failure after a second course[17-18]. Patients who were transplanted within 12 months of diagnosis had a more favourable 2 yr OS (96% vs 76%, p=0.07). In conclusion, the use of alemtuzumab in SCT for acquired AA is the first reported change in conditioning regimen to result in a major reduction in chronic GVHD compared with previously reported studies.

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AUTHORSHIP

Contribution:

J.C.M., V.G., Z.Y.L., G.J.M., A.P. contributed to the study design.
J.C.M., Z.Y.L., V.G, A.H., R.I., D.I.M., N.R., A.P., G.J.M., contributed to writing the paper.
V.G., Z.Y. L., V.P., N.R., D.I. M., M.K., M.S.I., R.I., J.H. contributed to the data collection.
V.G., Z.Y. L., V.P., N.R., D.I. M., M.K. contributed to patient recruitment.
Z.Y.L. analyzed the results and made the figures.
J.C.M. served as the principal investigator for this study.
All authors reviewed the paper.

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TABLE 1: PATIENT CHARACTERISTICS

Characteristic	No.	%
Median age (range)	35 (8-62)	
Patients aged < 50 yr	38	76%
Patients aged ≥ 50 yr	12	24%
Gender		
Male	26	52%
Female	24	48%
Disease severity		
Very severe AA	15	30%
Severe AA	25	50%
Non-severe AA	10	20%
AA aetiology		
Idiopathic	39	78%
Drug-induced	5	10%
Hepatitis	4	8%
Pregnancy	2	4%
Median interval Dx-HCT (mths) (range)		
SIB	6 (2-252)	
UD	10 (4-137)	
Prior ATG treatment		
Yes	33	66%
No	14	28%

Unknown	3	6%
Donor type		
Matched sibling	21	42%
Unrelated	29	58%
Stem cell source		
Bone marrow (BM)	24	48%
G-BM	7	14%
PBSC	14	27%
BM + PBSC	5	10%
Transplant period		
1999-2004	6	12%
2005-2010	44	88%
Sorror Score		
0 – 1	36	72%
≥ 2	6	12%
unknown	8	16%
PNH clone at time of HCT	9/34	26%
CMV status		
Donor+/Recipient+	14	28%
Donor-/Recipient-	24	48%
Donor+/Recipient-	6	12%
Donor-/Recipient+	6	12%
Total Alemtuzumab dose		
>60mg	20	40%
</=60mg	30	60%

FIGURE LEGENDS

FIGURE 1
Overall survival (OS) curves

1a) OS curves for the cohort stratified by donor type (MDS – matched sibling donor, UD – unrelated donor)

1b) OS curves for the cohort stratified by transplant co-morbidity index (HCT-CI) (HCT-CI 0-1 vs HCT-CI ≥2)

1c) OS curves for the cohort stratified by recipient age (recipient age <50 yrs vs ≥50 yrs)

1d) OS curves for the cohort stratified by stem cell source (BM – bone marrow, PBSC – peripheral blood progenitor cells)

FIGURE 2
Representative median peripheral blood chimerism results in patients following FCC conditioning [UF- unfractionated (n=33), CD3 (n=16), and CD15(n=16)]

Figure 1a

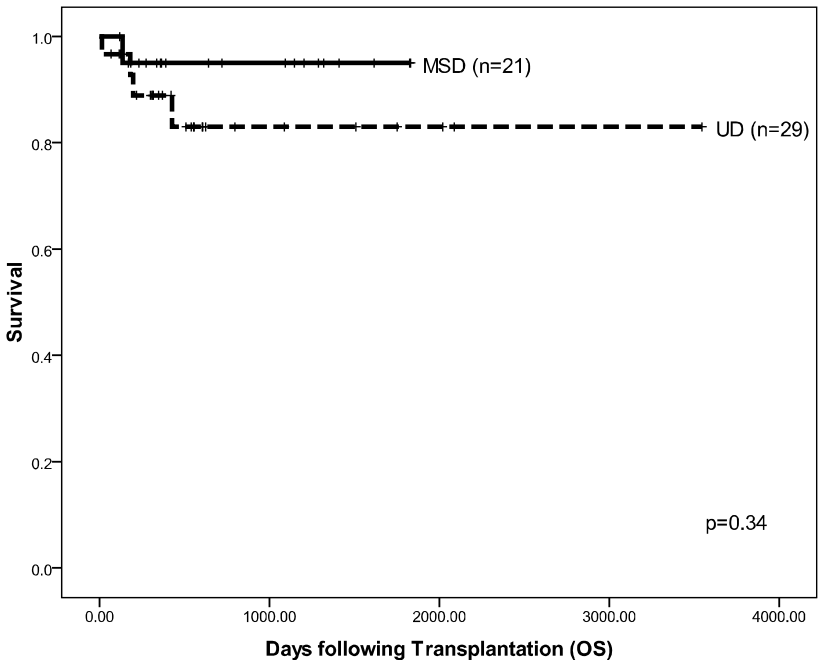


Figure 1b

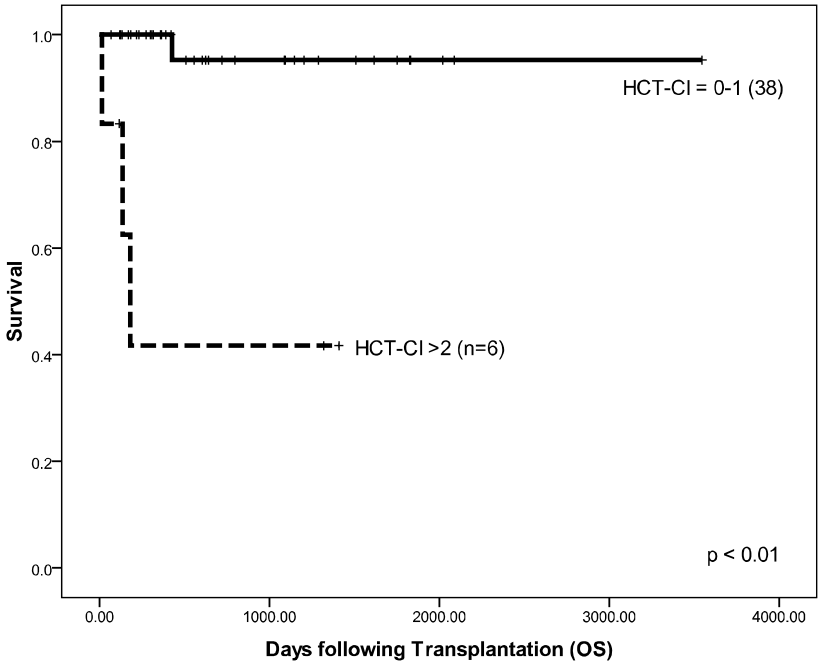


Figure 1c

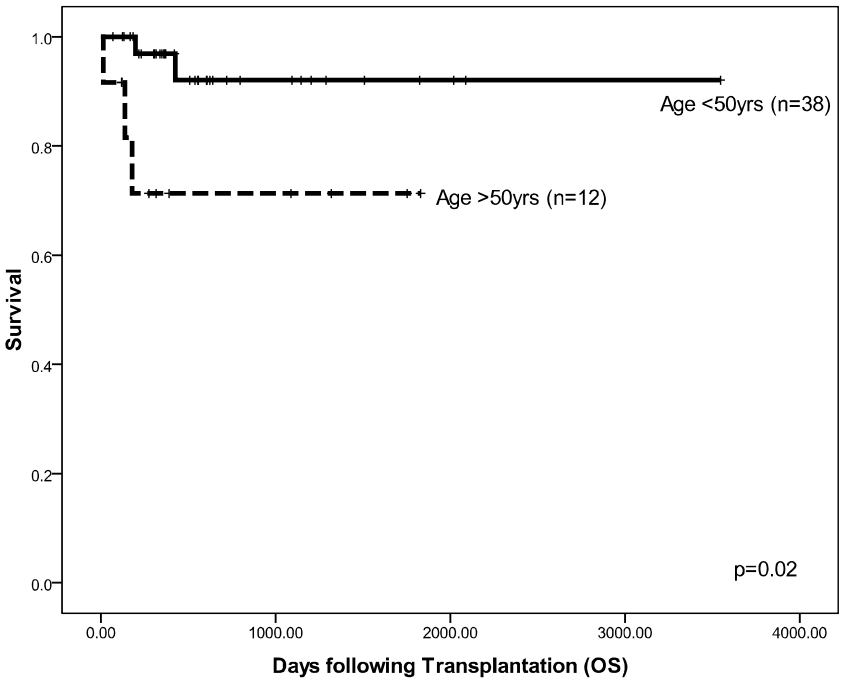
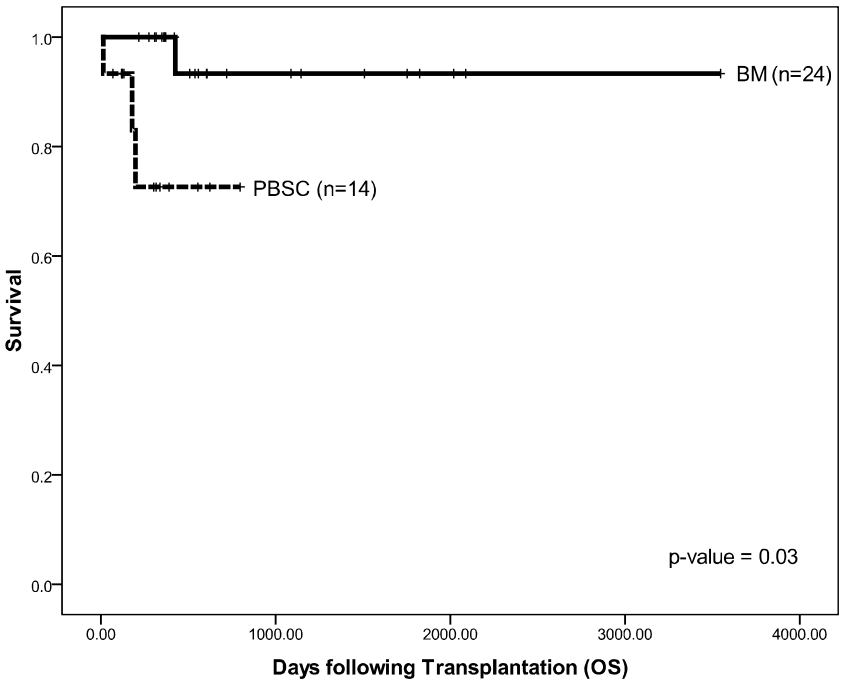


Figure 1d



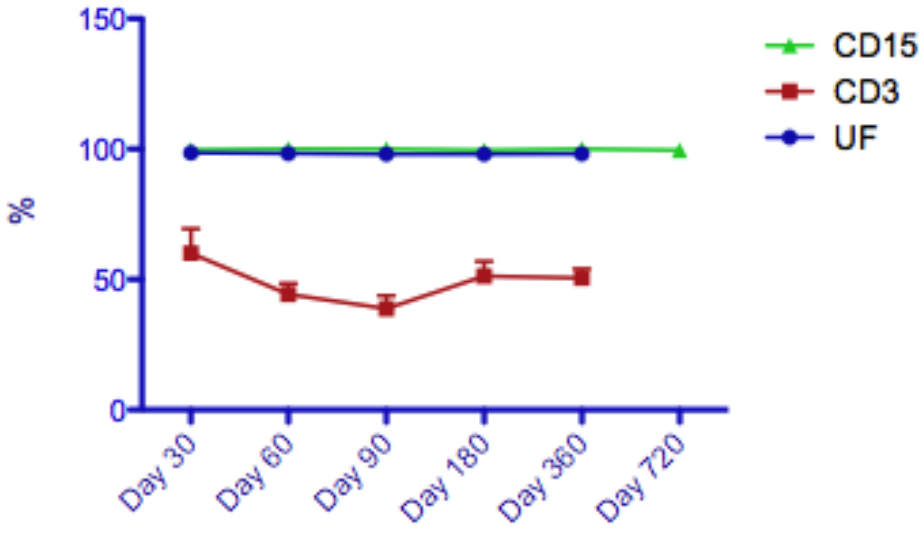

150
100
50
0
%
Day 30
Day 60
Day 90
Day 180
Day 360
Day 720
CD15
CD3
UF

FIGURE 2