SUPPLEMENTARY APPENDIX

# Rapid induction of single donor chimerism after double umbilical cord blood transplantation preceded by reduced intensity conditioning: results of the HOVON 106 phase II study

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**Supplemental File**

*Definitions*

High risk hematological malignancy was defined as (1) acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL) or myelodysplastic syndrome (MDS) with poor prognostic features in first complete remission (CR) (2) relapse AML/ALL/MDS in second or subsequent CR (3) chronic myeloid leukemia (CML) in second chronic phase (4) chronic lymphocytic leukemia (CLL) or low-grade non-Hodgkin lymphoma (NHL) responsive to at least third line chemotherapy.

The time to neutrophil recovery was defined as the number of days from transplantation to the first of 2 consecutive days with neutrophil counts ≥ 0.5 x $10^9$/l. Engraftment was defined as neutrophil recovery in combination with > 10% donor hematopoiesis in BM or, in the absence of BM results, in PB, as chimerism results of unseparated BM and unseparated PB were highly concordant. Secondary graft failure was defined as development of cytopenia without detection of donor markers after initial engraftment. Complete donor chimerism was defined as >95% donor hematopoiesis (single or both units); mixed chimerism was defined as 10-95% donor hematopoiesis and >5% recipient hematopoiesis. A unit was defined as “surviving unit” if finally > 95% of BM and/or PB cells originated from that unit. A unit was referred to as “non-engrafting unit” if in the end < 5% of BM and/or PB cells were derived from that unit. Acute GVHD was graded according to the Glucksberg criteria.

Progression-free survival (PFS) was calculated as the time from transplantation until progression/relapse (PD) or non-relapse mortality (NRM), whichever came first. Overall survival (OS) was determined from transplantation until death from any cause. Patients still alive at the date of last contact were censored.

*Transplant procedures*

The conditioning regimen consisted of cyclophosphamide (60 mg/kg) at day -7, fludarabine (40 mg/$m^2$) at days -6 to -3 and TBI 2 Gy at days -2 and -1. All patients received graft-versus-host

disease (GVHD) prophylaxis consisting of mycophenolate mofetil (MMF) 3 times a day from day 0 until day +30 and cyclosporin A, twice daily, from day -5 to at least day +90. Patients were hospitalized in laminar air flow rooms until neutrophil counts had reached 0.2 x $10^9$/l on two consecutive days. Beginning at day -7 until neutrophil recovery antibacterial and antifungal prophylaxis were given according to local protocols. Both units were infused at one day with a 2 hours' time interval in between in 6 patients. The majority of patients received their grafts on 2 subsequent days. This change in policy was made after several infusion reactions had been observed. Units were washed before infusion if the prefreeze RBC count exceeded 150 x $10^9$ per bag for major ABO-incompatible products or 300 x $10^9$ for all other units. RBC-depleted units were infused immediately after a direct-thaw procedure at the bedside. From day 0 until day +15 i.v. streptococcal prophylaxis was added. As from day 0 antiviral prophylaxis (valaciclovir) was given. Prophylaxis for Pneumocystis jirovecii (trimethoprim-sulfamethoxazole) was started after neutrophil recovery. Reactivations of cytomegalovirus (CMV) and EBV were treated according to protocol. Chimerism analysis, based on quantitative amplification of informative short tandem repeat (STR) regions by polymerase chain reaction (PCR), was performed on fresh unseparated peripheral blood (PB) cells, PB T-cells and unseparated bone marrow (BM) at 1, 2, 3, 6, 12 and 24 months post transplant. Analysis of early PB chimerism by PCR (days +11, +18 and +25) was performed retrospectively if cryopreserved samples were available.

*Analysis of early chimerism in leukocyte subsets*

Early chimerism in leukocyte subsets was analysed by the use of HLA-specific monoclonal antibodies (HLA-mAbs) in a subgroup of patients as described before[1]. Briefly, flow cytometry using HLA-mAbs was performed on PB if the presence of antigen mismatches between recipient and cord blood units allowed for the simultaneous detection of both recipient-derived and CBU-derived PB cells. This subgroup consisted of all patients that qualified for simultaneous 3 party detection after transplantation. Results were reported before as a part of a larger cohort of analysed patients[1].

*Additional analyses of cord blood units*

Absolute counts of viable CD34+ cells, lymphocyte subsets and NK-cells were measured by single platform flow cytometry using subset-specific mAbs and 7-aminoactinomycin D (7-AAD) or 4',6-diamidino-2-phenylindole (DAPI) staining. TNC viability was assessed with light microscopy using trypan blue dye exclusion. For CFU-GM and BFU-E analysis, $1x10^5$ and $2 x10^5$ cells were plated in 35 mm petridishes and cultured in MethoCult H84434 medium (StemCell Technologies, Vancouver, Canada). Cultures were incubated in a humidified atmosphere in 5% CO2 at 37ºC and counted after 14 days. Additional high resolution typing of HLA-A, -B, -C, DQB1 and -DPB1 was performed at the transplant centers if sufficient DNA was available.

**Reference**

1. Somers JA, Brand A, van Hensbergen Y, Mulder A, Oudshoorn M, Sintnicolaas K, et al. Double umbilical cord blood transplantation: a study of early engraftment kinetics in leukocyte subsets using HLA-specific monoclonal antibodies. Biol Blood Marrow Transplant 2013;19(2):266-273.

Supplemental Table 1. HLA match grade

| | unit vs recipient | | unit vs unit | |
|---|---|---|---|---|
| HLA-A,-B,-DRB1 selection criteria* | n=106 | | n=53 | |
| 3/6 | 0 | (0) | 1 | (2) |
| 4/6 | 61 | (57) | 39 | (74) |
| 5/6 | 41 | (39) | 8 | (15) |
| 6/6 | 4 | (4) | 5 | (9) |
| HLA-A,-B,-C,-DRB1 allele level, n (%) | n=103 | | n=50 | |
| 2/8 | 2 | (2) | 0 | (0) |
| 3/8 | 7 | (7) | 8 | (16) |
| 4/8 | 27 | (26) | 9 | (18) |
| 5/8 | 35 | (34) | 17 | (34) |
| 6/8 | 24 | (23) | 9 | (18) |
| 7/8 | 8 | (8) | 3 | (6) |
| 8/8 | 0 | (0) | 4 | (8) |
| HLA-A,-B,-C,-DRB1,-DQB1,-DPB1 allele level, n (%) | n=80 | | n=40 | |
| 3/12 | 1 | (1) | 1 | (3) |
| 4/12 | 3 | (4) | 3 | (7) |
| 5/12 | 9 | (11) | 3 | (7) |
| 6/12 | 12 | (15) | 7 | (18) |
| 7/12 | 22 | (28) | 8 | (20) |
| 8/12 | 22 | (28) | 12 | (30) |
| 9/12 | 10 | (12) | 2 | (5) |
| 10/12 | 1 | (1) | 0 | (0) |
| 11/12 | 0 | (0) | 3 | (7) |
| 12/12 | 0 | (0) | 1 | (3) |
| HLA-DRB1, -DQB1, -DPB1 allele level, n(%) | n=81 | | n=40 | |
| 0/6 | 0 | (0) | 1 | (3) |
| 1/6 | 3 | (4) | 1 | (3) |
| 2/6 | 11 | (14) | 9 | (22) |
| 3/6 | 32 | (40) | 11 | (28) |
| 4/6 | 21 | (26) | 8 | (20) |
| 5/6 | 12 | (15) | 9 | (22) |
| 6/6 | 2 | (2) | 1 | (3) |
| KIR ligand mismatch, n (%) | n=98 | | n=51 | |
| unidirectional (GVH) | 22 | (22) | 0 | (0) |
| unidirectional (HVG) | 28 | (29) | 0 | (0) |
| unidirectional (unit-vs-unit) | 0 | (0) | 21 | (41) |
| bidirectional | 6 | (6) | 7 | (14) |
| absent | 42 | (43) | 23 | (45) |

*HLA-A and -B: split antigen level; HLA-DRB1: allele level. Abbreviations: KIR: killer-cell immunoglobulin-like receptor; GVH: graft-versus-host; HVG: host-versus-graft