

Allogeneic stem cell transplantation using alemtuzumab-containing regimens in severe aplastic anemia

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Abstract Alemtuzumab, a humanized anti-CD52, IgG1 monoclonal antibody, is used to reduce graft-versus-host disease (GVHD) and aid engraftment after allogeneic haemopoietic stem cell transplant (HSCT). Its associated low incidence of GVHD makes it an attractive alternative to anti-thymocyte globulin (ATG) in transplant conditioning regimen for severe aplastic anaemia (SAA). We have reviewed the use of alemtuzumab-based conditioning regimen for HSCT in SAA and show that it results in sustained haematological engraftment, a very low incidence of chronic GVHD without an increase in viral infections. Intriguingly, alemtuzumab appears to induce tolerance post-HSCT with the findings of stable mixed T cell chimerism with full donor myeloid chimerism and the absence of chronic GVHD, and which persist on withdrawal of post-graft immunosuppression. Finally, its low toxicity profile may permit future application of HSCT to older patients with SAA who fail to respond to immunosuppressive therapy.

Keywords Alemtuzumab · Aplastic anemia · Haemopoietic stem cell transplant (HSCT)

Introduction

Allogeneic haemopoietic stem cell transplantation (HSCT) is the only potentially curative therapy for patients with acquired severe aplastic anaemia (SAA). HSCT is the

initial treatment of choice for newly diagnosed patients up to the age of 40–50 years in the presence of an HLA-matched sibling donor (MSD). Matched unrelated donor (MUD) HSCT is indicated after failure to respond to a course of immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and cyclosporin (CSA) [1, 2].

Survival has significantly improved over the past 3 decades, because of the introduction of CSA in the early 1980s, resulting in reduced graft rejection, and better supportive care with transplant regimens resulting in lower transplant related mortality. Improved HLA matching and better conditioning regimens have also contributed to improved outcomes after MUD HSCT. Long-term survival is seen in 80–90 % of patients following MSD HSCT [3–6] and 75–80 % for MUD HSCT in SAA [7–10].

However, the overall survival (OS) after HSCT for SAA is age-dependent, with worse outcomes in older patients [11, 12], and better outcomes in children [13–15]. Graft-versus-host disease (GVHD) also predicts for worse survival after HSCT in SAA [5]. There is no advantage for any degree of GVHD in SAA, in contrast to the beneficial impact of graft-versus-leukaemia (GVL) effect in myeloid malignancies. The historical use of TBI-based conditioning regimens reduced the risk of graft rejection, but increased GVHD, pneumonitis and serious long-term effects, such as second malignancies and reduced growth and development in children [16, 17]. ATG-based conditioning regimens are now most commonly used, to reduce GVHD and aid engraftment; for MSD HSCT; ATG is used with high dose cyclophosphamide (CY) 200 mg/kg for patients <30–40 years old, and for MUD HSCT, with fludarabine, and lower dose CY with or without low dose TBI (2 Gy). A recent retrospective EBMT study of 1,886 patients showed that the addition of ATG improved OS after MSD HSCT [6]; a previous prospective randomized study from the CIBMTR showed no

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significant difference in OS when ATG was added to CY 200 mg/kg, but that study was underpowered due to relatively low number of patients enrolled [4]. However, ATG-based conditioning is associated with up to 30 % incidence of chronic GVHD [3–6], and severe chronic GVHD impacts quality of life as well as OS (Table 1).

Alemtuzumab: the humanized form of CAMPATH-1 antibodies

The CAMPATH-1 monoclonal antibodies recognise CD52, a GPI-linked membrane protein, which is expressed on peripheral blood T and B cells, dendritic cells, monocytes, eosinophils and lower expression on neutrophils, but not expressed on CD34+ haemopoietic cells [18–21].

The early CAMPATH-1 antibodies, rat IgM (CAMPATH-1M) and rat IgG (CAMPATH-1G) were remarkably lytic with human complement. CAMPATH-1G and the humanized form CAMPATH-1H (alemtuzumab), also bind to human IgG Fc receptors and activate antibody-dependent cellular cytotoxicity (ADCC) [18, 22]. The CAMPATH-1 antibodies have long been used for prevention of GVHD and graft rejection in reduced intensity allogeneic HSCT for haematological malignancies, through the depletion of T lymphocytes in both donor and recipient [22–24]. Pharmacokinetic studies have shown that alemtuzumab is detectable in the plasma for several weeks after administration, resulting in the depletion of recipient auto-reactive lymphocytes and prevention of GVHD by depletion of donor alloreactive T cells [23, 25], resulting in a low incidence of graft rejection and GVHD, respectively.

The rationale for using alemtuzumab in AA HSCT

The ideal conditioning regimen in SAA is one that results in sustained engraftment, minimal regimen-related toxicity, the absence of both acute and chronic GVHD, and makes available the potential of HSCT for older patients, who were previously considered ineligible for this treatment. Alemtuzumab is particularly applicable to transplantation for non-malignant diseases, such as SAA, with the aim to induce allograft tolerance.

For patients being transplanted for leukaemia, donor alloreactive T cells contribute to a fine balance between risks of GVHD and graft-versus leukaemia, but in SAA there is no benefit of any graft-versus-disease effect. However, donor lymphocyte depletion may exacerbate impaired immune reconstitution and potentially increase the risk of viral infections post-transplant. In contrast to HSCT for haematological malignancy, graft failure

remains a significant problem in SAA, especially in patients, who have been heavily transfused and are allo-immunized to major and minor HLA antigens. Graft failure occurs in up to 10 % of MSD and 15 % of MUD HSCT patients [3, 6, 8, 10].

In the context of SAA, the knowledge that CD52 is a GPI-linked protein is of prime importance as a relatively large proportion (around 40–50 %) of patients with AA have an associated PNH clone, as detected by highly sensitive flow cytometry. Hence, before considering using alemtuzumab as part of the conditioning regimen for HSCT, if a PNH clone is detected in peripheral blood granulocytes and red cells, recipient T cells should also be examined for CD52 expression, as deficient expression of CD52 on recipient T cells may expose the patient to a high risk of rejection when alemtuzumab is used pre-transplant. The presence of a population of recipient T cells showing absent expression of CD52 would prompt the use of ATG instead of alemtuzumab, although there is no evidence base to define the size of such a population. In our clinical practice, we have used an arbitrary cut off of >10 % CD52-deficient T cells.

Early studies using CAMPATH-1 antibodies in SAA HSCT

The early single centre studies using CAMPATH-1G in the conditioning regimen for MSD HSCT for SAA showed graft failure rates of 24 %. The high graft rejection was associated with the initial use of alemtuzumab given both pre- and peri-transplantation in a cohort of 21 patients, in an attempt to achieve maximum GVHD prophylaxis [26]. However, subsequent patients receiving alemtuzumab only pre-transplant showed a reduction in graft rejection to 10–15 %, while maintaining a low incidence of GVHD [27]. Patients had either transient mixed chimerism or persistence of recipient (<20 %) cells. It was noted that the use of Campath-1G was associated with a high incidence of mixed chimerism which tips the balance away from GVHD. Interestingly, the high survival of early patients with graft failure was in part due to a high incidence of autologous recovery, seen in about 50 % of patients [26].

With the subsequent availability of the humanized form of CAMPATH-1, alemtuzumab, a small single centre study reported stable engraftment and a favourable impact on the incidence and severity of GVHD in patients receiving MUD HSCT for SAA, warranting further investigation of this drug [28].

In another single centre, observational study, the outcomes of 10 consecutive patients treated with alemtuzumab-based GVHD prophylaxis and 14 consecutive patients who received calcineurin inhibitor plus methotrexate-based GVHD prophylaxis were compared [29]. The

Table 1 Summary of studies using alemtuzumab in the conditioning regimen in HSCT for SAA

Authors	Years	N	Median age years (range)	Conditioning regimen	Graft failure	GvHD	Follow up/OS
Kanda et al. [40]	2013	15	34 (20–46)	Fludarabine 30 mg/kg/day × 4 Cyclophosphamide (CY) 25 mg/kg/day × 4 Alemtuzumab 0.16 mg/kg/day × 6 TBI 2 Gy (in the 13 MUD patients)	8.3 %	Acute Gd II–IV: 0	1-year OS: 83.3 %
Samarasinghe et al. [13]	2012	44	8.1 (3.8–19)	Fludarabine 30 mg/m ² × 5 CY total dose of 120 mg/kg over 2 days or 200 mg/kg over 4 days Alemtuzumab 0.9–1 mg/kg	0	Acute Gd I–II: 31.8 %; Gd III–IV 2.3 %; cGVHD: 6.8 %	5-year OS and FFS: 95.01 %
Marsh et al. [8]	2011	50	35 (8–62)	Fludarabine 30 mg/m ² × 4 days CY 300 mg/m ² × 4 days Alemtuzumab (median dose 60 mg)	12 %	Acute Gd I–II: 13.4 %; cGVHD: 8 %	2-year actuarial OS: 88 ± 5 % and FFS 80 ± 6 %
Novitzky et al. [30]	2013	30	19 (7–60)	Fludarabine 30 mg/m ² × 5 days CY 60 mg/kg × 2 days Alemtuzumab 1 mg/10 ¹⁰ mononuclear cells 'in the bag' (median 6.5 mg)	10 %	Acute: 0	OS at 1939 days : 100 %
Siegal et al. [29]	2008	10	40 (35–56)	Age < 40 years: CY 50 mg/kg × 4 days Alemtuzumab total dose 50 mg Age > 40 years: fludarabine 30 mg/m ² CY 10 mg/kg × 4 days or busulphan 3.2 mg/kg × 4 days ± TBI Alemtuzumab total dose 60 mg	11 %	Acute Gd I–II: 11 %; cGVHD: 0	1 year OS: 70 %
Gupta et al. [28]	2005	7	13 (8–35)	Adults: Fludarabine 30 mg/m ² × 4–5 days CY 300 mg/m ² × 4 days; Alemtuzumab 20 mg/day × 5 days Children: Fludarabine 30 mg/m ² × 4–5 days CY 20 mg/kg/day for 4 days Alemtuzumab 0.2 mg/kg/day × 5 days	0	Acute Gd I–II: 3/7 (42.8 %); cGVHD: 2/7 (28.5 %)	2 deaths (1 with adenovirus infection and 1 with cGVHD and CMV) 6 FFS at median 289 days (range 211–995)
Gupta et al. [27]	2004	33	17 (4–46)	CY 50 mg/kg × 4 days Campath-1G or Campath-1H 0.75–1 mg/kg (14 patients received Campath 1G post-transplantation as GVHD prophylaxis in the protocol)	24 %	Acute Gd II–IV: 14 %; cGVHD: 4 %	5-year OS 81 %

incidence of acute and chronic GVHD was significantly lower in alemtuzumab-treated patients as compared to conventionally treated patients. Engraftment time and rates of graft failure appeared similar in the two groups. Although more alemtuzumab-treated patients developed CMV reactivation none developed CMV disease.

An alternative approach was to use ex vivo T cell depletion by infusion of stem cell graft that has been T-depleted ex vivo with alemtuzumab, after reduced intensity conditioning with fludarabine and cyclophosphamide. In a series of 30 patients, there were no cases of acute or chronic GVHD, and all patients achieved initial engraftment, but there were 3 cases of late graft failure at 4 and 7 months, and a third following treatment of CMV with ganciclovir [30].

Multicentre, retrospective studies of alemtuzumab-based conditioning in SAA

The novel 'FCC' conditioning regimen of fludarabine (300 mg/m^2), cyclophosphamide (300 mg/m^2) from days -7 to -4 and alemtuzumab (median dose of 60 mg) given pre-transplantation from days -7 to -3 , was reported in a retrospective, multicentre study of 50 patients with acquired SAA [8]. The median age was 35 years (range 8–62), and 29 received a transplant from an UD and 21 from an MSD. All UD HSCT patients, apart from 2, were matched for HLA-A, -B, -C, -DRB1, and DQB1 using high-resolution DNA typing. Two patients were transplanted from 9/10 matched unrelated donors and received TBI 2 Gy in addition to FCC conditioning. The 2-year OS

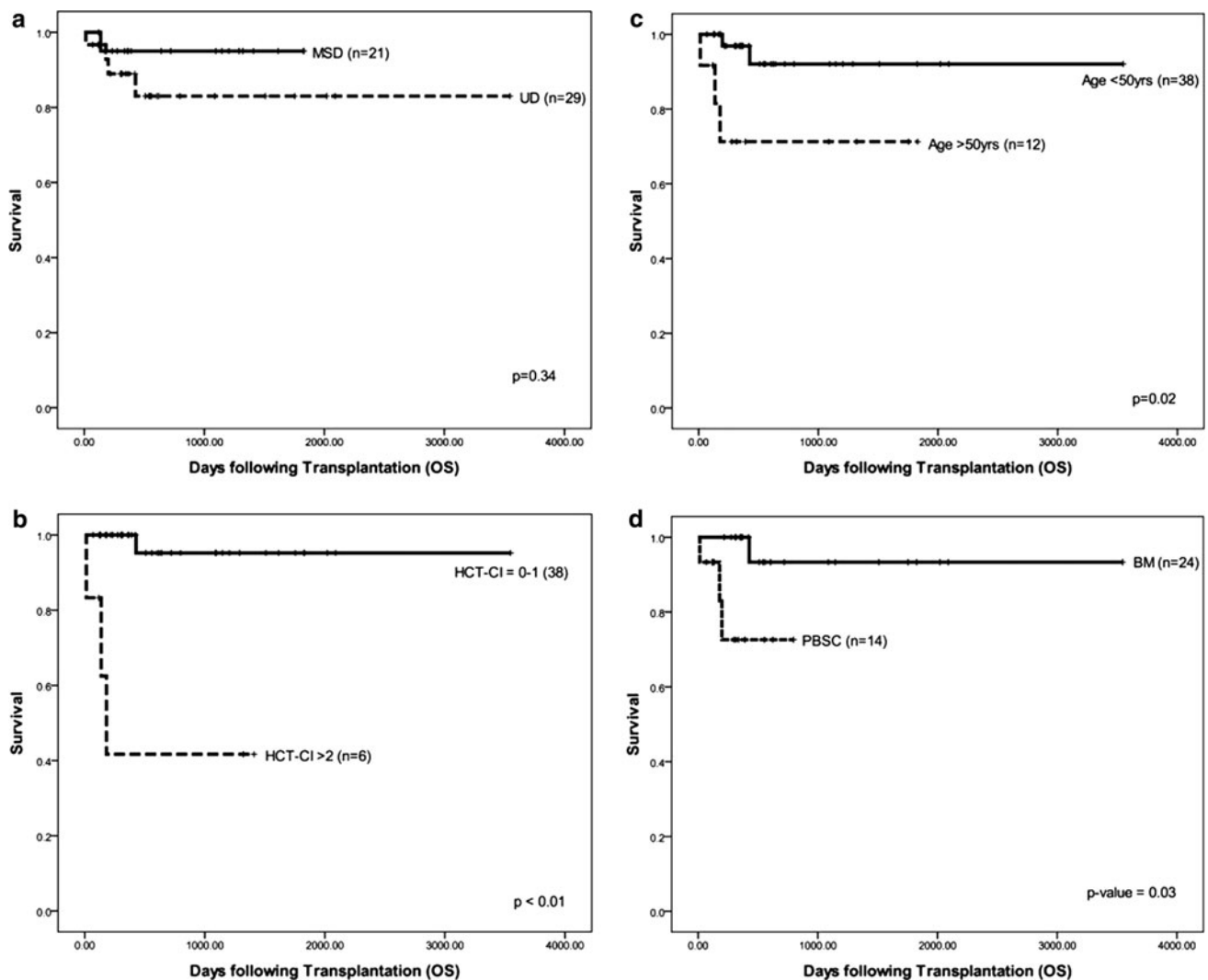


Fig. 1 Overall survival curves following HSCT using FCC conditioning, with stratification for **a** donor type, **b** HCT-CI, **c** age <50 years and **d** stem cell source. With permission of Marsh et al. [8]

was 95 % for MSD and 83 % for UD HSCT (see Fig. 1a). Cumulative incidence of graft rejection was 9.5 and 14.5 % for MSD and UD HSCT, respectively. The incidence of GVHD was very low: acute GVHD 13.7 % and chronic GVHD 4 % [8].

An important advantage of using alemtuzumab as part of conditioning for MUD HSCT in SAA, was that the addition of low dose (2 Gy) TBI to aid engraftment, was not required. In contrast, it is common practice to use TBI 2 Gy for fully matched UD HSCT for SAA with ATG-based conditioning, as a previous study reported a graft failure rate of 35 % when only fludarabine, cyclophosphamide and ATG were used in adults with SAA [9, 10].

Mixed donor chimerism, as assessed on unfractionated PB mononuclear cells, occurs frequently after HSCT for SAA [31]. Progressive mixed chimerism carries a high risk of graft rejection, but stable mixed chimerism (SMC) is associated with the absence of chronic GVHD and excellent survival. Assessment of chimerism in PB sub-populations, using the 'FCC' protocol, revealed stable mixed chimerism was frequently seen in CD3+ T cells alongside full donor myeloid (CD15) chimerism and normal haematological recovery (see Fig. 2) [8]. Stable mixed T cell chimerism was associated with the absence of chronic GVHD; persistence of mixed T cell chimerism following the withdrawal of post-graft immunosuppression with CSA, raises the likelihood that a state of tolerance is achieved post-HSCT. Further studies to define T cell sub-populations and immune reconstitution post-HSCT are indicated. For example, as shown in a recent study of reduced intensity HSCT for haematological malignancies using alemtuzumab with fludarabine and busulphan, increased numbers of effector CD4 T cells and their imbalance relative to regulatory CD4 T cells, were noted to be a signature of GVHD [32].

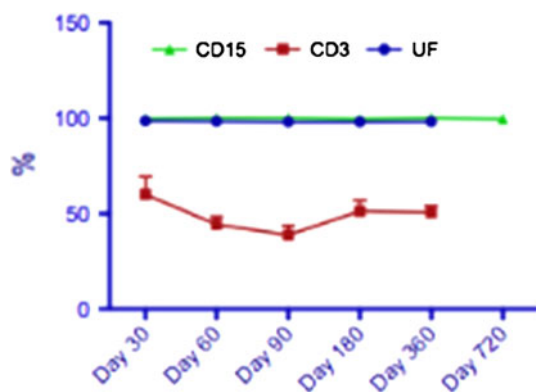


Fig. 2 Chimerism results post-HSCT using FCC conditioning. Representative median PB chimerism results shown in this figure. UF unfractionated ($n = 33$), CD15 ($n = 16$), CD3 ($n = 16$), with permission of Marsh et al. [8]

A second retrospective, multicentre study from the UK, evaluated 44 children who received MUD HSCT, of whom 40 had previously failed IST. The conditioning comprised 'FCC' with a higher dose of CY compared to the study discussed above. BM was used as the stem cell source in 59 % and PBSC in 41 %. There were no cases of graft failure and excellent overall and failure-free survival of 95 % at 5 years. Acute GVHD grades I–II occurred in 31.8 % and grades III–IV in one patient (2.3 %). Chronic GVHD was seen in only 3 patients (6.8 %), two with limited and one extensive. There was no association between stem cell source and acute or chronic GVHD [13].

A recent small, multicentre study from Japan, examined the effect of alemtuzumab dose in 15 patients with SAA, 12 from UD and 3 MSD, who also received fludarabine and CY (25 mg/kg \times 4), and 2 Gy TBI (except in the 3 MSD patients) [33]. The initial dose was 0.2 mg/kg/day \times 6 (in the first 3 patients) and the next dose was 0.16 mg/kg/day \times 6 in the next 3. The latter dose was the final dose in the rest of the patients. All patients engrafted, and there was one late graft failure. Mixed T cell chimerism of >10 % donor cells was observed in 50 % of patients. There were 2 cases of acute GVHD (both Gd I) and 2 cases of chronic GVHD. CMV reactivation occurred in all patients, but there was only one case of CMV disease. EBV reactivation occurred in 2 patients with no EBV PTLD. Two patients died (late graft failure and sepsis, respectively).

The first study to compare alemtuzumab with ATG-based conditioning regimens in HSCT for SAA has been performed by the British Society for Blood and Marrow Transplantation (BSBMT). In this retrospective, multicentre study of 159 patients, 88 were transplanted from MSD, 65 from MUD and 6 from alternative donors. Alemtuzumab was used in 103 and ATG in 55 patients. The results showed 1, 5 and 10 years OS of 89, 85 and 85 %, respectively, and there was no difference in 1-year OS between the alemtuzumab and ATG groups [34]. Subsequent analyses have shown better OS for MUD HSCT using alemtuzumab as compared to ATG and no difference for MSD. A lower risk of chronic GVHD was observed with alemtuzumab as compared to ATG (11 vs 26 %) (J. Marsh, unpublished data 2013). Prospective studies comparing alemtuzumab and ATG-based conditioning are now indicated.

The impact of stem cell dose and source

A low stem cell dose is associated with an increased risk of graft failure in HSCT for SAA, as initially based on the PB nucleated cell counts [35]. Evaluation of infused BM CD34+ cell dose showed that the risk of graft failure increased significantly with doses of $<2 \times 10^6$ /kg [36].

Thus, it is important to ensure an adequate stem cell dose, although data are lacking in terms of the optimal number of CD3+ cells that is not associated with an increase in the risk of GVHD.

The source of stem cells for SAA HSCT is also important, as studies reported to date, have shown that for ATG-based conditioning regimens, BM is the preferred source as compared to PBSC; the use of PBSC results in a higher incidence of chronic GVHD and worse OS [6, 37, 38]. One of the concerns for MUD HSCT has been the poor quality of bone marrow harvests in some instances, resulting in low stem cell dose harvested. The use of PBSC can enable a higher and at least adequate cell dose in most cases. Using alemtuzumab-based conditioning, the use of PBSC enables an adequate stem cell collection without increasing the risk of chronic GVHD; in that study, there was worse 2-year OS with PBSC as compared to BM, but no difference in failure-free survival [8]. However, the study was too small to compare the results of MSD with MUD HSCT in terms of stem cell source, and further studies on larger number of patients are required.

Infections

There are concerns about the risk of viral infections associated with the use of alemtuzumab. Some studies have reported a higher incidence of viral infections, including CMV and EBV, following the use of alemtuzumab-based conditioning regimens, when compared with ATG and methotrexate [39, 40]. However, no increased incidence of CMV, EBV or adenovirus infection or reactivation was reported in the retrospective multicentre adult and pediatric studies reported above [8, 13]. Nevertheless, a regular surveillance program for these viruses should be employed post-HSCT.

Outcomes in older patients

HSCT for SAA is less successful in older patients. Using conventional high dose CY and ATG conditioning regimen for MSD HSCT, OS falls to 50 % for people aged 40–50 years [11, 12]. A higher incidence of GVHD and organ toxicity may contribute to worse survival outcomes in older patients. Using the FCC protocol (see Fig. 1c), in the small sub-group of patients aged >50 years (range 50–62), 2-year OS was 71 % [8]. Thus, the low toxicity of the regimen, may allow older patients, who have previously failed IST treatment, to be considered for HSCT, although larger studies are indicated to explore this further. This study also reported for the first time, the importance of

assessing co-morbidities pre-transplant (see Fig. 1b), and this should now be evaluated further in the selection of older patients for HSCT.

Conclusions

Alemtuzumab in combination with fludarabine and cyclophosphamide (FCC) HSCT for acquired AA is associated with a very low incidence of chronic GvHD and excellent OS, without undue increase in graft failure and infections. Alemtuzumab-based reduced intensity conditioning regimens may be considered in older patients >50 years. Prospective randomized trials comparing alemtuzumab and ATG-based conditioning are needed both in matched sibling and unrelated donor setting to confirm the retrospective data and to better understand the potential state of tolerance post-HSCT seen with alemtuzumab.

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