

CD4⁺ T-cell alloreactivity after haploidentical hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is an important, potentially curative therapeutic modality in many hematopoietic disorders.¹ A fully human leukocyte antigens (HLA)-matched sibling or unrelated donor is the preferred source for stem cells.¹ However, finding such a donor may pose a problem, especially for non-Caucasian patients.¹ Stem cells from a haploidentical donor or umbilical cord blood can serve as an alternative for those lacking a fully matched donor. In the past, haploidentical HSCT was associated with two problems; a higher rate of graft failure and, a higher incidence of graft-versus-host disease (GvHD), both necessitating intensive immunosuppressive measures.² The development of conditioning regimens with intensified immunosuppressive therapy, including high-dose post-transplant cyclophosphamide (PT-Cy) therapy, improved results after haploidentical HSCT by ensuring engraftment, less GvHD, and a more favorable restoration of immune recovery.² We earlier showed the strong presence of alloreactive CD4⁺ T cells in most patients early after double umbilical cord blood transplantation (dUCBT) and their potential role in enhancing the graft-versus-leukemia effect.^{3,4} In this study, we addressed whether such HLA-class II specific CD4⁺ T cells are present early after haploidentical HSCT.

The trial was approved by the ethics committees of the participating institutions and was conducted in accordance with the Declaration of Helsinki. All participants had given written informed consent. A total of 22 patients with high-risk hematologic diseases, eligible for haploidentical HSCT were retrospectively included in this study. The primary endpoint was the proportion of evaluable patients with activated class-II specific CD4⁺ T cells. Patients received myeloablative conditioning, consisting of thiotepa (10 mg/kg), busulfan 6.4 or 9.6 mg/kg and fludarabine 150 mg/m², followed by transplantation of an unmanipulated bone marrow graft from a haploidentical family member, as previously described.² GvHD prophylaxis consisted of PT-Cy, tacrolimus, and mycophenolate mofetil. Peripheral blood from patients was collected 1 month after transplantation for isolation of peripheral blood mononuclear cells (PBMC). Engraftment was achieved in all patients at the time of sampling. A library of stimulator cells expressing a single HLA-class II allele (HLA-DRA1/B1, HLA-DQA1/B1 or HLA-DPA1/B1) was generated by retroviral transduction of HLA-class II negative HeLa cells.⁴ Post-transplantation PBMC were assayed for the presence of alloreactive T cells. The very few alloreactive T cells were propagated in a co-culture with irradiated HeLa cells expressing a single mismatched HLA-class II allele derived from the recipient with addition of a cytokine mixture. Subsequently, alloreactivity was measured by flow cytometry and expressed as fold increase (%CD137⁺/CD4⁺) of reactivity toward HeLa cells transduced with mismatched HLA-class II alleles relative to reactivity towards the not-transduced 'empty' HeLa cells, as described previously.⁴ In addition, alloreactivity towards matched 'control' alleles were included (*Online Supplementary Figure S4*).

The characteristics of the 22 patients and their grafts are presented in Table 1A and 1B, respectively. The majority of the patients were diagnosed with acute myeloid or lymphoblastic leukemia. Figure 1 shows immune recovery of natural killer (NK) cells, B cells, and

Table 1A. Patients' characteristics.

Number of patients	N=22	%
Diagnosis, n (%)		
Acute myeloid leukemia	9	41
Acute lymphoblastic leukemia	8	36
Myelofibrosis	4	18
Non-Hodgkin lymphoma	1	5
Plasma cell leukemia	1	5
Male gender, n (%)	16	73
Age, years, median (range)	63(27-73)	
EBMT-risk score, n (%)		
2-3	7	32
4-5	13	59
6-7	2	9

EBMT: European Blood and Marrow Transplantation.

Table 1B. Graft characteristics.

Number of infused cells	Median (range)
Nucleated cells (10 ⁶ /kg)	398.8 (261.5-534.1)
Viable CD34 ⁺ cells (10 ⁶ /kg)	3240.0 (1800.0-5800.0)
Viable CD3 ⁺ cells (10 ⁶ /kg)	36.2 (25.2-60.1)

CD4⁺ and CD8⁺ T cells after haploidentical HSCT. Normal values were not reached during the follow-up of 3 months. High resolution typing was performed for all 22 patients. *In vitro* tests could be performed for 11 out of the 22 patients for whom HLA-class II transduced HeLa cells were available (Figure 2). T-cell numbers increased a median of 1.6-fold (range, 0.3-36.2) in the HLA-class II allele-specific propagation cultures of the post-haploidentical HSCT PBMC samples. In four out of 11 (36%) patients tested, CD4⁺ T-cell alloreactivity towards HLA-class II mismatched alleles of the recipient was detected, in three cases towards DR alleles and in one case towards a DQ allele (Figure 2). The median CD4⁺ T-cell alloreactivity towards DR, DQ and DP alleles was increased 19.7-fold (range, 1.3-200.0), 1.6-fold (range, 1.0-3.8) and 1.4-fold (range, 1.0-1.6), respectively, as compared to reactivity towards the not transduced 'empty' HeLa cells. In the present study, a positive CD4⁺ T-cell response towards 'control' matched alleles was not observed for any of the tested alleles. The magnitude of the CD4⁺ T-cell response was significantly higher towards mismatched alleles than towards matched alleles, with the median fold increases being 1.7 (range, 1.0-200.0) versus 1.0 (range, 0.9-1.8), respectively ($P=0.03$; Mann-Whitney test). It should be noted that alloreactivity towards controls could not be determined for three patients because of an insufficient number of expanded T cells (Figure 2).

Both haploidentical HSCT and dUCBT are important alternative treatment modalities for patients in need of allogeneic HSCT but lacking a matched sibling or unrelated donor.¹ The early challenges of transplant complications related to graft failure and GvHD have been overcome with new strategies such as using two cord blood units in dUCBT and intensified immunosuppressive therapy including high dose PT-Cy in haploidentical HSCT.^{2,5} Haploidentical HSCT has a number of advantages in comparison to dUCBT, including unlimited donor availability and accessibility of post-transplant donor-derived immune cells. We earlier showed the presence of donor-derived CD4⁺ T cells specific for mismatched HLA class II

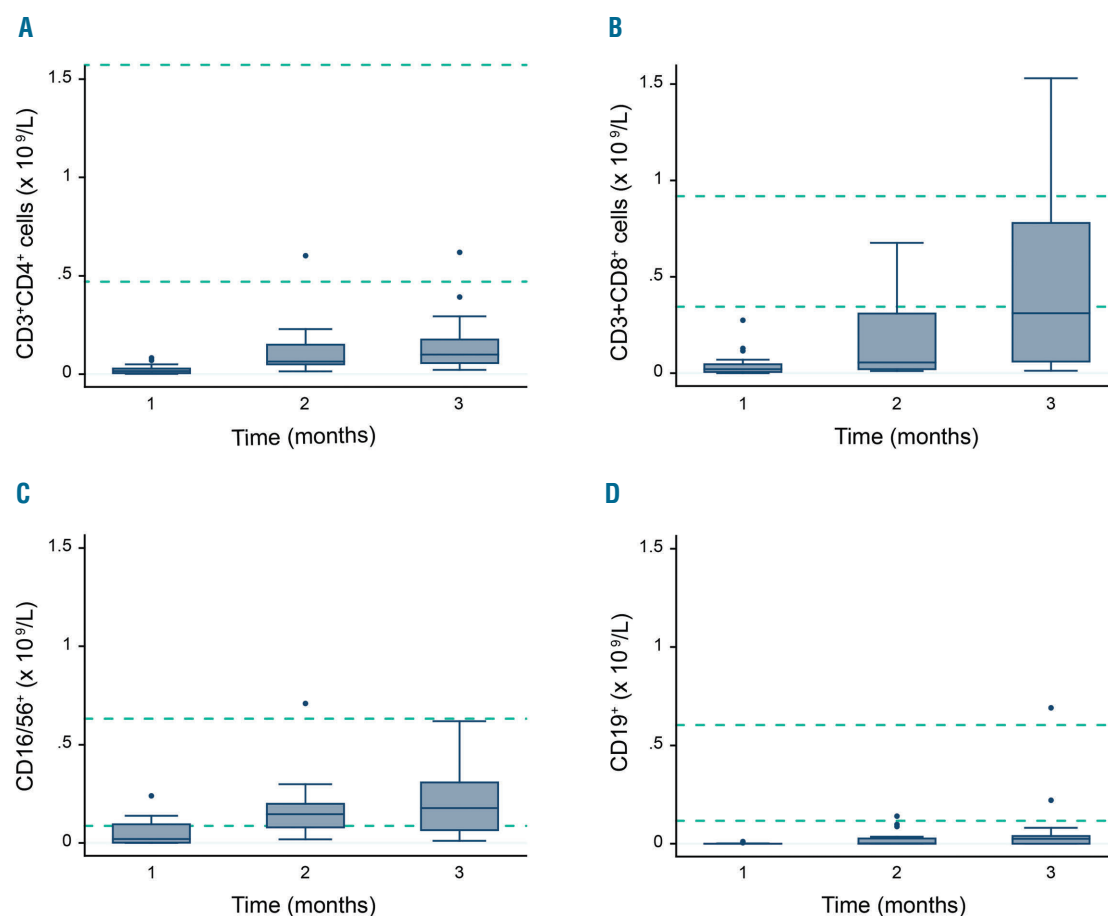


Figure 1. Immune recovery after allogeneic hematopoietic stem cell transplantation. (A) CD4⁺ lymphocytes, (B) CD8⁺ lymphocytes, (C) natural killer cells, (D) B-lymphocytes. Dashed lines represent the upper and lower limits of reference values.

alleles in 92% of patients after dUCBT. These alloreactive CD4⁺ T cells showed activity towards patient-derived leukemic cells, especially when the mismatch was shared between recipient and the 'loser' cord blood unit.^{3,4} In the present study, we set out to explore HLA-class II-specific CD4⁺ T-cell alloreactivity after haploidentical HSCT. Here we show the presence of such T cells for 36% of the tested alleles, which is much lower than the 92% detected after dUCBT, supporting our hypothesis that CD4⁺ T-cell alloreactivity may be less predominant after haploidentical HSCT.³

Current standard regimens of GvHD prophylaxis include the combination of a calcineurin inhibitor, cyclosporine, or tacrolimus, and either mycophenolate mofetil, or sirolimus, while prophylaxis in haploidentical HSCT consists of additional high-dose PT-Cy.⁶ Mycophenolate mofetil inhibits T-cell proliferation and adhesion, which hamper T-lymphocytic responses to allogeneic cells.⁷ Calcineurin inhibitors specifically inhibit intracellular signals responsible for T-cell activation after antigen binding, while PT-Cy induces apoptosis in alloreactive donor T cells after their activation and entry into the proliferative phase.^{8,9} Thus, it might further reduce the proliferation of early reconstituting alloreactive T cells with antileukemic potential. In addition, NK-cell recovery might also be compromised. Russo *et al.* recently studied NK-cell recovery and alloreactivity after haploidentical HSCT with PT-Cy.¹⁰ In that study, NK-cell proliferation was completely abrogated by day 8 after PT-Cy, suggesting selective elimination of cycling NK cells.

Importantly, donor NK cells harvested from patients at days 15 and 30 after haploidentical HSCT had a less mature phenotype. In addition these immature NK cells displayed less alloreactivity and an impaired antileukemic potential.

Overall, haploidentical HSCT with PT-Cy is characterized by a delayed early immune reconstitution, especially of CD4⁺ T cells, in comparison to the reconstitution following transplantation with donor cells from other sources, reaching normal levels after roughly 1 year.¹¹ Antigen-presenting cells play a central role in activating T cells and initiating immune responses. Several studies have shown that recovery of dendritic cells is also delayed after haploidentical HSCT and normal values are gradually achieved 1 year after transplantation.^{11,12} Delayed recovery of such antigen-presenting cells may lead to less presentation of recipient class II antigens and contribute to the relatively low numbers of alloreactive CD4⁺ T cells found in this study. A recent study explored dendritic cell recovery after dUCBT and matched sibling donor transplantation.¹³ Interestingly, umbilical cord blood recipients had better dendritic cell reconstitution in comparison to recipients of grafts from matched related donors, with normal values being reached at day 100 after transplantation. In addition, better dendritic cell recovery in the cord blood setting was associated with fewer relapses. Moreover, circulating class II-expressing cells after infusion of both cord blood units in a dUCBT might contribute to presenting mismatched HLA-class II to CD4⁺ T cells. Strong expression and/or a high number

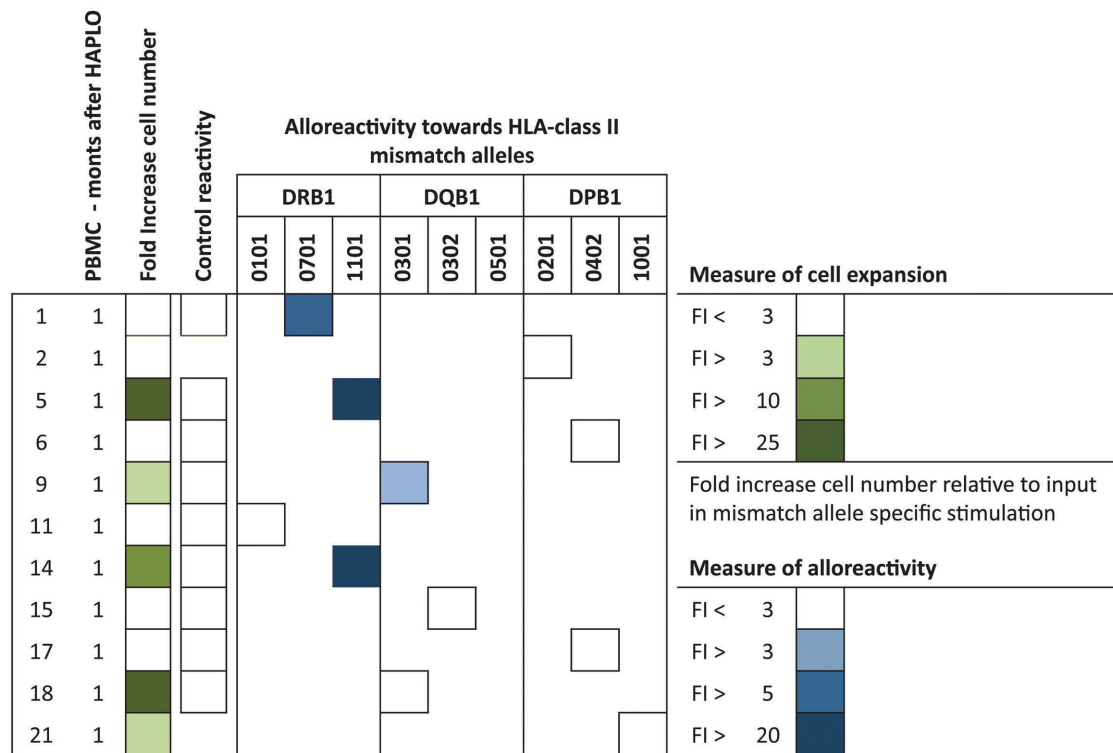


Figure 2. T-cell expansion, control reactivity and alloreactivity response. Eleven samples of peripheral blood mononuclear cells obtained from 22 patients 1 month after haploidentical hematopoietic stem cell transplantation were propagated towards mismatched HLA-class II alleles of the recipient in a T-cell and HeLa-cell co-culture, using a panel of HeLa cell lines, each transduced with a single HLA-class II allele. T-cell expansion of individual cultures at day 21 is presented as fold increase in cell number. Propagated T cells were subsequently tested for (allo)reactivity towards that same mismatched, and matched HLA-class II alleles. The response was quantified by CD137 upregulation on CD4⁺ T cells (%CD137⁺/CD4⁺) and presented for the alloreactivity as 'fold increase (%CD137⁺/CD4⁺)' of reactivity towards HeLa cells transduced with mismatched HLA-class II alleles relative to reactivity towards the not-transduced 'empty' HeLa cells. The level of T-cell expansion, control reactivity (towards matched alleles) and alloreactivity response are presented in a four-color grading scale. PBMC: peripheral blood mononuclear cells; HAPLO: haploidentical stem cell transplantation; HLA: human leukocyte antigen; FI: fold increase.

of antigen-presenting cells might evoke a stronger CD4⁺ T-cell response.

In conclusion, this study showed the presence of alloreactive CD4⁺ T cells early after haploidentical HSCT, although less frequently than previously observed in the dUCBT-setting.³ Intensified immunosuppression, especially PT-Cy, to prevent GvHD might blunt the CD4⁺ T-cell response, with possible loss of part of the graft-versus-leukemia activity. Our observations might help the understanding of the kinetics of immune responses in both haploidentical and cord blood transplantation. However, a prospective comparison would be needed to quantify the different immune responses in more detail. Moreover bone marrow was the sole source of the grafts in our study, whereas the use of PBSC as a transplant source might be associated with increased alloreactivity after transplantation.¹⁴

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doi:10.3324/haematol.2019.244152

Disclosures: no conflicts of interests to disclose.

Contributions: BK, AB, CHJL and JJC contributed to the study design; all authors provided study materials or patients; all authors were involved in collection and assembly of clinical data; BK, AB and JJC were involved in analyzing and interpreting the data and writing this report; and all authors reviewed and approved the final version of the manuscript.

References

- Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med.* 2006;354(17):1813-1826.
- McCurdy SR, Luznik L. How we perform haploidentical stem cell transplantation with posttransplant cyclophosphamide. *Blood.* 2019;134(21):1802-1810.
- Kalin B, ter Borg M, Wijers R, et al. Double umbilical cord blood transplantation in high-risk hematological patients: a phase II study focusing on the mechanism of graft predominance. *HemaSphere.* 2019;3(5):e285.
- Lamers CH, Wijers R, van Bergen CA, et al. CD4⁺ T-cell alloreactivity toward mismatched HLA class II alleles early after double umbilical cord blood transplantation. *Blood.* 2016;128(17):2165-2174.
- Barker JN, Weisdorf DJ, DeFor TE, et al. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. *Blood.* 2005;105(3):1343-1347.
- Luznik L, Fuchs EJ. High-dose, post-transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation. *Immunol Res.* 2010;47(1-3):65-77.
- Allison AC, Eugui EM. Mycophenolate mofetil and its mechanisms of action. *Immunopharmacology.* 2000;47(2-3):85-118.
- Liu J, Farmer JD, Jr., Lane WS, Friedman J, Weissman I, Schreiber SL. Calcineurin is a common target of cyclophilin-cyclosporin A and

- FKBP-FK506 complexes. *Cell*. 1991;66(4):807-815.
9. Eto M, Mayumi H, Tomita Y, Yoshikai Y, Nishimura Y, Nomoto K. Sequential mechanisms of cyclophosphamide-induced skin allograft tolerance including the intrathymic clonal deletion followed by late breakdown of the clonal deletion. *J Immunol*. 1990;145(5):1303-1310.
 10. Russo A, Oliveira G, Berglund S, et al. NK cell recovery after haploidentical HSCT with posttransplant cyclophosphamide: dynamics and clinical implications. *Blood*. 2018;131(2):247-262.
 11. McCurdy SR, Luznik L. Immune reconstitution after T-cell replete HLA-haploidentical transplantation. *Semin Hematol*. 2019;56(3):221-226.
 12. Pérez-Martínez A, González-Vicent M, Valentín J, et al. Early evaluation of immune reconstitution following allogeneic CD3/CD19-depleted grafts from alternative donors in childhood acute leukemia. *Bone Marrow Transplant*. 2012;47(11):1419-1427.
 13. Touma W, Brunstein CG, Cao Q, et al. Dendritic cell recovery impacts outcomes after umbilical cord blood and sibling donor transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2017;23(11):1925-1931.
 14. Bashey A, Zhang MJ, McCurdy SR, et al. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete haploidentical donor transplantation using post-transplant cyclophosphamide. *J Clin Oncol*. 2017;35(26):3002-3009.