Original Article

Long-term immune reconstitution following anti-CD52-treated or anti-CD34-treated haematopoietic stem cell transplant for severe T lymphocyte immunodeficiency.

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Re-submitted; 19-10-2007
Presented in abstract form at the 33rd Annual Meeting of the European Group for Blood and
Marrow Transplantation, Lyon, France, March 2007
MA Slatter and AR Gennery received a small travel bursary to attend the 33rd Annual Meeting of the European Group for Blood and Marrow Transplantation, Lyon, France, March 2007 from Miltenyi Biotec (Gladbach, Germany)

Word count: 3231
Abstract

**Background:** Results of treatment of severe T Lymphocyte immunodeficiencies by haematopoietic stem cell transplantation (HSCT) have improved. T cell depleted (TCD) haplo-identical transplants are successful if there is no HLA-identical donor. Methods to remove T lymphocytes include addition of anti-CD52 antibodies and CD34+ positive HSC-selection.

**Objective:** Assessment of long-term immune function is important following these treatments. We looked at immune reconstitution in 36 survivors > 2 years following HSCT for severe T Lymphocyte immunodeficiencies and compared engraftment quality between the two T lymphocyte depletion methods.

**Methods:** Chimerism, T and B lymphocyte subsets immunoglobulin level and specific antibody production at last follow up, were examined. The $\chi^2$ (Fishers exact test) and Wilcoxon rank sum analyses were used to compare the groups.

**Results:** Nineteen patients received anti-CD52-treated and 19 anti-CD34-treated HSC. More anti-CD52-treated patients had full donor myeloid chimerism ($p=0.025$). All patients developed full donor T lymphocyte chimerism. There was no difference in donor B lymphocyte chimerism, but significantly more anti-CD52-treated patients had class switched memory B lymphocytes ($p=0.024$), normal IgG levels and normal responses to tetanus and Haemophilus influenzae type B vaccination. More anti-CD52-treated patients with common gamma chain or JAK3 SCID had donor B lymphocytes.

**Conclusion:** Long-term T lymphocyte function is good with either treatment method, with low incidence of graft versus host disease. The results imply more incomplete donor chimerism in anti-CD34-treated patients with less B lymphocyte function.

**Clinical implications:** Retention of cells other than HSC in the manipulated marrow may result in more complete engraftment and immune function.
Capsule summary:
We show good long-term immune function to 19 years post-transplant following T lymphocyte-depleted marrow transplantation for severe T lymphocyte immunodeficiency: results suggest T lymphocyte-depletion method may influence establishment of donor chimerism.

Key words: Severe combined immunodeficiency, haematopoietic stem cell transplantation, T cell depletion, CD34 positive stem cell selection, immune reconstitution

Abbreviations
HSCT, haematopoietic stem cell transplantation
HSC, haematopoietic stem cell
GvHD, graft versus host disease
Hib, Haemophilus influenzae type B
TEE, Thymic emigrant equivalent
FISH, fluorescent in-situ hybridisation
CγC, common gamma chain
IL7Rα, interleukin 7 receptor alpha
JAK3, janus-associated kinase 3
SCID, severe combined immunodeficiency
Introduction

Severe combined immunodeficiencies are inherited genetic defects in T lymphocyte differentiation with or without defects in other lymphoid (B and natural killer cell) or myeloid lineages [1]. Conventional treatment for these disorders is haematopoietic stem cell transplantation (HSCT).

Improvements in tissue typing, advances in stem cell manipulation, new chemotherapy conditioning regimens as well as new methods for detecting and treating viral and fungal infection have led to improving survival and cure over the past two decades [2].

For many patients without an HLA identical relative or unrelated donor the use of T lymphocyte depleted haematopoietic stem cells (HSC) from an HLA mismatched donor has been successful with survival rates of 75-85% [3,4]. A number of methods have been employed to remove viable T lymphocytes from the graft including the use of E-rosette lectin depletion and in vitro CAMPATH-1M anti-lymphocyte antibodies (anti-CD52). Since the late 1990s European centres performing T lymphocyte depleted HSCT for patients with primary immunodeficiency have used CD34+ positive stem cell selection rather than T lymphocyte depletion. The most commonly used method, the Miltenyi CliniMACS® system, uses an organic iron bead attached to an anti-CD34 antibody to isolate purified CD34+ HSC from the other cells, by passing the HSC source through a magnetic column [5]. The purified CD34+ HSC fraction is infused into the patient. Using this method, 4 log depletions of T lymphocyte numbers can be achieved.

There are important differences between CD34+ positive stem cell selected and T lymphocyte depleted bone marrow. Whilst the residual T lymphocyte count in anti-CD52-treated marrow may be relatively high, very few of the T lymphocytes remain viable as they are still coated with anti-CD52 when infused into the recipient and are then destroyed by complement-mediated lysis. In the anti-CD34-treated HSC product, although the number of T lymphocytes infused into the patient may be very low, those that are infused are viable and could cause graft versus host disease
(GvHD). Additionally the anti-CD52-treated product will contain component blood cell precursors and cells already in early differentiation from the stem cell, as well as other stromal factors that may aid engraftment of HSC into the bone marrow space [6] thus achieving “better” engraftment.

We have previously published the results of the long term follow up of 19 children who received anti-CD52-treated marrow, transplanted between 1987-1998 [7]. It was subsequently noted that since changing to CD34+ positive stem cell selection of marrow, more patients failed to develop B lymphocyte function. Results of long-term follow up of all these patients are presented, particularly comparing the quality of engraftment in patients receiving anti-CD52-treated HSC with those given CD34+ selected HSC.
Methods

A retrospective review was performed of all patients undergoing HSCT for severe T Lymphocyte immunodeficiencies who had survived > 2 years treated at our centre with either anti-CD52-treated or anti-CD34-treated HSCT. Anti-CD52- and anti-CD34- treatment was performed on whole, freshly harvested marrow as previously described [5, 8].

The following parameters were analysed: myeloid chimerism, T lymphocyte number and chimerism, CD3+/CD4+/CD45RA+/CD27+ and CD3+/CD4-/CD45RA+/CD27+ (naïve) T lymphocyte numbers, B lymphocyte number and chimerism, memory B lymphocyte (CD19+/CD27+/IgM+) and class switched memory B lymphocyte (CD19+/CD27+/IgM-) number, and serum IgG, IgA and IgM level, as well as specific antibody levels to tetanus toxoid, *Haemophilus influenzae* type B (Hib) and pneumococcal polysaccharide antigens following vaccination.

Lymphocyte subset analysis was measured by 4 colour flow cytometry as previously described [9]. Briefly, lymphocyte surface marker studies were performed on fresh whole blood collected in EDTA, using appropriate markers (CD45 PerCP, CD3 FITC, CD4 APC, CD8 PE, CD19 APC, CD16/CD56 PE, CD3 PerCP/CD4 APC/CD45RA FITC/CD27 PE, CD19 PerCP/CD27 FITC/IgM APC/IgD PE (Becton Dickinson, UK Ltd, Oxford)), and analysed on a Becton Dickinson FACSCalibur flow cytometer. The T and B lymphocyte numbers were defined as normal or low using age-specific reference ranges. Class-switched memory B lymphocytes were defined as <1% or > 1% of the B cell population. The markers CD3+/CD4+/CD45RA+/CD27+ and CD3+/CD4-/CD45RA+/CD27+ were used as surrogates for recent thymic emigrant T lymphocytes (thymic emigrant equivalent TEE) [10]. Briefly, the CD3 subset was gated and CD4+/CD45RA^BRIGHT^+/CD27+ lymphocytes identified. It was assumed that the majority of CD4-lymphocytes in this population were CD8+. By identifying CD4-CD45RA+CD27+ lymphocytes,
effector cells (which are CD45RA+CD27-), were excluded. We have previously demonstrated that
the CD45RA^{BRIGHT}+/CD27+ lymphocyte population correlates with CD45RO- lymphocytes
(unpublished observations). Lymphocytes were described as present or absent. Lymphocyte
responses to stimulation with phytohaemagglutinin were performed using standard tritiated
thymidine uptake methods and measured as a percentage of the response of a healthy adult control
[7]. Immunoglobulin concentrations were measured by rate nephelometry, and specific antibody
responses to tetanus toxoid, Haemophilus influenzae type B conjugate vaccine and pneumococcal
polysaccharide vaccine antigens were measured by Enzyme-Linked ImmunoSorbent Assay [11].
Immunoglobulin levels were defined as normal or low using age-specific reference ranges and
specific antibody levels were defined as present or absent after vaccination.

Cells were separated into separate lineages prior to measuring chimerism. Whole blood was stained
with CD3, CD19 or CD15 micro beads and cell lines were separated using an autoMACS®
automated bench-top magnetic cell sorter (Miltenyi Biotec Ltd, Surrey, UK). Chimerism was
measured in sex-mismatched cases by XY-fluorescent in-situ hybridisation (FISH) using standard
cytogenetic techniques. Briefly, interphase FISH was performed using a Vysis 2-colour CEP
X/CEP Y probe set according to the manufacturers protocol. Where donor and recipient were same
sex, chimerism was measured by short tandem repeat marker analysis of genomic DNA, as
previously described [12] – sensitivity was accurate to within 5%. For the purposes of analysis,
chimerism was defined as donor, mixed or recipient in specific cell lineages.

Patients, when given cytoreductive chemotherapy, were treated in accordance with the European
Group for Blood and Marrow Transplantation/European Society for Immunodeficiencies Inborn
Errors Working Party treatment guidelines current at time of transplantation. No patients received
radiotherapy. Nucleated cell doses in the stem cell products were compared between groups, as
CD34+ cell doses were not available for many of the earliest patients who received anti-CD52-
treated marrow.

The $\chi^2$ (Fishers exact test) and non-parametric Wilcoxon rank sum analyses were used to detect
statistical differences between the groups (GB-Stat PPCworks 6.5.4, Dynamic Microsystems Inc,
Silver Spring, MD) – a 2 sided $p$ value of $\leq 0.05$ was considered to be significant.

Patient Characteristics

From May 1987 until the end of August 2004, 72 patients with severe T Lymphocyte
immunodeficiencies were transplanted with 57 (79%) survivors; 47 patients underwent T
lymphocyte depleted HSCT, of whom 36 survive (77%). Prior to 1999, marrow from HLA-
mismatched donors was depleted in vitro with anti-CD52 antibody (27 patients). Subsequently
marrow has been CD34+ stem cell selected as described above (22 patients). All patients surviving
> 2 years from transplantation for severe T Lymphocyte immunodeficiencies, who were still alive at
the time of the study, were included.
Results

Patient characteristics are detailed (Table 1); there were 19 long term survivors in each group. Two patients (12, 13) in the anti-CD52 group were re-transplanted using CD34+ HSC. There were 8 deaths in the anti-CD52-treated group (3 fungal infection, 3 parainfluenzae pneumonitis, 1 cytomegalovirus pneumonitis, 1 idiopathic pneumonitis; median time at death 2.5 months post-HSCT, range 5 days – 7 months), and 3 in the anti-CD34-treated group (1 hepatic veno-occlusive disease, 1 Epstein Barr viral infection, 1 adenovirus infection; time at death 3 days – 1 month post-HSCT). There were no deaths associated with GvHD in either group. Of the survivors, 10 had common gamma chain deficient (CγC) severe combined immunodeficiency (SCID), 8 had T-B+NK+ SCID (3 confirmed interleukin 7 receptor alpha chain (IL7Rα) defects), 8 had recombinase activating gene 2 or artemis deficiency, 3 had adenosine deaminase SCID, 3 had janus-associated kinase (JAK) 3 deficiency, 2 had zeta-associated protein 70 kinase deficiency and 4 had other phenotypes. There was no significant difference in the number of patients in each group who received fully myeloablative cytoreductive chemotherapy with busulphan 16mg/kg and cyclophosphamide 200mg/kg.

There was no significant difference in age at transplantation between the groups (anti-CD52-treated group median 7 months, range 1.25-17 months; anti-CD34-treated group median 6 months, range 1-25 months). The anti-CD52-treated group had significantly longer follow up than the anti-CD34-treated group (median 12.83 years, range 1.17 – 18.92 years versus median 5.92 years, range 2.67 – 8.17 years, p <0.0001). The anti-CD52-treated group received significantly higher nucleated cell doses (median 5 x10⁷/kg range 0.4 – 525 x10⁷/kg versus median 0.13 x10⁷/kg, range 0.0114 – 0.57 x10⁷/kg, p < 0.0001) and T lymphocyte doses (median 225 x10⁷/kg range 0 – 6690 x10⁷/kg versus median 15 x10⁷/kg, range 2.2 – 180 x10⁷/kg, p = 0.0001) respectively. Three patients in each group received a ‘boost’ infusion of T lymphocyte depleted HSC from the original donor, without further chemotherapy, for incomplete chimerism [13]. There was no significant difference in survival
between the groups (70% vs 86%, p = 0.19) and no late deaths (> 12 months post-HSCT) in either
group. Only four patients in the anti-CD52-treated HSC group, and 1 in the anti-CD34-treated HSC
group had grade I acute GvHD of the skin, and one patient in each group had mild localised chronic
GvHD of the skin.

Chimerism and Immune Reconstitution

There was no significant difference in the number of patients who achieved some donor myeloid
chimerism between the two groups (Table 2). However, significantly more patients who received
anti-CD52-treated HSC had full donor myeloid chimerism (7 vs 1, p=0.025) (figure 1). More anti-
CD34-treated HSC patients with some donor myeloid chimerism received a fully myeloablative
dose of busulphan (16mg/kg) than anti-CD52-treated HSC patients (8 vs 2, p=0.03). All patients in
both groups developed full donor chimerism in the T lymphocyte lineage. Following immune
reconstitution, all anti-CD34-treated HSC patients had TEE cells compared to 15/19 in the anti-
CD52-treated HSC group. Neither the B lymphocyte number, nor the number of patients with at
least some donor B lymphocyte chimerism at last follow up was significantly different between the
two groups. However, more patients who had received anti-CD52-treated HSC had class switched
memory B lymphocytes (15 vs 9, p=0.024). There was no difference between the groups in the
number of patients who had normal levels of IgM or IgA at latest follow up. However, significantly
more patients who had received anti-CD52-treated HSC had normal IgG levels (16 vs 10, p =
0.036). Correlating with this, at time of last follow-up, more patients in the anti-CD52-treated HSC
group had achieved a sustained response to tetanus toxoid vaccination (16 vs 10, p=0.036) and to
Hib conjugate vaccine (16 vs 9, p= 0.019). There was no difference between groups in patients
mounting a response to pneumococcal polysaccharide antigen. More patients in the anti-CD34-
treated HSC group had CγC/JAK3 deficient SCID (4 vs 9, p=0.09). None of these patients with
CγC/JAK3 deficient SCID in either group received busulphan 16mg/kg, and there was no difference
in the number of these patients receiving no conditioning compared to busulphan 8mg/kg. The
numbers of CγC/JAK3 SCID patients were too small to make confident comparisons, but
significantly more CγC/JAK3 SCID patients had donor B lymphocytes in the anti-CD52-treated
HSC group (3/4 vs 2/9, p=0.014).

Complications

Four patients developed a single significant invasive infection > 2 years following transplantation.

Four developed planar warts, only 2 of whom had CγC or JAK3-deficient SCID – in no patient
were they severe or persistent. Four developed autoimmunity, 2 of whom had autoimmune thyroid
disease (Table 1). There was no late GvHD, except for patient 13 who had chronic skin GvHD after
the first transplant. Six patients had mild to moderate behaviour-related problems, only one of
whom had adenosine deaminase SCID.
This study adds to those previously reporting long term follow up of SCID patients receiving T lymphocyte depleted HSCT [14, 15], but is the first to compare quality of immune reconstitution after anti-CD52- or anti-CD34-treated HSCT in severe T lymphocyte disorders. Our overall survival of 77% compares favourably with other reported series [4, 16]. Overall survival was better in the anti-CD34-treated HSC group, but there was no significant difference between the two groups. This improvement over time is likely to reflect improvement in transplant care, including more accurate diagnoses, earlier detection of viral and fungal infections by PCR, with new, more effective treatments, and better supportive care, as has been demonstrated in other studies [2], rather than differences in the method of marrow manipulation. Whilst follow up was longer in patients receiving anti-CD52-treated HSC, no deaths occurred beyond 12 months in either group.

The T lymphocyte depletion method employed to manipulate the graft does not appear to affect T lymphocyte chimerism. Interestingly, there were more patients in the anti-CD34-treated HSC group who had TEE T lymphocytes. A recent study has demonstrated that TEE lymphocytes may be found in the absence of myeloid engraftment, particularly in patients with CγC/JAK3 SCID [14], of which there were more in our anti-CD34-treated HSC group: our study appears to support this observation. We did not have data comparing the speed of T lymphocyte immune reconstitution between the two marrow manipulation methods, particularly with appearance of TEE T lymphocytes. However, previous studies have indicated that the appearance of recent thymic emigrants is similar for patients who received replete or TCD HSCT [17].

Although, overall, there was no statistical difference in B lymphocyte donor chimerism between the two groups, there was a trend to more complete B lymphocyte donor chimerism in the anti-CD52-treated HSC group. Over a longer period of time, humoral function may improve so that those transplanted longest have better antibody responses. It was not possible to investigate this further, as
the early data in the anti-CD52-treated HSC patients was incomplete. However, all but 3 patients (8, 12, 13) in the anti-CD52-treated group had discontinued immunoglobulin replacement by 4 years post HSCT [7]. It therefore seems unlikely that many of our patients who received anti-CD34-treated HSC, but remain on immunoglobulin treatment, will develop humoral immunity in the future. More likely, antibody function post-transplant may depend on the type of SCID and the chimeric state [18]. Recipient B lymphocytes in IL7Rα deficient SCID are functionally normal, whereas those from CγC or JAK3 deficient SCID are intrinsically defective [19]. Therefore, unless donor B lymphocyte chimera is achieved in patients with CγC or JAK3 deficient SCID, normal humoral function is unlikely as interaction with donor T lymphocytes does not result in immunoglobulin class switching and antigen-specific IgG production in recipient B lymphocytes [20]. Our results are difficult to interpret, as more patients in the anti-CD34-treated HSC group had CγC/JAK3 SCID with recipient B lymphocytes. There were more patients with CγC/JAK3 SCID who achieved donor B lymphocyte chimerism in the anti-CD52-treated HSC group, despite the fact that there was no difference in the conditioning regimens employed for the CγC/JAK3 deficient patients, and none received full myeloablative chemotherapy. Patient 1, with CγC deficiency has normal humoral immunity, apparently with recipient B lymphocytes — it may be that the methods of detecting donor chimerism were not sensitive enough to detect very low levels of chimerism (<5%), or that there is a significant population of donor B lymphocytes in lymphoid tissue. More sensitive methods of detecting microchimerism may detect donor cells in this patient [19]. Patient 8, with IL7Rα deficiency does not make immunoglobulin, although donor T lymphocytes would be expected to interact with recipient B lymphocytes. In this patient, T lymphocyte engraftment is poor, with few TEE and a restricted T lymphocyte repertoire (data not shown); there may be too few T and B lymphocyte interactions for effective humoral immunity. Patient 14, with Artemis deficiency has complete donor B lymphocyte engraftment with recipient myeloid engraftment. Donor early B lymphocyte pre-cursors may have a competitive advantage over recipient B
lymphocyte pre-cursors which are blocked at an early stage in B lymphocyte development, enabling chimerism of donor mature B lymphocytes.

There is an impression of better donor stem cell engraftment with overall more complete donor myeloid and B lymphocyte engraftment in patients who received anti-CD52-treated HSC. This implies superior stem cell engraftment and perhaps better long-term immune function; a continuing longitudinal study is needed to see if there is a greater waning of immune function in the anti-CD34-treated HSC group compared to the anti-CD52-treated HSC group. This observation is perhaps surprising in that fewer patients in the anti-CD52-treated HSC group received full myeloablative conditioning with busulphan 16mg/kg, which is more likely to result in myeloid engraftment. The depletion of most additional marrow cells, other than CD34+ HSC may have unintended drawbacks for engraftment, as some of the other cellular elements may aid marrow engraftment of stem cells. Other methods of marrow manipulation such as CD3/CD19 depletion may result in decreased GvHD risk, without compromising engraftment [21].

As new information appears, the determinants of successful outcome of transplantation for SCID are becoming clearer. Long-term T lymphocyte immunity appears to require early, sustained thymic output of donor T lymphocytes [14-16, 22, 23]. There is growing evidence of a correlation between myeloid chimerism, TREC\textsuperscript{s} and naïve T lymphocytes, although that may be less important for patients with \textgamma C/JAK3 SCID [14, 16, 17]. Further very long-term studies are required to confirm these observations, but if there is no good evidence of newly emerging thymic T lymphocytes early post-HSCT, then long-term T lymphocyte engraftment and function is likely to be poor [16]. In these instances, early intervention with further therapeutic measures is more likely to be successful, before thymic atrophy is established [22, 23]. As for B lymphocyte function, our study shows the correlation between class switched memory B lymphocytes and antigen-specific IgG production, and these markers should be considered an indication of long-term B lymphocyte function. The
advantages and disadvantages of chemotherapy continue to be debated [1, 4,19]. The role of cytoreductive chemotherapy in achieving long term immune reconstitution is becoming more clear [24], and is confirmed in our study. It is of note that all patients who require continued immunoglobulin replacement received a less myeloablative dose of busulphan 8mg/kg. Whilst the short-term risks of gene therapy, particularly for CγC SCID have been recognised [25], it will be important to compare long-term outcomes for HSCT and gene therapy. In particular, comparison between conditioned and non-conditioned HSCT with gene therapy will be required, and the molecular defect for which treatment has been given, as well as the method of HSC manipulation should be considered as our data suggest this may effect the outcome.

Autoimmunity was seen in 4 of our patients and has been previously reported, as has late occurring infection [15, 24, 26]. The mechanism of autoimmunity in this setting is unclear, presumably multifactorial, but there may be a role for subsets of T lymphocytes [27]. Other than a requirement for continuing immunoglobulin replacement, no other significant long term sequelae were observed.

Whilst in the first years following TCD HSCT as treatment for severe T lymphocyte deficiency, long-term survival was the aim of successful treatment, quality of engraftment, long-term immunoreconstitution and quality of life are now as important [1, 16, 22, 23]. Our results confirm that mismatched T lymphocyte depleted HSCT for severe T lymphocyte immunodeficiencies remains a successful technique with 77% overall survival, and good T lymphocyte function, and 71% with good humoral immunity. Both techniques of marrow manipulation seem effective, and vastly reduce the risk of GvHD. There is an impression that the B lymphocyte function may be better after anti-CD52-treated HSC but further studies with more patients are needed to ascertain whether T lymphocyte depletion rather than haematopoietic stem cell selection results in more complete immune function, and in particular, in better B lymphocyte function.
References


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Figure 1 Number of Patients with Recipient, Mixed or Donor Chimerism in Myeloid and B Lymphocyte Lineages who received anti-CD52-treated or anti-CD34-treated haematopoietic stem cells.