Preservation of adaptive immunity after nonmyeloablative hematopoietic cell transplantation in adults with sickle cell disease (PROTECT study)

In patients with sickle cell disease (SCD), nonmyeloablative matched sibling donor (MSD) hematopoietic cell transplantation (HCT) with alemtuzumab (1 mg/kg) and low-dose (3 Gy) total body irradiation (TBI) typically results in stable mixed chimerism with donor T-cell chimerism of >50%. In contrast, haploidentical HCT with reduced intensity conditioning (RIC), including antithymocyte globulin (ATG), thiotepa, and post-transplantation cyclophosphamide (PTCy) typically results in full donor chimerism. Previous studies, primarily focusing on malignant hematologic conditions, have demonstrated that patients undergoing allogeneic HCT lose their vaccination-derived immunity after transplant.[1-3] Consequently, comprehensive post-transplant revaccination is standard practice for all recipients of allogeneic HCT.[4, 5]

We hypothesized that mixed chimerism after nonmyeloablative MSD transplant for SCD results in better long-term preservation of recipient-derived adaptive immunity than haploidentical transplant with full donor chimerism, potentially eliminating the need for revaccination. We evaluated preservation of vaccination-derived immunity in adults with SCD after either nonmyeloablative MSD or RIC haploidentical transplant.

To confirm that the antibody response measured after transplant is recipient-derived and not donor-derived, we also investigated immune responses to hepatitis B virus (HBV) vaccinations in a subgroup of patients receiving hematopoietic stem cells from donors who had not been exposed to HBV (vaccination). Finally, we characterized the dynamics of immune reconstitution within the first 24 months post-transplant by measuring lymphocyte subsets.

Adult patients (>18 years) with SCD who underwent allogeneic HCT at Amsterdam UMC were eligible for inclusion. Patients received either nonmyeloablative MSD transplant or RIC haploidentical transplant, as previously described.[6,7] All MSD recipients received hydroxyurea/azathioprine preconditioning for three months prior to HCT. Other pre-transplant treatment as well as indications for transplant were comparable between MSD and haploidentical recipients (*Online Supplementary Table S1*).

This study was approved by the institutional review board (2021 091/NL77161.018.21) and carried out in accordance with the principles of the Declaration of Helsinki 2013, and registered at clinicaltrials.gov (identifier: NCT05200338). The primary objective was to evaluate the durability of immune responses against pre-transplant vaccinations (measles, rubella, and pneumococcus), comparing nonmyeloablative MSD transplantation (mixed chimerism) to RIC haploidentical transplant (full chimerism) in SCD patients. Secondary objectives included response to HBV vaccination in a subgroup of patients to determine whether the post-transplant immunity was recipient-derived, and assessment of immune reconstitution. Antibody titers against measles, rubella and *Streptococcus pneumoniae* were collected at baseline (pre-transplant) and +6, +12, and +24 months post-transplant. Patients receiving revaccination were censored for that specific pathogen beyond this timepoint. The standard revaccination protocol is described in *Online Supplementary Table S2*. Immune reconstitution was assessed by measuring lymphocyte subsets ($CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, and natural killer [NK] cells) and serum IgG levels. Patients receiving immunomodulatory therapies, such as rituximab, were censored from immune reconstitution assessment after the first dose. We compared preservation of immunity and immune reconstitution between groups using Fisher's exact test and Mann-Whitney U test, respectively (R, version 2024.12.1). $P<0.05$ was considered statistically significant.

Twenty-four MSD transplant recipients and 14 haploidentical transplant recipients were included (Figure 1). For the HBV subgroup analysis, 6 patients scheduled for MSD transplant, 6 patients scheduled for haploidentical transplant, and 6 non-transplant control SCD patients were included (Figure 1). Seven of the 18 patients included in the HBV subgroup analysis had already been vaccinated against HBV and did not require additional doses, while 11 subjects received Engerix-B® HBV vaccination (*Online Supplementary Table S3*). Given that vaccine-derived pneumococcal immunity typically wanes after five years, the timing of pre-transplant vaccination is relevant for post-transplant durability. In the MSD group, patients had received their pneumococcal vaccination a median of three years (range: 1-6) prior to transplant, compared to two years (range: 0-5) in the haploidentical group. The median follow-up period was 43 months (interquartile range [IQR]: 31-71). In the MSD group, median chimerism at one year post-transplant was 68% (IQR: 59-79) in the T-cell fraction and 100% (IQR: 92-100) in the myeloid fraction. In the haploidentical group, median T-cell and myeloid chimerism were both 100% (IQR: 100-100) one year post transplant. Sirolimus was tapered from 12 months post-transplant and discontinued in all patients at a median 15 months (IQR: 14-16) post-transplant. The duration of sirolimus use was comparable between the MSD (median: 15 months, IQR: 14-18) and haploidentical (median: 14 months, IQR: 13-16)

groups. Four patients in the haploidentical group developed chronic graft-*versus*-host disease (GvHD), one with skin GvHD resolved readily with topical therapy, the other 3 were still using systemic immunosuppression at the time of these analyses (18-34 months post-transplant).

For measles, 21 patients in the MSD group were seropositive at baseline (Figure 2A). During post-transplant follow-up, 18/21 patients (85.7%) preserved immunity, while 3 fell below the

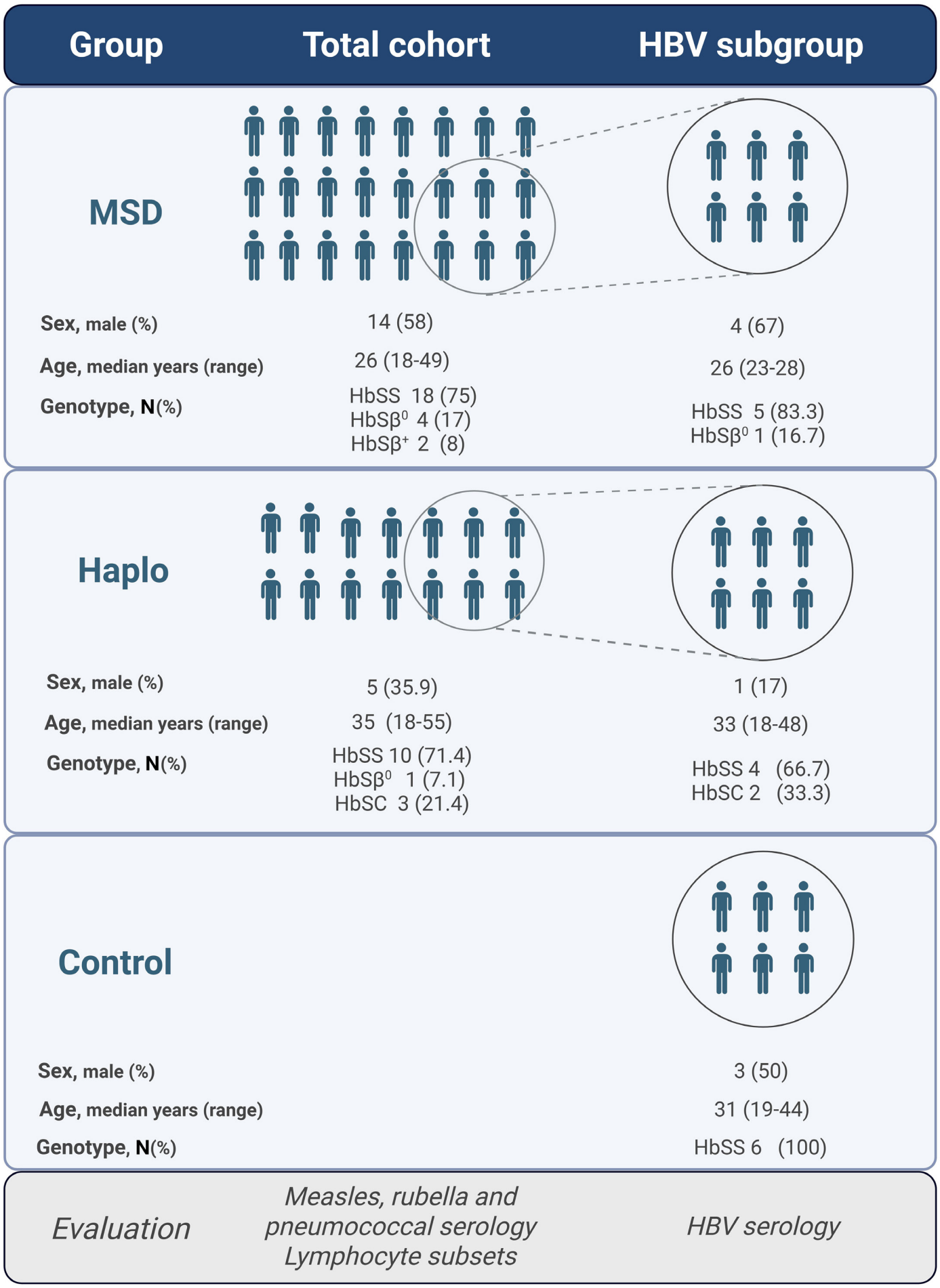


Figure 1. Baseline characteristics and cohort composition. The study includes two cohorts. The total cohort includes all matched sibling donor (MSD) (N=24) and haploidentical (Haplo) (N=14) transplant recipients from Amsterdam UMC. This total cohort involves serology for measles, rubella, and *Streptococcus pneumoniae*, as well as lymphocyte subset evaluation. Within the total cohort, a hepatitis B virus (HBV) subgroup (circled) also underwent HBV serology assessment, including MSD recipients (N=6), haploidentical recipients (N=6), and non-transplant sickle cell disease (SCD) controls (N=6). Created with Biorender.com

protective threshold, one of whom had received rituximab post-transplant. In the haploidentical group, 10 patients were seropositive at baseline, with 7/10 (70%) preserving their immunity during post-transplant follow-up, while 3 lost their immunity. This difference was not statistically significant (*P*=0.36).

For rubella, 20 patients in the MSD group were seropositive at baseline, and 19 (95%) of them remained seropositive during post-transplant follow-up (Figure 2B). The only subject who lost immunity had received rituximab shortly after transplant. In the haploidentical group, 12 patients were seropositive at baseline, with 8 of them (66.7%) remaining seropositive and the other 4 (33.3%) gradually losing their immunity after transplant. Preservation of immunity against rubella was better in the MSD group than in the haploidentical group (*P*=0.053).

For pneumococcal strains, 19/20 (95%) patients in the MSD group preserved immunity during follow-up. Nine patients who did not receive a revaccination demonstrated immunity at 24 months post-transplant. In the haploidentical group, only 1/6 (16.7%) of the evaluable patients preserved immunity after transplant (*P*=0.005) (Figure 2C). The number of evaluable patients varied for each pathogen due to differences in baseline serostatus and revaccination timing.

All 12 subjects in the HBV subgroup analysis were anti-HBs seropositive at baseline (Figure 2D). In the months following

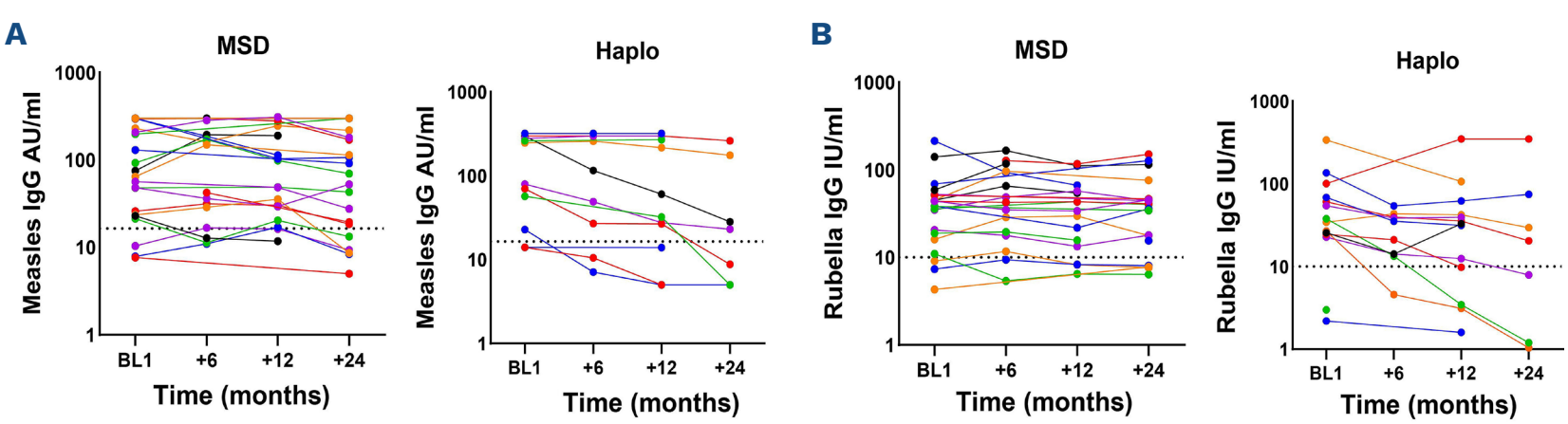


Figure 2. Preservation of immunity against measles, rubella and *Streptococcus pneumoniae*. Course of measles (A) and rubella (B) specific IgG titers in the months following hematopoietic cell transplantation (HCT) in matched sibling donor (MSD) (left) and haploidentical (Haplo) (right) groups. Protective threshold is indicated by the dotted line. Measles seropositivity cutoff value >16.5 AU/mL. Rubella cutoff value >10 IU/mL. Each line represents an individual subject. (C) Course of immunity against *Streptococcus pneumoniae* infection at baseline (BL), +6, +12 and +24 months after HCT in MSD and haploidentical transplant recipients. Each row represents an individual subject. Subjects without any data regarding pneumococcal immunity and subjects with only baseline available are not shown. For the single value ELISA, subjects with a titer above 37 mg/L are considered immune. For the multiple subvariant test, subjects with antibody titers above 1.0 μg/mL for 6 or more pneumococcal serotypes are considered immune. (D) Course of anti-hepatitis B (HB) titers in the matched sibling donor (left), haploidentical (right) and non-transplant control patient (below) subgroups. Protective threshold is indicated by dotted line (> 10 mIU/mL). Each line represents an individual subject. Anti-HB: antibodies against HB.

HCT, antibody titers of 3/6 MSD patients (50%) decreased below the protective threshold. Two of these patients (subjects 1 and 3) had received rituximab due to Epstein-Barr virus reactivation. In the haploidentical HBV subgroup 2/6 subjects (33.3%) lost protective immunity. In non-transplant control patients, 5/6 subjects were seropositive, with one non-responder at baseline. At 12 months post vaccination, one subject lost immunity (Figure 2D). There were no significant differences in immunity preservation between the three groups (P>0.05).

At baseline, median CD19$^+$ cell counts and total IgG levels were higher than reference values in both transplant groups. In the months following HCT, decreases in lymphocyte counts and IgG levels were observed in both groups (Figure 3). Between +12 and +24 months, all subsets recovered to levels within or above reference values. The MSD group maintained significantly higher levels of total IgG. Absolute CD3$^+$ and CD4$^+$ cell counts at +3 months were significantly higher in patients receiving haploidentical transplants (following ATG and PTCy) than in patients receiving MSD transplant (following alemtuzumab): CD3$^+$ 0.35x10^9/L *versus* 0.11x10^9/L, respectively, P=0.008; CD4$^+$ 0.30 x10^9/L *versus* 0.05x10^9/L, respectively, P=0.001.

In line with our hypothesis, analysis of MSD transplants (mixed chimerism) showed stable antibody titers against measles (85.7%), rubella (95%), and *Streptococcus pneumoniae* (95%) for up to 24 months after transplant. The MSD transplant recipients who did lose immunity had low baseline titers or were treated with rituximab post-transplant. The proportion of patients preserving their pre-transplant immunity was smaller in the haploidentical group compared to the MSD group. Although the majority of haploidentical transplant recipients (full donor chimerism) preserved immunity against measles (70%) and rubella (66.7%) until two years post-transplant, a significant proportion (1/3) of these patients has a period of susceptibility to these infections as the live attenuated vaccine against measles, mumps and rubella (MMR) is generally given after two years post-transplant.

Our findings demonstrate better preservation of immunity compared to earlier studies in patients undergoing allogeneic HCT for malignant hematologic conditions.[1,8] Previous studies showed an association between the intensity of the conditioning regimen and antibody titers, with more profound loss of immunity in subjects who had received myeloablative conditioning. This probably contributes to the difference found in our cohort, in which patients receiving MSD transplant were treated with nonmyeloablative conditioning and those receiving haploidentical transplant with a more

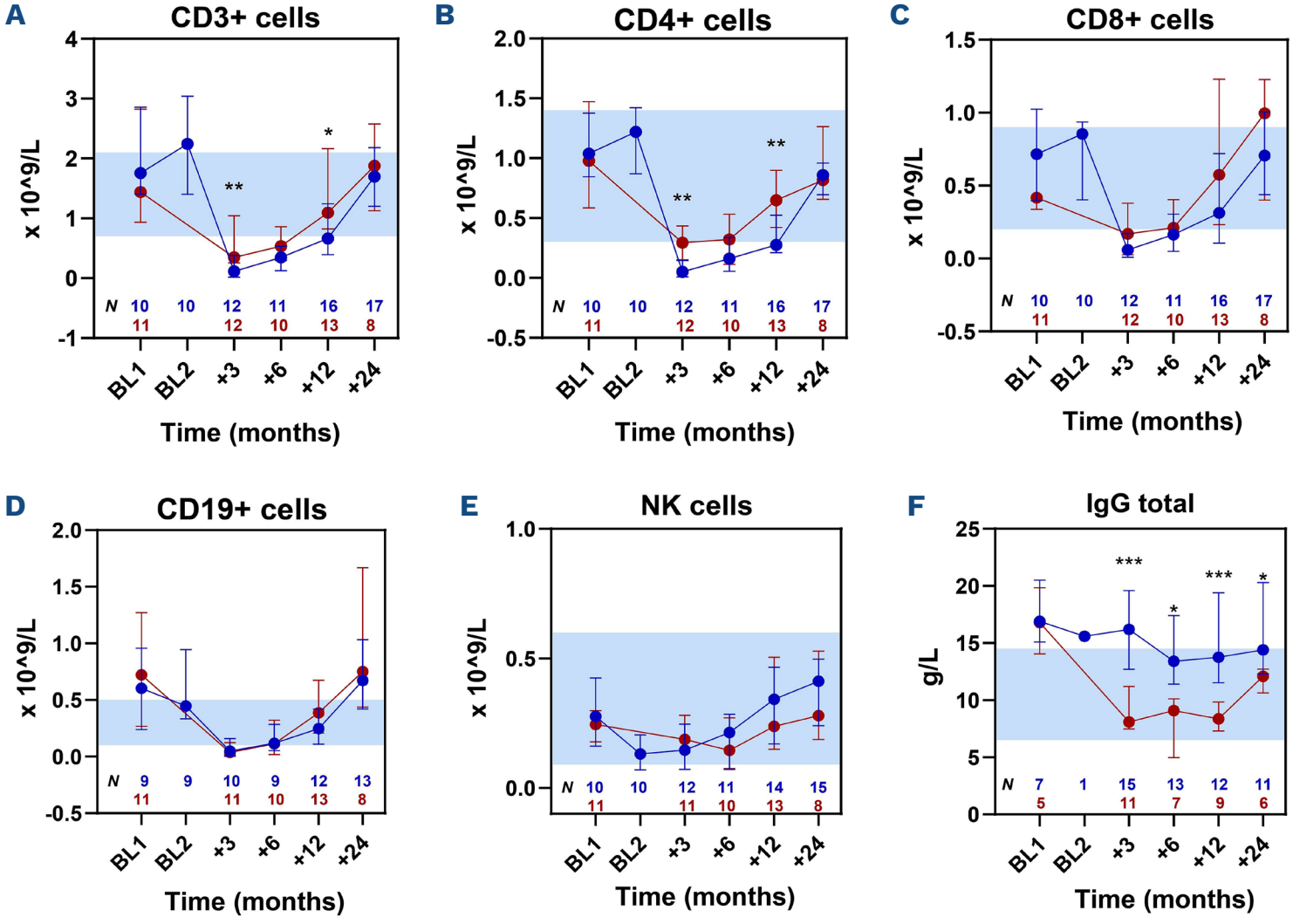


Figure 3. Immune reconstitution after transplantation. Time courses of absolute CD3$^+$ (A), CD4$^+$ (B), CD8$^+$ (C), CD19$^+$ (D), natural killer (NK) (E) subsets, and total IgG (F). Data points show medians for matched sibling donor (MSD) (blue) and haploidentical (red) groups; error bars represent interquartile range (IQR). Blue box indicates normal reference values. Numbers show sample size (N) per group at each timepoint. P values are calculated using the Mann-Whitney U test: *P<0.05, **P<0.01, ***P<0.001. BL1: baseline; BL2: after preconditioning, but before conditioning (MSD only, haploidentical patients received no preconditioning).

intensive RIC.[6,7] Furthermore, in contrast to patients with malignancies, SCD patients undergoing HCT are not heavily pretreated with chemotherapy, which might have significant impact on lymphocytes involved in preservation of immunity. In the HBV subgroup analysis, preservation of anti-HBV response between the transplant groups and the non-transplant SCD patients was comparable, indicating that the preservation of pre-transplant immunity is recipient-derived, as none of the donors had been exposed to HBV through vaccination. Similarly, the persistence of anti-pneumococcal immunity also indicates post-transplant preservation of recipient-derived immunity as donors had not been vaccinated against *Streptococcus pneumoniae*. While we cannot rule out transfer of some donor immunity for measles and rubella, the better immunity preservation in patients with mixed chimerism (MSD recipients) compared to full chimerism (haploidentical recipients) argues against donor-derived immunity.

Our findings suggest that revaccination might not be needed or can be postponed until at least 24 months post-transplant in SCD patients undergoing nonmyeloablative MSD transplant resulting in mixed chimerism. Delayed revaccination might be beneficial since a more mature immune system probably results in improved vaccination response post-transplant. For the haploidentical transplant recipients, the variability in immunity preservation between subjects and pathogens limits recommendations.

Immune reconstitution dynamics in adult SCD patients undergoing HCT have not been well characterized previously. ATG and PTCy both affect immune reconstitution, with PTCy being associated with a more profound decline in NK, T and B cells in the first month after transplant.[9] Alemtuzumab has been shown to have a stronger depleting effect on $CD8^+$ cells than ATG, possibly explaining the differences found in $CD8^+$ cells at +3 months between the MSD and haploidentical groups.[10] Despite these different approaches, immune reconstitution was generally swift in both transplant groups. Limitations of the study include the small sample size and revaccination schedules precluding long-term assessment. Importantly, vaccine-derived immunity can be lost up to ten years post-transplant.[11-13]

In conclusion, we demonstrate that in SCD patients, nonmyeloablative MSD transplant leads to more durable preservation of pre-transplant immunity compared to haploidentical transplant with RIC. Immune reconstitution was comparable between both groups. While revaccination programs might be delayed to at least two years post-transplant, a prospective study using titer-guided revaccinations might establish whether revaccination after nonmyeloablative MSD transplant in SCD patients can be entirely abandoned.

Authors

Elisabeth Dovern,[1,2*] Maud Zwolsman,[1-3*] Abraham Goorhuis,[2,4] Bart J. Biemond,[1,2,5] Katja C. Wolthers[6] and Erfan Nur[1-3,5]

[1]Department of Hematology, Amsterdam UMC, University of Amsterdam; [2]Amsterdam Institute for Infection and Immunity, Amsterdam UMC; [3]Department of Blood Cell Research, Sanquin Research and Landsteiner Laboratory; [4]Department of Infectious Diseases, Amsterdam UMC; [5]Cancer Center Amsterdam, Amsterdam UMC and [6]Department of Medical Microbiology and Infection Prevention, Amsterdam UMC, Amsterdam, the Netherlands

**ED and MZ contributed equally as first authors.*

Correspondence:
E. NUR - e.nur@amsterdamumc.nl

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Contributions

EN conceptualized the study, designed the research protocol, recruited all study participants, interpreted data, and drafted the manuscript; AG conceptualized the study; ED conceptualized the study, designed the research protocol, recruited all study participants, vaccinated patients, collected and interpreted data, and drafted the manuscript; MZ collected data, performed the statistical analyses, created the figures and tables, interpreted data, and drafted the manuscript; KW delivered the virus IgG titers. All authors critically reviewed the manuscript.

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Data-sharing statement

The data supporting the findings of this study are available on reasonable request from the corresponding author.

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