Early determinants of long-term T-cell reconstitution after hematopoietic stem cell transplantation for severe combined immunodeficiency

Running title: T-cell reconstitution after HSCT for SCID

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**Authors’ contributions**

Laboratory experiments were performed by Mette Hazenberg, Helene Roelofs and Sigrid Otto. Robbert Bredius and Jaak Vossen were responsible for patient care. Routine clinical laboratory data were produced under supervision of Maarten van Tol, and data management was performed by Els Jol-van der Zijde and Jeroen Heidt. Taco Kuijpers collected material from healthy controls. José Borghans and Robbert Bredius undertook the biostatistical analyses. Jaak Vossen, Robbert Bredius and Maarten van Tol were responsible for the general design of the study, and Mette Hazenberg, Frank Miedema, Helene Roelofs and Willem Fibbe for the design of the specific parts on TRECsl and telomere lengths, respectively. Together with José Borghans these authors were involved in the interpretation of the results and general outline of the paper. José Borghans and Maarten van Tol wrote the article.
Abstract

The immune system of patients with severe combined immunodeficiency (SCID) reconstitutes to a large extent during the first years post-hematopoietic stem-cell transplantation (HSCT). It was suggested, however, that accelerated loss of thymus output may cause impaired immune function at the long term. To address this issue, we studied SCID patients who underwent allogeneic HSCT 5 to 32 years earlier, and identified early determinants of long-term T-cell reconstitution. A variety of immune parameters were analyzed both early (1-4 years) and late (5-32 years) after HSCT. Late after HSCT, a clear distinction could be made between a group of 8 patients with impaired T-cell reconstitution and 11 patients with good immune reconstitution. Importantly, in patients with decreased long-term T-cell reconstitution, T-cell recovery was already poor early after HSCT, demonstrating that long-term immune failure was not caused by accelerated loss of thymus output or long-term graft failure, but resulted from poor early grafting. The number of T-cell receptor excision circles (TRECs) early after HSCT was most predictive for long-term T-cell reconstitution. Frequent monitoring of T-cell immunity and TREC numbers early after HSCT may thus serve to timely identify patients who will fail to reconstitute properly and who may need additional treatment.
Introduction

Severe combined immunodeficiency (SCID) is a group of inherited disorders characterized by severely impaired cellular and humoral immunity, which causes an increased risk of persistent opportunistic infections and is generally fatal at very early age if untreated.\textsuperscript{1,2} Since 1968 SCID patients have been successfully treated using allogeneic hematopoietic stem cell transplantation (HSCT).\textsuperscript{3,4} Ideally, stem cells are obtained from HLA-identical siblings, giving a current 3-year survival rate of approximately 85\%.\textsuperscript{5} HLA-haplo-identical parental donors are used with increasing success.\textsuperscript{5,6} Several factors, including the lack or proper prevention of graft-versus-host disease (GvHD),\textsuperscript{7} types of SCID in which B cells are present,\textsuperscript{8} and transplantation in the neonatal period\textsuperscript{9} are associated with better outcome after HSCT. Moreover, T-cell reconstitution in the first 6 months after HSCT was found to be strongly associated with survival.\textsuperscript{7}

Generally, T-cell immunity restores within about a year post-HSCT, while B-cell immunity restores more slowly.\textsuperscript{7,10-12} It remains unclear, however, whether the various T-cell subsets in SCID patients normalize similarly within the first years post-HSCT when compared to healthy age-matched controls. Reconstitution of naive T cells in SCID patients was found to coincide with normalization of the size of the thymus.\textsuperscript{9,13} Due to thymus-dependent T-cell generation, not only naive T-cell counts but also T-cell diversity increase during the first year post-HSCT.\textsuperscript{14}

Despite considerable T-cell reconstitution during the first years after HSCT in the majority of SCID patients, it has been suggested that T-cell immunity may be impaired later in life because of long-term graft failure or accelerated loss of thymus output.\textsuperscript{14,15} Until now only few studies of long-term T-cell reconstitution after HSCT for SCID have been performed, and longitudinal data are scarce. Buckley et al.\textsuperscript{6} reported normal T-cell responses to mitogens and normal total T-cell counts in a cross-sectional study of 89 SCID patients who had been treated with HLA-matched or haplo-identical HSCT up to 17 (median 5.6) years earlier. However, Patel et al.\textsuperscript{15} found that the average number of naive T cells and
the level of T-cell proliferation in response to mitogens decreased with time post-transplantation in a group of 83 SCID patients who were investigated up to 14 years post-transplantation. To study whether these changes could be due to an accelerated loss of thymus output, T-cell receptor excision circles (TRECs) were measured. TRECs are extra-chromosomal DNA circles, which are formed during T-cell receptor rearrangement in the thymus and are not replicated upon T-cell division. TRECs were investigated in 51 SCID patients of whom 16 reached a follow-up of at least 5 years post-HSCT and 3 were evaluated longitudinally. Because the average TREC content of peripheral blood mononuclear cells (PBMC) in SCID patients after HSCT declined faster than the typical age-related TREC decline in healthy controls, it was proposed that the thymus of SCID patients may not be capable of sustaining sufficient output, possibly causing long-term infectious problems.\textsuperscript{15}

Recent clinical follow-up of long-term survivors after HSCT for SCID in our transplant unit has suggested an increased incidence of infections and autoimmune disorders late after HSCT. We, therefore, set out to study T-cell immunity longitudinally in all SCID patients who were transplanted in our clinic between 5 and 32 years earlier, to establish whether T-cell immunity or thymus-dependent T-cell lymphopoiesis showed an accelerated decrease with age. By analyzing blood samples that were collected early (between 1 and 4 years) and late (between 5 and 32 years) after HSCT, and by comparing the data with age-matched healthy control values, we were able to define early determinants of long-term T-cell reconstitution.
Methods

Cohort of SCID patients
Between 1968 and 1997 35 SCID patients were treated with allogeneic HSCT at the Pediatric Transplant Unit of Leiden University Medical Center (LUMC); 21 of them were alive 5 years post-HSCT. Nineteen of these 21 patients and their parents could be contacted and all were willing to participate in a study to evaluate their current state of acquired immunity. The cohort is heterogeneous with respect to the type of SCID, the graft donor, the manipulation of the bone marrow (BM) graft, conditioning, the occurrence and grade of GvHD, and GvHD prophylaxis (see Table 1). Three patients showed a failure of engraftment and were retransplanted 4-6 months after the first attempt. The date of second HSCT was considered as the starting point of follow-up in these cases. Total follow-up ranged from 5 to 32 (median 12) years post-HSCT. Informed consent was obtained from all participants or their parents. The Review Board for medical ethics of the LUMC approved the study.

Samples
PBMC from SCID patients and their donors were analyzed at two time points: a late time point during follow-up, between 5 and 32 (median 12) years post-HSCT (late FU) and an early time point between 1 and 4 (median 1.5) years post-HSCT (early FU). As controls, blood samples were taken from healthy Dutch adults (n=10), children who were visiting the Academic Medical Center outpatient clinic for various conditions that were not related to immunopathologic or infectious diseases (n=26), or HSC donors at the Pediatric Transplant Unit of the LUMC (n=137). Viable PBMC were stored in liquid nitrogen until analysis.

Peripheral blood lymphocyte (sub)populations and T-cell proliferation
Naive (CD45RA-) and memory (CD45RA+) T-cell counts within the CD3+CD4+ and CD3+CD8+ T-cell subsets, as well as CD19+/CD20+ B cell counts and CD3+CD16/56+ NK cell counts were determined by flow cytometry as described previously. T-cell proliferation rates were measured ex vivo by flow
cytometric analysis of Ki-67 nuclear antigen expression in naive and total CD4+ and CD8+ T cells, as described previously.18 Proliferative responses of PBMC to the T-cell mitogen phytohemagglutinin (PHA, 5 μg/ml) were measured by ³H-thymidine incorporation. ³H-thymidine was added to the cultures after 3 days of stimulation, and its incorporation was analyzed 16 hours later. Chimerism of the different cell lineages was determined by XY-fluorescence in situ hybridization (FISH) or by polymerase chain reaction (PCR) amplification of short tandem repeats as previously described.19,20

**TRECs and telomere lengths**

DNA was purified from CD4+ or CD8+ T cells using the QIAamp Blood Kit according to manufacturer's instructions (Qiagen, Hilden, Germany). Signal joint TREC content of these fractions was quantified using real-time PCR as described previously.21 TREC content was expressed as the number of TREC copies per CD4+ or CD8+ T cell, assuming that 1 μg DNA represents 150 000 T cells. Telomere length was determined in naive (CD45RA+) and memory (CD45RA-) CD4+ T cells by a combination of immunostaining and flow-FISH analysis.22

**Statistical analysis**

Immune parameter differences between the two groups were analyzed using the Mann-Whitney U test. The proportion of the different SCID phenotypes, conditioning and levels of chimerism in the two groups were compared using the Student’s t-test, using pooled variances if there was no significant difference between the variances of the two groups (based on Levene’s Test for equality of variances). P-values <0.05 were considered significant.
Results

T-cell reconstitution long term post-HSCT

The median number of naive CD4$^+$ T-cells in SCID patients long-term (5-32, median 12 years) post-HSCT was found to be lower than that of age-matched healthy controls, as was reported previously. However, close analysis of the data revealed a clear distinction between a group of 11 SCID patients with normal and a group of 8 patients with decreased naive CD4$^+$ T-cell counts at late follow-up (Figure 1a). In most cases, decreased naive CD4$^+$ T-cell counts were accompanied by low total CD4$^+$ T-cell counts (Figure 1a), and relatively low naive and total CD8$^+$ T-cell counts (Figure 1b). At late follow-up, the median telomere length in naive (p=0.001) and memory (p=0.002) CD4$^+$ T cells, the number of CD19$^+$/CD20$^+$ B cells (p=0.001), and the in vitro response of PBMC to the T-cell mitogen PHA (p=0.045) were also found to be significantly higher in patients with good compared to poor naive CD4$^+$ T-cell reconstitution. The numbers of CD3$^-$CD16/56$^+$ NK cells in both groups were not statistically different (p=0.16).

To analyze if the distinction between the two groups of patients could be related to thymus output, we measured TREC contents in CD4$^+$ and CD8$^+$ T cells. At late follow-up, only individuals with poor T-cell reconstitution had reduced CD4$^+$ TREC contents compared to age-matched healthy controls (data not shown). Differences in TREC contents may reflect differences in thymus output, but may also be due to TREC dilution by peripheral T-cell proliferation. When the level of T-cell proliferation was analyzed by measuring Ki-67 expression in naive and total CD4$^+$ T cells at late follow-up, we found no significant differences between patients and healthy controls, but patients with poor T-cell reconstitution had significantly higher levels of Ki-67 expression than individuals with good T-cell reconstitution (p=0.006). Since increased T-cell proliferation rates could thus be the cause of lower TREC contents in individuals with poor long-term T-cell reconstitution, we also analyzed the total numbers of TRECs in CD4$^+$ and CD8$^+$ T cells per μl blood, which are not influenced by T-cell proliferation, as a more direct measure of thymus output. Again, a clear distinction between the two
groups was observed: CD4⁺ TREC numbers were decreased in patients with impaired T-cell reconstitution at late follow-up (p<0.001), while they were comparable to healthy age-matched controls in individuals with good long-term T-cell reconstitution (p=0.495, Figure 1c). CD8⁺ TREC numbers were also significantly (p<0.001) higher in patients with good compared to poor long-term immune reconstitution (data not shown). This suggests that the number of thymus emigrants in patients with poor long-term T-cell reconstitution was reduced.

Differences in T-cell reconstitution are already evident early after HSCT

To study the possibility that poor T-cell reconstitution at the long term was due to an accelerated decline in thymus output or to long-term graft failure, as was previously suggested,¹⁴⁻¹⁵ we performed a longitudinal, retrospective analysis of T-cell immunity and thymus output in our cohort of SCID patients by also investigating a time point early (1 to 4, median 1.5 years) after HSCT. It appeared that individuals with poor T-cell reconstitution at late follow-up already had significantly (p=0.002) lower naive CD4⁺ T-cell counts at early follow-up compared to patients with good long-term T-cell reconstitution (Figure 2a and Table 2). In analogy, CD4⁺ TREC numbers per μl blood in patients with poor long-term T-cell reconstitution were already significantly lower (p=0.002) early after HSCT compared to patients with good long-term T-cell reconstitution (Figure 2b and Table 2). The median period between HSCT and the time point of early follow-up was 1.5 years for both groups of patients, and could thus not explain the differences in short-term T-cell reconstitution between the groups. Taken together, these data suggest that poor long-term T-cell reconstitution was neither caused by an accelerated loss of thymus output nor by long-term graft failure. In 11 out of 19 SCID patients, long-term T-cell reconstitution and thymus output after HSCT did not differ from healthy age-matched controls, while in the other group of patients with poor T-cell reconstitution and decreased thymus output at late follow-up, both impairments were already evident early after HSCT.

Determinants of long-term T-cell reconstitution

Besides naive CD4⁺ T-cell counts and CD4⁺ TREC numbers, we investigated if any of the other immune parameters at early follow-up or patient characteristics such as the type of SCID correlated
with good or poor T-cell reconstitution at late follow-up (Table 2). Total CD4$^+$ T-cell counts, CD19$^+$/CD20$^+$ B-cell counts, and TREC in CD8$^+$ T cells at early follow-up were significantly lower in patients with poor compared to good long-term T-cell reconstitution. Of note, naive CD4$^+$ T-cell counts at 1 to 4 years post-HSCT were significantly reduced in both groups of SCID patients when compared to healthy age-matched controls ($p<0.001$). At early follow-up there was no significant difference between both groups of patients in any of the CD8$^+$ T-cell subsets, memory CD4$^+$ T-cell counts, NK-cell counts or Ki-67 expression (Table 2). Also the number of nucleated cells in the graft did not differ between both groups of patients (data not shown). Remarkably, patients with poor T-cell reconstitution at late follow-up had been transplanted at a significantly younger age compared to patients with good long-term T-cell reconstitution. SCID phenotypes without NK cells (SCID NK$^-$) correlated significantly with good outcome, while SCID types without B cells (SCID B$^-$) correlated with poor T-cell recovery (Table 2). The number of reported clinical complications and infectious problems did not differ significantly between the two groups of patients (Table 1).

Despite the numerous significant correlations between early parameters and T-cell reconstitution at late follow-up, hardly any of these parameters could be used for early identification of individual patients at risk for poor long-term T-cell reconstitution. For example, 3 individuals who developed good T-cell reconstitution at late follow-up, had naive CD4$^+$ T-cell counts at early follow-up in the same range as individuals with poor long-term T-cell reconstitution (see Figure 2a). Similarly, a clear distinction between individuals with poor and good long-term immune reconstitution could not be made on the basis of total CD4$^+$ T-cell counts or CD19$^+$/CD20$^+$ B-cell counts at early follow-up, age at transplantation or SCID phenotype (data not shown). The parameters that most clearly allowed for discrimination between individuals of the two groups of patients at early follow-up were the CD4$^+$ TREC content and number (Figure 2b). Of the few patients in whom telomere length could be measured at early follow-up, naive CD4$^+$ T-cell telomere lengths tended to be shorter in patients with poor (n=2) compared to good (n=7) long-term T-cell reconstitution (Table 2).
Discussion

In our cohort of 19 SCID patients we found no evidence for an accelerated decline of T-cell immunity, thymus output or telomere length with time post-HSCT. In more than half of the patients immune parameters at long-term follow-up were comparable to healthy age-matched controls, even up to 25 years post-HSCT, while in others who showed poor T-cell reconstitution at the long term, these immune parameters turned out to be already low early after HSCT. Thus, our data do not confirm the suggestion put forward by Sarzotti et al.\textsuperscript{14} and Patel et al.\textsuperscript{15} that T-cell immunity in transplanted SCID patients may be impaired later in life because of long-term graft failure or an increased rate of deterioration of thymus function. Our study points out that on average long-term T-cell immunity in transplanted SCID patients may indeed not be as good as in healthy age-matched controls, but that this is merely due to a subset of patients who already reconstituted poorly early post-HSCT. Sarzotti et al.\textsuperscript{14} previously reported that after an initial normalization of T-cell diversity in SCID patients during the first year post-HSCT, T-cell diversity generally decreased during the next 10 years, concomitant with decreasing TREC values and naive T-cell numbers. This was interpreted as an indication that thymus output deteriorated at a faster pace in transplanted SCID patients compared to healthy controls.\textsuperscript{14} Of note, however, the decrease in T-cell diversity that was reported was restricted to the memory CD8\textsuperscript{+} T-cell population, and may thus be related to antigen-driven clonal expansion rather than decreasing thymus output.

Patel et al.\textsuperscript{15} have performed a cross-sectional study on thymus function in a cohort of SCID patients, of whom the majority had received a T-cell depleted bone marrow graft from a haplo-identical donor. No chemotherapy before transplantation and no GvHD prophylaxis were given. The percentage of patients with subnormal PBMC TREC contents early (1-4 years) and late (> 5 years) after HSCT was 18\% (5 out of 28 cases) and 32\% (7 out of 22 cases), respectively. The low frequency of patients with low TREC contents early after HSCT, compared to our study cohort (42\%, 8 out of 19), might be related to the relatively high proportion of SCID-X1 patients in that study, the lower age at
transplantation, and the fact that neither chemotherapy nor immunosuppressive treatment was applied, which is likely to have an effect on thymus function. The possible effect of myelosuppressive or myeloablative conditioning on the engraftment of long-term progenitors remains a matter of debate. In this respect, it is interesting that Patel et al.\textsuperscript{15} observed a rapid decline of PBMC TREC contents in SCID patients transplanted without conditioning, whereas such an accelerated decrease was not observed in the present study.

It is generally thought that T-cell immunity normalizes within 1 year post-HSCT in the majority of SCID patients. However, in comparison with age-matched healthy controls, naive CD4\(^+\) T-cell counts in SCID patients were still significantly reduced at a median follow-up of 1.5 years post-HSCT, both in patients who did and who did not reconstitute well at the long term. In this respect, our data suggest that in a subgroup of SCID patients further improvement of T-cell recovery may occur relatively late after HSCT, differentiating the statement\textsuperscript{7} that the absence of normal T-cell function $\geq$2 years after HSCT could be an indication for a second transplant procedure. Remarkably, a young age of the patients at the time of HSCT did not predict good long-term T-cell reconstitution in our cohort. On the other hand, it was previously found that thymus-dependent recovery of naive T cells 1-2 years after HSCT is superior in patients who were transplanted in the neonatal period.\textsuperscript{9} The observation that the SCID NK\(^-\) phenotype correlated with good long-term T-cell reconstitution, supports the concept that host NK cells may interfere with the development of lymphoid precursors and, thereby, impair the recovery of the immune system.\textsuperscript{8} On the other hand, our observation that the SCID B\(^+\) phenotype correlated with good outcome confirms previous studies that showed faster normalization of T- and B-cell function in B\(^+\) compared to B\(^-\) SCID post-HSCT.\textsuperscript{5,7} In this respect, it is of note that 4 out of 5 SCID-X1 patients (common $\gamma$-chain defect) are within the group with good T-cell reconstitution. However, overall, we did not find specific genetic defects to be related with good or poor T-cell reconstitution post HSCT, because of the heterogeneity of our SCID population, the small number of patients per genetic defect, and an unknown defect in 7 cases.
Our analysis demonstrated that CD4⁺ TREC contents and TREC numbers early post-HSCT were predictive for long-term T-cell reconstitution. Decreased TREC contents at short-term follow-up in our study may be due to a lack of thymus output or to an increased rate of T-cell proliferation, possibly related to GvHD or infections. Since we found that also the total number of CD4⁺ TRECs per μl of blood was decreased in patients with poor T-cell reconstitution, and that Ki-67 expression at early follow-up did not differ between patients with good and poor long-term T-cell reconstitution, we conclude that thymus output early after HSCT plays a crucial role in long-term T-cell reconstitution. Differences in thymus output between patients might be due to differences in the capacity of the originally vestigial thymus to be repopulated by progenitor cells, to differences in the quality and quantity of donor BM-derived progenitor cells, or to their capacity to repopulate the precursor compartment early after HSCT. The observation that T-cell reconstitution after HSCT correlated well with B-cell reconstitution would favour the latter two explanations, although we cannot exclude the possibility that poor maturation of naive B cells in secondary lymphoid organs was a direct consequence of poor T-cell reconstitution.

Taken together, our study shows that stable and long-lasting T-cell reconstitution is to be expected in SCID patients who reconstitute well early after HSCT. The group of SCID patients as a whole seems to have an increased risk of autoimmune or infectious problems at older age compared to other HSCT patients. Unexpectedly, in our study cohort these problems were not clearly associated with the investigated immunological parameters (see also Laffort et al.), which complicates clinical decision making on additional treatment. Previous studies have shown, however, that T-cell reconstitution is strongly associated with survival. Our analysis shows that TREC values measured at about 2 years post-HSCT may, therefore, provide an important criterion when considering re-transplantation or boost with donor stem cells in patients who do not reconstitute well at the short term. This strategy has been shown to be successful in 3 out of 4 patients who were retransplanted 3-5 years after the first HSCT. However, the recently published failure of gene therapy for SCID-X1 at older age may point to age constraints on the regenerative capacity of the hypoplastic thymus of SCID patients.
constraints should be taken into account when considering a second transplant with the purpose to restore the T-cell pool.
Acknowledgements

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Legends to Tables and Figures

Table 1. Patient Characteristics

UPN: unique personal number; SCID genotype: ? = unknown, AR = autosomal recessive, XL = X-chromosome linked, ADA = adenosine deaminase deficiency, \( \gamma_c \) = common gamma chain deficiency, IL-7R\( \alpha \) = IL-7 receptor alpha chain deficiency, Artemis = DNA-repair defect due to mutations in the Artemis gene, RAG1-2 = Recombinase-activating gene (RAG1 or RAG2) deficiency; 2nd HSCT: denotes the 3 patients who were transplanted twice; Age at HSCT is given in months, early and late follow-up (FU) are given in years post-HSCT; Donor: IRD = HLA-identical family donor, ORD = haplo-identical family donor, MUD = matched unrelated donor; TCD graft: Alb. Gradient = Albumin gradient, E-rosetting = rosetting of T cells to sheep erythrocytes; Conditioning: TBI = total body irradiation, CY = cyclophosphamide, BU = busulphan; GvHD prevention: MTX = methotrexate, CsA = cyclosporin A; Clinical status: spastic dipl = spastic diplegia, retard = retardation; Infections: URTI = upper respiratory tract infections, ENT = ear, nose and throat infections, pneum = pneumonia, resp tract = respiratory tract.

Table 2. Early prediction of long-term T-cell reconstitution after HSCT for SCID

Median parameters of the whole SCID cohort at early follow-up (1-4 years post-HSCT) were compared with age-matched healthy controls (*: p-value). Within the SCID cohort, median parameters of immunity at early follow-up and HSCT-related variables were compared between the groups of patients with poor and good T-cell reconstitution at late follow-up (**: p-value). NK-cell chimerism in the group with poor long-term immune reconstitution was based on 7 patients because hardly any NK cells were present in one of the patients. Conditioning: BU = busulphan, CY = cyclophosphamide. Significant differences are denoted by p-values in boldface.
Figure 1. Immune parameters at late follow-up, between 5 and 32 years post-HSCT

(a) CD4\(^+\) T-cell counts in the different subsets, (b) CD8\(^+\) T-cell counts in the different subsets, and (c) CD4\(^+\) TREC numbers. Open circles denote healthy controls; black circles and grey triangles denote SCID patients at long-term follow-up with poor and good long-term reconstitution of the naive CD4\(^+\) T-cell population, respectively.

Figure 2. Longitudinal changes in naive CD4\(^+\) T-cell counts and CD4\(^+\) TREC numbers between early and late follow-up

Naive CD4\(^-\) T-cell counts (a) and CD4\(^+\) TREC numbers (b) were followed at early (1-4 yrs post-HSCT) and late (>5 yrs post-HSCT) follow-up. Black circles and grey triangles denote SCID patients with poor and good reconstitution of the naive CD4\(^+\) T-cell population at late follow-up, respectively. Open circles denote control values which were taken from age-matched (1-4.5 years and 5-32 years old, respectively) healthy individuals. The two open triangles in panel b represent the only child with good long-term immune reconstitution despite low TREC numbers at early follow-up. During early follow-up this child was in an extremely poor clinical state and suffered from mal-absorption, which may explain the abnormal immune pattern at this time point.
Figure 1a

- Control
- Poor long-term reconstitution
- Good long-term reconstitution

Graphs showing data distribution for different cell counts per μl against age.
Figure 1b

- Control
- Poor long-term reconstitution
- Good long-term reconstitution

- Naive CD8+ T-cell counts (per μl)
- Memory CD8+ T-cell counts (per μl)
- Total CD8+ T-cell counts (per μl)

Age
Figure 1c
Figure 2

a

b
Table 1: Patient Characteristics

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<th>UPN</th>
<th>gender</th>
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<th>SCID Genotype</th>
<th>2nd HSCT</th>
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Subgroup with good reconstitution (N=11)

17  F  T-B+NK+/- γc (AR)  v  33  IRD  Alb. gradient  -  -  -  -  -  warts  25.5
50  F  T-B+NK- γc (AR)  v  18  IRD  E-rosetting  TBI/CY  MTX  -  -  -  -  -  1.5  18.3
56  F  T-B+NK- γc (AR)  v  9  ORD  E-rosetting  CY  -  2  extensive  vasculitis  -  1.5  17.4
59  M  T-B+NK+ γc (AR)  v  9  ORD  E-rosetting  -  -  2  extensive  -  -  2.9  17.9
96  F  T-B-NK+/- ADA  8  ORD  E-rosetting  BU/CY  CsA  1  -  spastic dipl/retard  -  1.0  15.1
112 M  T-B-NK- γc (XL)  v  5  ORD  E-rosetting  BU/CY  CsA  1  limited  -  -  2.5  13.8
199 M  T-B-NK- γc (XL)  10  ORD  E-rosetting  BU/CY  MTX  -  -  -  -  -  URTI/ENT  1.6  8.5
220 F  T-B+NK+ γc (AR)  v  18  MUD  E-rosetting  BU/CY  MTX  -  -  -  -  -  1.0  8.3
221 F  T-B+NK+ γc (AR)  v  18  MUD  E-rosetting  BU/CY  MTX  -  limited  -  -  1.0  8.3
228 M  T-B-NK+/- ADA  7  ORD  E-rosetting  BU/CY  CsA  -  -  -  -  -  1.3  7.7
304 M  T-B-NK- γc (XL)  9  ORD  E-rosetting  BU/CY  CsA/MTX  2  limited  -  -  1.7  6.1

Subgroup with poor reconstitution (N=8)

2  M  T-B-NK- γc (XL)  5  IRD  Alb. gradient  -  -  -  -  -  lymphoedema  warts/pneum  3.9  32.2
53 M  T-B+NK+ IL-7Rα  v  12  ORD  E-rosetting  CY  CsA  -  -  -  -  -  warts  1.6  16.9
108 M  T-B+NK+ ? (AR)  10  ORD  E-rosetting  BU/CY  CsA  -  -  -  -  -  resp tract  1.0  13.8
131 M  T-B-NK+/- ADA  1  IRD  no  BU/CY  CsA  -  -  -  -  -  1.7  11.9
168 F  T-B-NK+ Artemis  8  ORD  E-rosetting  BU/CY  CsA  -  -  spastic dipl  -  3.5  10.3
183 F  T-B-NK+ RAG1-2  4  ORD  E-rosetting  BU/CY  CsA  -  -  -  -  -  1.0  6.3
242 M  T-B-NK+/- ADA  1  IRD  no  -  CsA  -  -  learning problems  -  1.4  7.2
306 M  T-B-NK+ RAG1-2  2  IRD  no  -  -  2  limited  -  -  1.3  5.0
Table 2: Early prediction of long-term T-cell reconstitution after HSCT for SCID

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (N=19)</th>
<th>SCID cohort (N=19)</th>
<th>p-value</th>
<th>Poor reconstitution (N=8)</th>
<th>Good reconstitution (N=11)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Counts of lymphocytes (per μl):</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naive CD4⁺ T cells</td>
<td>1432</td>
<td>341</td>
<td>&lt;0.001</td>
<td>86</td>
<td>630</td>
<td>0.002</td>
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<tr>
<td>Memory CD4⁺ T cells</td>
<td>303</td>
<td>222</td>
<td>0.016</td>
<td>253</td>
<td>185</td>
<td>0.711</td>
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<tr>
<td>Total CD4⁺ T cells</td>
<td>1662</td>
<td>789</td>
<td>0.001</td>
<td>451</td>
<td>1254</td>
<td>0.003</td>
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<tr>
<td>Naive CD8⁺ T cells</td>
<td>603</td>
<td>300</td>
<td>0.041</td>
<td>146</td>
<td>323</td>
<td>0.153</td>
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<tr>
<td>Memory CD8⁺ T cells</td>
<td>150</td>
<td>75</td>
<td>0.246</td>
<td>69</td>
<td>80</td>
<td>0.672</td>
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<tr>
<td>Total CD8⁺ T cells</td>
<td>697</td>
<td>552</td>
<td>0.412</td>
<td>419</td>
<td>640</td>
<td>0.328</td>
</tr>
<tr>
<td>Total CD19⁺/CD20⁺ B cells</td>
<td>677</td>
<td>381</td>
<td>0.059</td>
<td>70</td>
<td>649</td>
<td>0.005</td>
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<tr>
<td>Total CD3⁻CD16/56⁺ NK cells</td>
<td>193</td>
<td>127</td>
<td>0.014</td>
<td>119</td>
<td>129</td>
<td>0.625</td>
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<tr>
<td><strong>Ki-67 expression (%) in:</strong></td>
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<tr>
<td>Total CD4⁺ T cells</td>
<td>3.0</td>
<td>6.1</td>
<td>0.07</td>
<td>6.4</td>
<td>5.8</td>
<td>0.25</td>
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<tr>
<td>Naive CD4⁺ T cells</td>
<td>1.1</td>
<td>2.6</td>
<td>0.24</td>
<td>3.2</td>
<td>2.0</td>
<td>0.27</td>
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<tr>
<td><strong>TRECs:</strong></td>
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<tr>
<td>Content in CD4⁺ T cells</td>
<td>0.20</td>
<td>0.03</td>
<td>0.006</td>
<td>0.007</td>
<td>0.11</td>
<td>0.005</td>
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<tr>
<td>Content in CD8⁺ T cells</td>
<td>0.10</td>
<td>0.04</td>
<td>0.082</td>
<td>0.002</td>
<td>0.10</td>
<td>0.013</td>
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<tr>
<td>Number in CD4⁺ T cells/μl blood</td>
<td>410</td>
<td>23</td>
<td>0.001</td>
<td>1.4</td>
<td>105</td>
<td>0.002</td>
</tr>
<tr>
<td>Number in CD8⁺ T cells/μl blood</td>
<td>101</td>
<td>16</td>
<td>0.039</td>
<td>0.2</td>
<td>51</td>
<td>0.005</td>
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<tr>
<td>Telomere length in:</td>
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<td>1.6</td>
<td>0.079</td>
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<tr>
<td>Memory CD4⁺ T cells</td>
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<td>1.3</td>
<td>0.240</td>
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<table>
<thead>
<tr>
<th>Other parameters:</th>
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<tbody>
<tr>
<td>Age patient at HSCT (in yr)</td>
<td>0.7</td>
<td>0.4</td>
<td>0.7</td>
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<tr>
<td>Age donor at HSCT (in yr)</td>
<td>24.2</td>
<td>15.9</td>
<td>27.9</td>
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<tr>
<td>SCID phenotype (NK-)</td>
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<td>1</td>
<td>8</td>
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<tr>
<td>SCID phenotype (B-)</td>
<td>6</td>
<td>5</td>
<td>1</td>
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<tr>
<td>B-cells predominantly donor origin</td>
<td>11</td>
<td>4</td>
<td>7</td>
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<tr>
<td>NK cells predominantly donor origin</td>
<td>11</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Monocytes predominantly donor origin</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Myeloablative conditioning (BU/CY)</td>
<td>11</td>
<td>4</td>
<td>7</td>
</tr>
</tbody>
</table>
Early determinants of long-term T-cell reconstitution after hematopoietic stem cell transplantation for severe combined immunodeficiency

Jose A Borghans, Robbert G Bredius, Mette D Hazenberg, Helene Roelofs, Els C Jol-van der Zijde, Jeroen Heidt, Sigrid A Otto, Taco W Kuijpers, Willem E Fibbe, Jaak M Vossen, Frank Miedema and Maarten J van Tol