A phase II study to assess engraftment and engraftment kinetics after double cord blood transplantation with a reduced-intensity conditioning regimen in patients eligible for allogeneic stem cell transplantation lacking a matched unrelated donor.

PROTOCOL

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Signature of Investigator Date

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By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

1 Scheme of study

Age ≥ 18 and ≤ 65 years

AML/ ALL/ MDS/ CML/ AA
(high-risk disease)
Eligible for alloMUD-SCT
lacking a matched unrelated donor
and 2 matched (≥ 4/6) UCB units available

↓

Registration

↓

reduced intensity conditioning

Cyclophosphamide 60 mg/kg
Fludarabine 4x40 mg/m^2
TBI 2 x 2 Gy

↓

double UCBT

↓

Follow-up

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3 Synopsis

Study phase	Phase II
Study objectives	Evaluation of engraftment and progression-free survival following double cord blood transplantation after a reduced intensity conditioning regimen in adult patients
Patient population	Patients 18-65 years inclusive
Study design	Prospective
Duration of treatment	Expected duration of treatment is approximately 6 months (from conditioning regimen to stop immunosuppression).
Number of patients	40
Adverse events	Adverse events will be documented if observed, mentioned during open questioning, or when spontaneously reported
Planned start and end of recruitment	Start of recruitment: II 2008 End of recruitment: IV 2010

4 Investigators and study administrative structure

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5 Introduction

5.1 Allogeneic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (alloSCT) following myeloablative or nonmyeloablative cytotoxic therapy has proven to be a powerful treatment modality for adults and children with distinct hematological malignancies [1]. Apart from the antitumor/antileukemic effects of conditioning therapy, the T-cell and NK-cell mediated graft-versus-tumor effect is essential for complete eradication of disease. In case of relapse after alloSCT with stem cells from an HLA-identical sibling or matched unrelated donor (MUD), donor lymphocyte infusion, with or without preceding chemotherapy, has proven to be an important second chance for cure [2]. Both stem cells obtained from bone marrow or G-CSF-mobilized peripheral blood stem cells are applied. Compared to bone marrow (BM), larger grafts are obtained by G-CSF mobilized peripheral blood [3]. Nowadays peripheral blood stem cells are the preferred source of stem cells since it is known that the time to engraftment is dependent on the number of CD34+ cells.
AlloSCT is conducted with myeloablative as well as nonmyeloablative (reduced-intensity) conditioning. Myeloablative conditioning contributes to the eradication of the underlying hematological malignancy and provides immune suppression, which is essential to prevent graft rejection. The role of reduced-intensity conditioning (RIC) is mainly confined to provide sufficient immune suppression to prevent graft rejection. It has been shown that treatment related mortality (TRM) can significantly be reduced in older recipients of alloSCT following reduced-intensity conditioning [4]. While HLA-identical sibling donors are preferred, HLA-matched unrelated donors are used with increasing frequency in several hematological malignancies [5]. The chance of finding a suitable matched unrelated donor in the registries is approximately 70% for patients with a Caucasian background [6]. In general it takes several months before a suitable donor can be identified [7]. Chances to find a matched unrelated donor are limited for non-Caucasian people and estimate approximately 30-40% [6]. Alternative stem cell sources include haplo-identical family donors and umbilical cord blood (UCB). Especially with UCB transplantation clinical experience has been obtained on a large scale.

5.2 Umbilical cord blood transplantation

In 1988 the first umbilical cord blood transplantation (UCBT) was conducted in a child suffering from Fanconi's anemia [8]. Currently, UCBT with related or unrelated UCB is an important alternative way of alloSCT in children with hematological malignancies and certain non-malignant diseases [9]. Results after UCBT are at least comparable to results after MUD-BMT. A CIBMTR retrospective analysis compared the outcome of 492 unrelated bone marrow transplantations

(BMT) and 508 UCBT in children with ALL or AML [10]. Median follow up of survivors was 59 months for BMT and 45 months for UCBT. As compared to matched unrelated BMT, a lower transplant related mortality (TRM), treatment failure and overall mortality were observed after matched UCBT. Risks were however similar to matched unrelated BMT after high cell dose UCBT with a mismatch at 1 locus. The risk of acute graft-versus-host disease (GVHD) was lower after matched UCBT, whereas the risk of chronic GVHD was lower after matched or mismatched UCBT as compared to matched BMT. Estimated chances to find a suitable cord blood unit (CBU) have increased to 99%, 71% and 13% for a 4/6 matched, 5/6 matched and 6/6 matched CBU, respectively for Caucasian patients because more mismatches than currently acceptable for matched unrelated donors seem acceptable in UCBT [11]. For non-Caucasian patients, estimated chances to find a 5/6 or 4/6 matched UCB are 48-60% and 99%, respectively [11]. Another advantage is the shorter time needed for a search: a matched UCB unit can be available within several weeks [7]. At this moment, the major disadvantages of UCBT are the relatively small number of progenitor cells in the graft, causing a delayed engraftment and the lack of donor lymphocytes in case of relapse.

Single UCB transplantation in adults

Rocha et al. showed that UCB from an unrelated donor may be an alternative source of hematopoietic stem cells for adults with acute leukaemia who lack an HLA-matched related or volunteer matched unrelated donor [12]. After myeloablative conditioning the outcome in patients of at least 15 years of age with acute leukemia who received a single cord UCBT compared well to that after MUD-BMT. Median time to neutrophil recovery was 26 days after UCBT as compared to 19 days after MUD-BMT. Primary graft failure occurred in 20% after UCBT and in 7% after BMT. Secondary graft failure after UCBT was comparable (1 of 77 vs. 5 of 528) to what could be observed after MUD-BMT. The cumulative incidence of acute GVHD grades II, III and IV was 26% after UCBT and 39% after BMT. The risk of chronic GVHD was not significantly different between the two groups. Overall survival and leukaemia-free survival were also comparable in both groups. Infection was the main cause of death after UCBT, whereas deaths related to GVHD were significantly more common in the bone marrow group. There was no significant difference in TRM between the two groups (2 years cumulative incidence 44% vs. 38% for UCB and BM recipients respectively). Also, the risk of relapse was not significantly different in the two groups (23% in both groups). Laughlin et al. compared outcome of alloSCT from unrelated donors in adults with leukaemia who had received UCB or BM with either none or one HLA-mismatch [13]. TRM, treatment failure and overall mortality were similar after transplantation with UCB or alloSCT with a bone marrow graft with one HLA-mismatch. No differences in the rate of recurrence of leukaemia were observed among the 2 groups. Recently Takahashi et al. described the clinical outcomes of

171 adults with haematological malignancies who received an unrelated UCBT, related BMT or related PBSCT [14]. A significant delay in engraftment occurred after cord blood transplantation. However, overall engraftment occurred in 91% after UCBT and 97% after BMT/PBSCT. Incidences of acute GVHD grades III-IV and chronic extensive GVHD were lower after UCBT. TRM, relapse rate and disease free survival were similar in both groups.
The major problem after a single UCBT in adults appears to be primary graft failure and a delayed hematopoietic recovery caused by the small number of hematopoietic stem cells in cord blood grafts. Several approaches are currently explored to improve engraftment.
Preclinical studies have shown that cotransplantation of human mesenchymal stem cells with CD34-selected mobilized human peripheral blood stem cells may enhance human myelopoiesis and megakaryocytopoiesis in NOD/SCID mice [15]. It was speculated that mesenchymal stem cells provide critical growth factors and/ or adhesion receptors for human cell development. Noort et al. found that human fetal lung-derived mesenchymal stem cells promote engraftment of UCB stem cells in NOD/SCID mice [16]. Clinical data suggest that co-infusion of a cord blood unit and a low number of haplo-identical CD34+ cells may result in a shortened period of post transplant neutropenia [17]. This could be caused by transient engraftment of the haploidentical hematopoietic stem cells. An alternative approach is ex vivo expansion of progenitor cells from UCB [18, 19], but this approach has not been applied clinically. Recently, several clinical studies have shown that double UCBT is a safe and promising approach in adults [20, 21].

Double UCB transplantation in adults

Many adults are not eligible for single UCBT because infusion of a certain minimum number of cryopreserved total nuclear cells (TNC) is considered to be required to ensure engraftment [22]. Preclinical studies suggested that co-transplantation of two units of (partially) HLA-matched cord blood may overcome the limitation of the low cell dose in single UCBT [23]. Wagner et al. observed that patients who received a double UCBT did as well as patients transplanted with a single adequately sized umbilical cord blood graft with respect to rates of TRM, acute GVHD, neutrophil and platelet engraftment, disease free survival and overall survival after a myeloablative conditioning regimen [24]. Barker et al. reported 50 patients transplanted with a double CBU (cord blood unit) after myeloablative conditioning [24]. The median time to neutrophil recovery was 24 days; the incidence of acute GVHD gr III and IV was 20%. Kai et al. found similar results [25]. In a retrospective case-control study of myeloablative single UCBT versus double UCBT a shorter time to engraftment (20 vs 17 days for single and double UCBT) and a better overall survival (18 % vs 72% respectively for single and double UCBT at 12 months) were observed [26]. Infection was the primary cause of death at 100 days for recipients of a single UCBT (9/20 patients), whereas no infection-related deaths were observed after double UCBT in that study. It has been shown that

(double) cord blood transplantation after reduced-intensity conditioning is feasible as well [27, 28]. So far, various reduced-intensity conditioning regimens have been used. Miyakoshi et al. reported 30 patients transplanted with a single CBU after a conditioning regimen composed of fludarabine, melphalan and TBI 4Gy [29]. The cumulative incidence of complete donor chimerism at day 60 was 93% with a median time to neutrophil engraftment of 17.5 days. TRM within 100 days was 27% and the estimated 1-year overall survival was 32.7%. Brunstein et al. described results of 110 patients transplanted either with one or two CBU's, depending on total amount of TNC/kg per unit [30]. The conditioning regimen consisted of fludarabine, cyclophosphamide and TBI 2Gy. Neutrophil recovery was achieved in 92% at median of 12 days. TRM was 26% at 3 years. Survival and event-free survival (EFS) at 3 years were 45% and 38%, respectively. The probability of EFS was better using two units (39%) vs. one unit (24%).
Sustained hematopoiesis is usually derived from a single donor after double UCBT [20, 31]. This predominance can be found shortly or later after transplantation. So far, the distinct contributing factors which lead to predominance of the prevailing cord blood graft are not known. It is suggested that T-cell mediated immune effects, a NK-mediated effect or KIR mismatch play a role [32, 33]. CD3-content, type of HLA-mismatch, order of infusion, cell dose and ABO-mismatch do not seem to have an overruling effect on engraftment [20].

5.3 Selection of umbilical cord blood units

The major criteria for the selection of cord blood units are the total nucleated cell number and degree of matching for histocompatibility leukocyte antigens (HLA). Most studies report the total nucleated cell count (TNC) of the CBU measured before cryopreservation. The number of CD34 positive cells in the UCB is sometimes also used, but enumeration of CD34+ cells varies between centers and varies over time and therefore is not instrumental in the selection of CBU's [34]. Thus, although TNC is not directly reflecting the number of haematopoietic stem cell progenitors, at present it is the best available surrogate parameter and therefore will be used for the selection of CBU's in this study.
Which dose of TNC should be aimed for? In a study of 562 single UCBT of which 102 (18%) were performed in adults, the rate of myeloid engraftment was strongly dependent on the number of TNC infused [35] in relation to match degree. Median time to myeloid engraftment decreased from approximately 35 days in recipients of a CBU containing 0.7-2.4x10^7 TNC/kg to approximately 17 days in recipients receiving >10x10^7 TNC/kg, without reaching a plateau. Gluckman et al. analyzed 550 myeloablative UCBT in the Eurocord registry [36]. Most patients suffered from hematological malignancies, 35% were adults. The hazard of neutrophil recovery was found to be related to the TNC content of the CBU at freezing. Neutrophil recovery (neutrophil count > 0.5 x 10^9/l) at day 60 was reached in 80% of patients when the TNC dose was ≥ 4.0 x 10^7/kg in comparison to 69%

when the TNC dose was < 4.0 x 10^7/kg (p=0.0008). Also, a high number of infused TNC was predictive of platelet recovery. Although it is now generally accepted that the minimum TNC dose in single UBCT is a TNC of 2.0-2.5 x 10^7/ kg [22], these data raise the question whether a higher cell dose might be more optimal.

HLA-matching

HLA-matching is less critical for CBU's than for haematopoietic stem cells from unrelated adult donors, where typically an 8/8 match for A, B, Cw and DRB1 at the 4-digit level is required. In UCBT, HLA-matching is performed for the HLA-A and HLA-B locus at the serological split-antigen resolution level and at the HLA-DRB1-locus at the 4-digit resolution level. Thus, matching is performed for 6 HLA-antigens at 3 loci. From general experience, most transplant centers consider 2 out of 6 HLA-mismatches the maximum acceptable number of mismatches. Only a few studies have addressed the question as to whether the number of HLA-mismatches correlates with engraftment, the incidence of GVHD, TRM, relapse and survival. In a study of 562 UCBT, Rubinstein et al. reported that myeloid engraftment was more often successful in donor-recipient pairs without HLA-mismatches (100% engraftment) than in those with 1-3 HLA-mismatches (69-78% engraftment) [35]. In the Eurocord study of 550 patients it was found that with increasing number of mismatches engraftment was impaired so that the hazard of neutrophil recovery was linearly related to the number of HLA disparities i.c. up to 3 mismatches [36].

Cell dose versus HLA-match

The relative importance of cell dose versus HLA-matching in selecting CBU's is not known. There are no published data with regard to the relative importance of cell dose versus HLA-match grade. Also, it has been speculated that the negative effects of HLA-mismatches might be overcome by an increased cell dose, albeit with an increased risk of grade III-IV GVHD [36].

5.4 Conclusion

Currently, double UCBT seems the most promising approach to overcome the limitations of restricted number of progenitor cells in adult patients who qualify for alternative donor transplantation and lack a properly matched volunteer unrelated donor. Therefore, it is our intention to evaluate this approach prospectively in a cohort of adult patients. Double UCBT will be preceded by a reduced-intensity conditioning regimen. Endpoints will include graft failure, as well as time to engraftment of different cell lineages. In addition, immunological monitoring will be performed in order to evaluate whether parameters can be identified that predict which graft ultimately prevails.

6 Study objectives

To assess engraftment and engraftment kinetics after double cord blood transplantation preceded by a reduced-intensity conditioning regimen in patients eligible for allogeneic stem cell transplantation lacking a matched unrelated donor.
To evaluate immune reconstitution, acute and chronic GVHD, chimerism, toxicity, time to treatment failure, progression-free survival and overall survival after double unit UCBT.
To study patient-versus-graft, graft-versus-patient and graft-versus-graft interactions

7 Study design

This is a prospective phase II study. Patients lacking a matched unrelated donor and patients for whom a matched unrelated donor cannot be identified within 2 months and for whom an allogeneic transplant is urgently needed, are eligible for double UCBT if two suitable UCB units are available. Transplantation will be preceded by a reduced-intensity conditioning regimen, irrespective of patient age. The choice for a standard reduced-intensity conditioning regimen (even in patients below the age of 40 years) gives the opportunity to create a homogenous patient population and to gain experience with a certain conditioning regimen in a short period of time in this small patient category. Also, a reduced-intensity conditioning regimen is less toxic in this heavy pretreated patient group compared to a myelo-ablative regimen, the neutropenic period is expected to be shortened and autologous recovery in case of graft failure will occur. Post grafting immunosuppression is performed by mycophenolate mofetil (30 days) and cyclosporine A (90 days, taper thereafter).

8 Study population

8.1 Patient selection

8.1.1 Eligibility criteria

- Age 18-65 years inclusive
- Meeting the criteria for a MUD allo SCT and high risk disease * (see below)
- Lacking a sufficiently matched volunteer unrelated donor or lacking such a donor within the required time period of ≤ 2 months in case of urgently needed alloSCT
- Availability of 2 sufficiently matched UCB grafts with a total nuclear cell count > 4 x 10^7/kg (see paragraph 8.2).
- WHO performance status ≤ 2

- Written informed consent

*High risk disease as defined by:

- AML with -5, -7, EVI-1-expression or complex karyotype in first CR
- Relapsed AML/ MDS in second or subsequent CR
- ALL with t(9;22), t(4;11), t(1;19) or with high WBC at diagnosis (B-ALL > $30x10^9$/l, T-ALL > $100x10^9$/l) in first CR, or no CR after first induction but in CR after rescue chemotherapy
- Relapsed ALL in second or subsequent CR
- CML in second chronic phase after treatment for CML blast crisis
- VSAA or SAA relapsing after or failing immunosuppressive therapy

Patients with the following diseases may be included if considered high risk disease:

- Relapse AML with t(8;21) or inv16 in second or subsequent CR, with poor risk according to Breems prognostic score (appendix A)
- AML/MDS in patients 61-65 years inclusive, in first CR
- CML in second chronic phase after treatment for acceleration phase
- Lymphocytoplasmacytoid lymphoma, responsive disease after at least third line chemotherapy
- Folliculair NHL, responsive disease after at least third line chemotherapy
- CLL, responsive disease after at least third line chemotherapy

8.1.2 Exclusion criteria

- Relapse APL
- Primary myelofibrosis
- Bilirubin and/or transaminases > 2.5 x normal value
- Creatinine clearance < 40 ml/min
- Cardiac dysfunction as defined by:
  - Reduced left ventricular function with an ejection fraction $\leq$ 45% as measured by MUGA scan or echocardiogram (another method for measuring cardiac function is acceptable)
  - Unstable angina
  - Unstable cardiac arrhythmias
- Pulmonary function test with VC, FEV1 and/ or DCO < 50%
- Active, uncontrolled infection
- History of high dose total body irradiation

- HIV positivity

8.2 Cord blood selection

TNC dose in selected CBU's has a strong influence on both the rate and success of myeloid engraftment. We conclude that the commonly recommended minimum TNC dose of 2 – 2.5 x 10^7 /kg is probably still too low for consistent succesful and rapid engraftment. Therefore, in this study we will evaluate the results UCBT using a higher TNC dose which will be obtained by the consequent use of double cord blood units and strict selection criteria with regard to cell dose. Also, the TNC dose for each CBU will be adjusted for the presence of HLA-mismatches, whenever this is feasible.

CBU's will be selected according to the following criteria:

1. The total amount of total nucleated cells present in both CBU's together must be > 4.0 x 10^7/kg recipient body weight.
2. The minimum amount of total nucleated cells present in each CBU must be > 1.5 x 10^7/kg recipient body weight.
   Preferably, the minimum number of TNC in the CBU is higher as more HLA-mismatches are present as follows:
   a if 0/6 mismatches, then TNC >1.5 x 10^7/kg
   b if 1/6 mismatches, then TNC >2.0 x 10^7/kg
   c if 2/6 mismatches, then TNC >3.0 x 10^7/kg.
3. HLA-matching is performed for HLA-A and HLA-B at the serological split resolution level and for HLA-DRB1 at the 4-digit resolution level. The minimal match grade required is a 4/6 match both between recipient and CBU's and between CBU's.
4. Absence of HLA-antibodies in the recipient directed against HLA class I and class II mismatches on the cord blood cells is required.
5. Preferably, RBC- and plasma reduced CBU's are selected.
6. Preferably, ABO-compatible or minor ABO-mismatched CBU's are selected.

Note: If recipient anti-A/anti-B titer >1:512, (NaCl gel card (Diamed, Switzerland); incubation either at room temperature (IgM) or after treatment with 0,01M DTT at 37° C (IgG), major incompatible non-RBC reduced / non-plasma reduced CBU's are not administered.

9 Treatments

9.1 Allogeneic transplantation

9.1.1 Conditioning regimen

A reduced-intensity conditioning regimen is used.

Scheme:	day -7:	cyclofosfamide 60 mg/kg i.v.
	days -6, -5, -4, -3:	fludarabine 40 mg/m^2 i.v.
	days -2, -1:	TBI 2 Gy

9.1.2 GVHD prophylaxis

Cyclosporin A

Cyclosporin A (CsA) is given at 1.5 mg/kg iv. b.i.d., first dose at day -5. Adjust CsA to through levels between 250-350 µg/ml until day +90. Gradual taper after day +90 until day +180. When dose adjustments are necessary it should be aimed at maintaining the blood levels in the upper part of the therapeutic range. Drugs that may affect CsA levels include: steroids, fluconazole, ketoconazole, cimetidine (may increase CsA levels).

Mycophenolate Mofetil

Mycophenolate Mofetil (MMF) is given at 10 mg/kg p.o./ i.v. 3 dd, first dose at day 0. Stop at day +30.

CsA doses will be given with a 12 hours time interval
MMF doses willl be given with a 8 hours time interval.
Since significant nausea may accompany the conditioning regimen and this immunosuppression, regularly scheduled antiemetic therapy is recommended for all patients for at least a week after the transplant.

9.1.3 Conditioning regimen and immunosuppression schedule

Conditioning regimen and immune supression schedule

Day	- 7	- 6	- 5	- 4	- 3	- 2	- 1	0	+ 1
Cyclophosphamide	X								
Fludarabine		X	X	X	X				
TBI						X	X		
UCBT								X	X
Cyclosporine A			X	X	X	X	X	X	X →
MMF								X	X →
(val)aciclovir								X	X →
benzylpenicilline									X →

9.1.4 Special management orders during hospitalisation

Infections should be controlled before start of the conditioning regimen. Selective decontamination (SD) consisting of anti-bacterial agents (for example ciprofloxacin, 500 mg b.i.d. p.o. or 400 mg b.i.d. i.v.) and antimycotic agents (for example fluconazole 400 mg p.o./ i.v.) are given. Surveilance cultures of oral cavity, rectum and vagina are monitored twice a week.
Streptococcal prophylaxis (for example benzylpenicillin 1 g i.v. 6 dd) is given commencing day 0 to at least day +15. Antiviral prophylaxis is given commencing day 0 to at least day 360.
After SCT, CMV-PCR and EVB-PCR are monitored twice a week. Patients are hospitalized until neutrophil counts are at least > 0.2 x 10^9/l on two days in a row. PCP prophylaxis (co-trimoxazole 480 mg) is started after hematological reconstitution and will be given for at least 360 days.
Patients who do not engraft can discontinue infection prophylaxis at 3 months post-transplant. After this time period the immune suppressive effect of the conditioning regimen has resolved.

Specific investigations during hospitalisation:

- Surveillance cultures according to bacteriology guidelines
- Daily interim history and physical examination
- Daily platelet count
- WBC and differential at least every other day
- BUN, creatinin, sodium, potassium, calcium, glucose daily during chemotherapy, and thereafter at least twice weekly

- ASAT, ALAT, alkaline phosphatase, γ-GT, bilirubin and LDH at least twice weekly
- Monitoring of CMV-PCR and EVB-PCR twice a week
- Chest X-ray once during the first week, thereafter as clinically indicated

9.2 Cord blood processing and infusion

Depending on the existence of major ABO-incompatibility between CBU and recipient and the number of prefreeze RBC CBU's will undergo a careful washing procedure after thawing or will be infused immediately after a direct-thaw procedure.
Major ABO-incompatible CBU's will undergo a post-thaw washing procedure if the total prefreeze RBC count exceeds 150 x 10^9 . Minor ABO-incompatible or ABO-compatible CBU's will undergo a post-thaw washing procedure if the total prefreeze RBC count exceeds 300 x 10^9. In all other cases CBU´s will be infused immediately after a direct-thaw procedure. Grafts will be infused on two consecutive days (day 0 and day +1). An ABO compatible graft will be given first.
At the end of each day screening for hemolysis (haptoglobin, bilirubin, LDH, creatinin, CBC, DAT and plasma-HB) will be performed.

9.3 Treatment of GVHD

9.3.1 Treatment of acute GVHD

- Optimise CsA levels, if necessary give CsA intravenously.
- Prednisone (1 mg/kg b.i.d.) in case of severe and progressive GVHD gr 2-4. Prednisone should be given at 2 mg/kg for 10 days and, in case of response, tapered by 50% dose reduction every 5 days thereafter.
- If GVHD is not improving after 5 days treatment with prednisone, methylprednisolone 30 mg/kg b.i.d is given.
- Anti-thymocyte globulin may be given (optional) if GVHD is not improving within 14 days after start methylprednisolone.

9.3.2 Treatment of chronic GVHD

Limited: Cyclosporine A + prednisone 20 mg b.i.d.
If localized to skin only: corticosteroid ointment.

Extensive: Cyclosporine A, prednisone, if necessary: resume MMF

CsA and prednisone therapy has to be supported by antibiotic prophylaxis with co-trimoxazole and antiviral prophylaxis with valacyclovir.

9.4 Treatment of viral reactivation

9.4.1 Treatment of CMV-reactivation/ CMV-disease

CMV-reactivation:
Start with pre-emptive therapy if PCR-CMV ≥ 500 copies/ ml.
Treatment: valganciclovir 450 mg dd, first day 900 mg

CMV-disease:
Ganciclovir 5 mg/kg b.i.d., followed by valganciclovir 900 mg b.i.d..
CMV-immunoglobulin 50U/kg day 1, 4 and 8

9.4.2 Treatment of EBV-reactivation/ EBV-LPD

EBV-reactivation:
Start with pre-emptive therapy if PCR-EBV ≥ 1000 copies/ ml.
Treatment: rituximab 375 mg/m^2 i.v.
EBV-LPD:
Rituximab 375 mg/m^2 i.v.
Discontinuation of all immune suppressive therapy

9.5 Infection prophylaxis

Patients will receive prophylaxis for PCP, toxoplasmosis and HSV as per standard practice. Prophylaxis will be discontinued 1 year post-transplant, unless the patient is receiving treatment for chronic GVHD (prophylaxis should be extended). HSV prophylaxis should be started at day 0. Standard PCP and toxoplasmosis prophylaxis should be started at the time of hematological reconstitution after transplantation.
Standard CMV monitoring should commence at the time of transplant and should continue until at least day +360. CMV-prophylaxis should be given concomitantly with corticosteroid treatment (prednisone ≥ 40 mg/day or equivalent). Standard EBV monitoring should commence at the time of transplant and should continue until at least day +360.

The following vaccination scheme is recommended (to start with when immune suppressive therapy has been stopped):

Months after UCBT	6	12	13	14	24	
Prevenar		X			X	
Pneumovax				X		
Act-Hib		X			X	
DTP		X			X	
Meningotec/NeisVac		X			X	
Influvac	X*					Annually

* Depending on season; independent of concomitant use of immune suppressive therapy

10 End of protocol treatment

1. Completion of protocol treatment
2. No compliance of the patient (especially refusal to continue treatment)
3. Major protocol violation
4. Progression/relapse
5. Death

11 Required clinical evaluations

11.1 Pre-transplant evaluation at entry

Medical history	
Physical examination	Including WHO-performance status
Hematology	Hb, MCV, leukocyte count incl differential count, platelets
Blood chemistry	sodium, potassium, BUN, creatinin, liver enzymes, total bilirubin, albumin, LDH, calcium, glucose
Coagulation tests	PT, APTT
ABO-Rh-D and complete RBC blood groups, anti-A, anti-B, DAT, anti-HLA-antibodies (class I and II)	
Serological tests	CMV, EBV, HIV, hepatitis B/C, HTLV1+2, toxoplasma, lues
HbF, HbA	
Total counts of B-, T-, CD4, CD8 and NK cells	
Chest X-ray	
ECG	
Cardiac ejection fraction	
Pulmonary function test	Incl. VC, FEV1, TLC, diffusion capacity
Assessment of chimerism by VNTR/STR	
Bone marrow examination	Incl. markers of MRD
Evaluation of disease status	
Sampling for biological studies	Peripheral blood: 10 ml, 1-2 weeks pre SCT

Pretransplant investigations should include the following:

Medical history

A complete history with full details of

- Prior treatment and response
- Infections

Physical examination

Standard physical examination including body weight and height with special attention for:

- determination of WHO-performance status
- Findings related to underlying malignancy and prior treatment
- Infections

Hematology

Hemoglobin
Leukocyte count, differential count
Platelets
MCV
Total counts of B-, T-, CD4, CD8 and NK cells.

Chemistry
Sodium
Potassium
BUN
Creatinin
Liver enzymes
Total bilirubin
Albumin
LDH
Calcium
Glucose

Coagulation tests
PT
APTT

Additional blood analysis
ABO-D and complete blood groups
Anti-A, anti-B titer (see 8.2)
Anti-HLA-antibodies (class I and class II)
DAT
HbF, HbA
Total counts of B-, T-, CD4, CD8 and NK cells
CMV
EBV
HIV
HTLV
Hepatitis screen
Toxoplasma

Lues

Specific investigations

Chest X-ray

ECG

Cardiac ejection fraction

Pulmonary function test including VC, FEV1, TLC and diffusion capacity

Assessment of chimerism by VNTR/STR: pretransplant samples to be sent for evaluation of posttransplant chimerism

On indication: CT-scan

Bone marrow examination

Morphology

Markers of MRD: immunophenotyping, cytogenetics, molecular analysis

On indication: bone marrow biopsy

Sampling for biological studies

10 ml peripheral blood (heparinized) to be sent to Sanquin Blood Bank region Southwest, Leiden (see appendix G)

11.2 Post transplant evaluation

Chimerism will be evaluated on days +30, +60, +90, +180, +360 and then yearly until 5 years post-transplant. Chimerism studies will be done in peripheral blood, in peripheral blood T-cells and in bone marrow aspirate for mononuclear cells.

	Months after UCBT	1	2	3	6	12	24 **
1.	WHO performance	X	X	X	X	X	X
2.	Physical examination	X	X	X	X	X	X
3.	Haematology	X	X	X	X	X	X
4.	Chemistry	X	X	X	X	X	X
5.	ABO/haptoglobin	X	X	X	X	X	X
6.	HbF, HbA	X	X	X	X		
7.	PCR EBV/CMV	X	X	X	X	X	X
8.	PB chimerism	X	X	X	X	X	X
9.	CD3 chimerism	X	X	X	X	X	X
10.	BM chimerism/ morphology	X	X	X	X	X	X
11.	PB sampling for biological studies	X	X	X	X	X	X
12.	BM sampling for biological studies	X	X	X	X	X	X
13.	BM MRD *	X	X	X	X	X	X
14.	IMRE	X	X	X	X	X	X
15.	Disease evaluation			X	X	X	X

* if MRD-marker is present

** chimerism studies, cryopreservation and IMRE at least yearly thereafter until 5 years post-transplant

1. According to WHO classification, appendix B.
2. Careful examination including weight, signs of toxicity, infection, GVHD and VOD.
3. Hematology: complete blood cell counts (CBC) daily from day 0 until ANC>0.5 x 10^9/l for 2 days after nadir reached. Thereafter at each outpatient clinic visit.
4. Chemistry: electrolytes, albumin, glucose, creatinine, BUN, bilirubin, ALT/AST, alkaline phosphatase at each outpatient clinic visit.
5. In case of ABO-incompatibility: ABO-blood group, haptoglobin monthly.

6. HbF, HbA weekly, starting at day 0 (before SCT), continue until returned to reference value ; At every outpatient clinic visit when visit frequency is less than once a week
7. During hospitalisation 2x/week, thereafter every outpatient clinic visit for at least one year.
8. Heparinized peripheral blood to perform chimerism analysis by DNA (VNTR/STR).
9. Heparinized peripheral blood to perform CD3 selection followed by chimerism analysis by DNA (VNTR/STR).
10. Bone marrow aspirate for morphology; heparinized bone marrow to perform chimerism analysis by DNA (VNTR/STR).
11. (see appendix G)
    - For chimerism studies: weekly, starting at day 11-14 until single chimerism is reached: 10 ml (after hematological recovery: 6 ml)
    - At 3, 6, 12 months: cryopreservation of plasma (thereafter every 6 months)
    - At 3, 6, 12 months: cryopreservation of peripheral blood (thereafter every 6 months)
12. (see appendix G)
    - For chimerism studies: at 1, 2, 3, 6, 12, 24 months: 10 ml bone marrow
13. Bone marrow aspiration for MRD depending on presence of MRD markers.
14. Total counts of B-, T-, CD4, CD8 and NK cells.
15. Complete evaluation including physical examination, blood counts and radiology, depending on underlying disease.

11.3 Evaluation of CBU's

Data of CBU's will be collected pre-selection, after selection and after thawing.

Additional information will be collected by a qualification cord blood form.

		Pre-selection	After selection	After thawing *
1.	HLA-typing	A, B, DRB1		High-resolution
2.	TNC, viability	X		X
3.	Total RBC count	X	X	
4.	RBC / plasma depl.	X	X	
5.	CBU volume	X		
6.	Total CD34+ cell number (HPC)		X	X, viability
7.	Total CD3+ cell number (T-cells)			X, viability
8.	Total CD19+ cell number (B-cells)			X, viability
9.	Total CD3- CD16/56+ cell number (NK cells)			X, viability
10.	CFU-GM			X
11.	BFUe			X
12.	CMV	X		
13.	VNTR/STR			X
14.	Bacteriol cultures	X		X
15.	ABO-Rh blood group	X		
16.	Sex donor	X		
17.	Year of collection	X		
18.	Number of bags Volume of bags	 X	X	
19	Sampling for biological studies			X (see appendix G)

* without and with washing

1. Pre-selection confirmation of HLA-typing (at least A, B serological split-level and DRB1 at 4-digit level) will be performed by the providing cord blood bank or an EFI accredited HLA laboratory. Also HLA 4-digit typing for A, B, DQB1 and Cw will be performed if sufficient DNA is available after thawing

2. The prefreeze TNC as measured by the cord blood bank will be used for selection of CBU's. TNC will also be determined after thawing.
3. The prefreeze RBC as measured by the cord blood bank will be used for selection of CBU's. RBC will be measured after thawing as well.
4. Information about RBC-or plasma depletion is necessary and has to be provided by the cord blood bank for selection of CBU's.
5. The volume of CBU's will be provided by the cord blood bank.
6. The total amount of viable CD34-positive cells and CD34+ cells/kg recipient will be determined after thawing.
7. Total T-cell number will be measured after thawing.
8. Total B-cell number will be measured after thawing.
9. NK-cell number will be measured after thawing.
10. CFU-GM will be performed after thawing.
11. BFUe will be performed after thawing.
12. CMV-serology of the mother is provided by the cord blood bank.
13. Evaluation of markers for VNTR/STR of each CBU will be performed after thawing.
14. Bacteriological cultures are performed prefreeze (by the cord blood bank) and after thawing.
15. ABO-Rh blood group will be provided by the cord blood bank.
16. Sex of the donor will be provided by the cord blood bank.
17. Year of collection of CBU will be provided by the cord blood bank.
18. The number of bags and volume per CBU will be provided by the cord blood bank.

12 Toxicities

Toxicities will be scored according to the NCI Common Terminology Criteria for Adverse Events, CTCAE version 3.0, published June 10, 2003

12.1 Umbilical cord blood transplant

Side effects include low blood count, infections, bleeding and failure of the donor stem cells to grow. Supportive care with red cell and platelet transfusions and antibiotic therapy will be necessary. Graft-versus-host disease (inflammation of skin, liver, gastrointestinal system and/or other tissues) may also occur and require treatment with immune suppressing drugs. In addition, organ damage may occur as a result of radiation or the treatment with immune suppressing drugs. There is a risk that the patient will reject the UCB grafts and that donor cells will not be detected after transplant. Transplant-infusion related problems could occur including intravascular hemolysis and symptoms of DMSO-toxicity such as nausea and flushing, hypotension, cardiac arrhythmia and respiratory arrest.

12.2 Conditioning regimen

The expected side effect of TBI is myelosuppression. Cyclofosfamide can cause a chemical cystitis. Secondary leukemias are seen after treatment with cyclofosfamide. The main side effects of fludarabine include lowering of blood counts and infections. Hemolytic anemia has occurred in patients treated with fludarabine. This conditioning regimen may lead to a cytopenia > 10 days.

12.3 Immune suppressive therapy

Mycophenolate mofetil

Side effects include a reversible fall in blood cell count and gastrointestinal symptoms such as nausea, vomiting, diarrhea, and abdominal discomfort. Cases of intestinal bleeding have also been reported.

Cyclosporine A

The immediate effects of this drug may include nausea or vomiting when given orally. Other side effects include the possibility of developing hypertension, tremor, increased hair growth and possibly an effect on mental function. These effects are generally reversible upon decreasing the dose of the drug. An occasional patient has had a seizure but it is unclear whether cyclosporine, other drugs, or a combination of drugs was responsible. Some patients given intravenous cyclosporine for the treatment of GVHD experienced painful sensation in hands or feet or both. The

pain subsided with the improvement of the GVHD or when the cyclosporine was switched from the intravenous to the oral form.
Patients may experience a change of liver or kidney function, in which case, the dose may be reduced or possibly even stopped for a while. This effect on kidneys seems to increase when other nefrotoxic drugs are given at the same time, especially certain antibiotics. Occasionally the kidney damage is severe enough to require the use of an artificial kidney machine (hemodialysis). During treatment cyclosporine blood levels will be monitored to determine if there are increased risks of side effects that warrant changing the dose.

12.4 Graft-versus-host disease

Acute GVHD gr II-IV and chronic GVHD have been reported in 20-50% and 40-70%, respectively after UCBT. Skin involvement will be assessed by biopsy with percentage of body surface area involved recorded. GI symptoms suspect for GVHD will be evaluated by biopsy as indicated. Acute GVHD and chronic GVHD will be graded according to established criteria (appendix E)

13 (Serious) adverse events

13.1 Definitions

Adverse event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Serious adverse event (SAE)

A serious adverse event is defined as any untoward medical occurrence that at any dose results in:

1. Death.
2. A life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed).
3. Hospitalization or prolongation of hospitalization.
4. Significant / persistent disability.
5. A congenital anomaly / birth defect.
6. Any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above).

Note that ANY death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Suspected unexpected serious adverse reaction (SUSAR)

All **suspected** Adverse Reactions which occur in the trial and that are both **unexpected** and **serious**.

Suspected adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product).

13.2 Adverse event

13.2.1 Reporting of adverse events

Adverse events will be reported from the first study-related procedure until 30 days following the last dose of any drug from the protocol treatment schedule or until the start of subsequent systemic therapy for the disease under study, if earlier.
Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.
Adverse Events have to be reported on the Adverse Events CRF. Adverse Events will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 3.0 (see appendix F).
Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of CTCAE grade > 2, diseases under treatment, chronic diseases and long term effects of past events as present at the time of baseline assessment.

All Adverse Events have to be reported, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline concomitant diseases CRF
- AE's of CTCAE grade 1en 2
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
- Progression of the disease under study; complaints and complications as a result of disease progression remain reportable Adverse Events

13.2.2 Follow up of adverse events

All adverse events will be followed clinically until they have been resolved, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
Follow up information for grade 3 or 4 adverse events considered at least possibly related to the investigational medicinal product by the investigator should be reported on the AE CRF until recovery or until 6 months after the last dose of IMP, whichever comes first.
Follow up information for all other adverse events should be reported on the AE CRF until recovery or until 30 days after the last dose of any drug from the protocol treatment schedule, whichever comes first.

13.3 Serious Adverse Events

13.3.1 Reporting of serious adverse events

Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days following the last dose of any drug from the protocol treatment schedule or until the start of subsequent systemic therapy for the disease under study, if earlier.
Serious Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

SAEs must be reported to the HOVON Data Center by fax **within 24 hours** after the event was known to the investigator, using the SAE report form provided. This initial report should contain a minimum amount of information regarding the event, associated treatment and patient identification, as described in the detail in the instructions for the SAE report form. Complete detailed information should be provided in a follow-up report within a further 2 business days, if necessary.

All Serious Adverse Events have to be reported, with the exception of:

- Hospitalization for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory tests, bone marrow sampling) that are not related to an adverse event. Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- Prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- Hospitalization for a procedure that was planned prior to study participation (i.e. prior to registration or randomization). This should be recorded in the source documents. Prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.

13.3.2 Causality assessment of Serious Adverse Events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event CRF. The assessment of causality is made by the investigator using the following

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

13.3.3 Follow up of Serious Adverse Events

All serious adverse events will be followed clinically until they are resolved or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
Follow up information on SAE's should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

13.3.4 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the Co-investigator
The Co-investigator will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR)

13.4 Reporting Suspected Unexpected Serious Adverse Reactions

The HDC safety desk, on behalf of the sponsor, will ensure the reporting of any SUSARs to the Ethics Committees (EC), the Competent Authorities (CA) and the investigators in compliance with applicable laws and regulations.

Expedited reporting of SUSARs will occur no later than 15 days after the HOVON Data Center had first knowledge of the serious adverse event. For fatal or life-threatening cases this will be no later than 7 days for a preliminary report, with another 8 days for a complete report.

14 End points

Primary endpoint is:

- The cumulative incidence of graft failure (appendix D)

Secondary endpoints are:

- Time to neutrophil engraftment
- Time to lymphocyte engraftment
- Time to platelet engraftment
- Time to red blood cell transfusion independence
- Count of total CD3+, CD4+ and CD8+ cells and CD3-CD16/56+ cells at 3, 6, 12 and 24 months after UCBT
- Incidence and grade of acute GVHD
- Incidence of chronic GVHD
- Incidence of infections
- Transplant related mortality (TRM)
- Progression free survival (PFS, i.e. time from transplantation until progression/relapse or death from any cause, whichever comes first)
- Overall survival (OS) calculated from transplantation. Patients still alive or lost to follow up are censored at the date they were last known to be alive

15 Registration

Eligible patients should be registered before start of treatment. Patients need to be registered at the HOVON Data Center by one of the following options:

- Trial Online Process (TOP, https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.
- By faxing the completed registration/randomization CRF +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET
- By phone +31.10.7041560 Monday through Friday, from 09:00 to 17:00 CET

The following information will be requested at registration:

- Protocol number
- Institution name
- Name of caller/responsible investigator
- Patient's name code
- Sex
- Date of birth
- Date written informed consent
- Specific items patient gives consent for (see ICF)
- Eligibility criteria

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial). Patient study number will be given immediately by TOP or phone and confirmed by fax or email.

16 16 Data collection

16.1 CRF's

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF are derived from the protocol and will include at least:

- inclusion and exclusion criteria;
- baseline status of patient including medical history and stage of disease;
- timing and dosage of protocol treatment;
- adverse events;
- parameters for response evaluation;
- any other parameters necessary to evaluate the study endpoints;
- survival status of patient;
- reason for end of protocol treatment.

Each CRF page will be identified by a pre-printed trial number, and a unique combination of patient study number (assigned at registration), hospital and patient name code (as documented at registration) to be filled out before completing the form.

The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be dated and signed by the local investigator upon completion. All CRF entries must be based on source documents. The CRF and written instructions for completing the CRF will be provided by the HOVON Data Center.
Copies of the CRF will be kept on site. The original CRF pages must be sent to the HOVON Data Center at the requested time points. How and when to send in forms is described in detail in the CRF header and the CRF instructions.
All data from the CRF will be entered into the study database by the HOVON Data Center.

17 Statistical considerations

17.1 Patient numbers and power considerations

This phase II trial follows an optimal Simon 2-stage [37].The sample size calculation is based on the percentage of patients with a primary graft failure at day 60 post-transplant (PGF_{60}). A percentage more than 25% is considered too high ($H_0 : p \geq 0.25$), and a percentage less than than 10% as desirable ($H_1: p \leq 0.10$). The probability of accepting a treatment as worth further study, while in fact it is not (i.e., H_0 is true), is limited to 10% ($\alpha = 0.10$). The probability of rejecting a treatment for further study, while in fact it is (i.e., H_1 is true), is limited to 20% ($\beta = 0.20$). These characteristics imply a sample size of 34 patients (details of the sample size calculation can be found in [Simon]). However, in order to overcome dropout, 40 patients will be included.

17.2 Statistical analysis

All analyses will be done in accordance with the intention-to-treat principle, restricted to eligible patients.

17.2.1 Efficacy analysis

- With respect to the main endpoint:
  A binomial probability test will be used to evaluate whether this regimen is worth further study, i.e., differs significantly from 25%. A 90% confidence interval for the percentage PGF_{60} will be presented ($\alpha = 0.10$).
- With respect to the secondary endpoints:
  Actuarial survival curves for all time-to-event endpoints will be computed using the Kaplan-Meier method, and 95% CI will be constructed. All analyses of secondary endpoints are exploratory. Hence, no conclusions will be drawn from them.

17.2.2 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events and infections.

17.2.3 Additional analyses

Additional analyses may involve the analysis of prognostic factors with respect to engraftment, PFS, and OS. Logistic and Cox regression analysis could be used for this purpose.
Before any additional analysis will be performed, a separate analysis plan will be discussed with the principal investigator. Any such analysis should, however, be considered as exploratory, i.e. hypothesis generating, and not confirmatory.

17.3 Interim analysis

One interim analysis will be performed when D60 engraftment data of the first 13 patients are available. In line with Simon the trial will be discontinued early if 3 or more patients had a primary graft failure. This stopping criterion implies a probability of early termination of this regimen, when indeed H_0 is true, of 67%. No conclusions with respect to the acceptance of this regimen for further study will be drawn at the interim analysis.

18 Ethics

The study protocol and any amendment that is not solely of an administrative nature will be approved by an Independent Ethics Committee or Institutional Review Board.

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.

19 Trial insurance

The sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this

responsibility to a national co-sponsor. Proof of insurance will be submitted to the Ethics Committee.
In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial.

20 Patient information and consent

Written informed consent of patients is required before registration. The procedure and the risks and the options for therapy will be explained to the patient.

21 Publication policy

Trial results will always be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication.
The final publication of the trial results will be written by the Principal Investigator, the Co-investigator(s) and the trial statistician on the basis of the statistical analysis performed by the trial statistician. A draft manuscript will be submitted for review to:

- All co-authors
- The chair of the relevant HOVON working group, who is entitled to share and discuss the manuscript with working group members
- An industry partner if so agreed in the contract between HOVON and company

After revision the final manuscript is submitted to the HOVON secretary for review of compliance with this policy.
After approval by the HOVON board the manuscript will be sent to a peer reviewed scientific journal.

Authors of the main manuscript will include the Principal Investigator, the Co-investigator(s), investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion rate), the trial statistician and the trial manager. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.
Authors of correlative manuscripts (e.g. results of side studies) will include the Principal Investigator, the Co-investigator(s), and those persons who have made a significant contribution to the published results.

Interim publications or presentations of the study may include demographic data, overall results and prognostic factor analyses, results for secondary endpoints, but no comparisons between randomized treatment arms for the primary endpoint may be made publicly available before the recruitment is discontinued.

Investigators participating in the trial have a right to publish results from data they collected for the study. The Principal Investigator, the Co-investigator(s) and the trial statistician must approve any such publication, abstract or presentation based on patients included in this study. This is applicable to any individual patient or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study endpoints unless the final results of the trial have already been published.

Glossary of abbreviations

(in alphabetical order)

AA	Aplastic Anaemia
AE	Adverse Event
ALAT	Alanine aminotransferase/glutamic pyruvic transaminase/GPT
AML	Acute Myeloid Leukemia
ALL	Acute Lymphoid Leukemia
ANC	Absolute Neutrophil Count
APL	Acute Promyelocytic Leukemia
APTT	Activated Partial Thromboplastin Time
ASAT	Aspartate aminotransferase/glutamic oxaloacetictransaminase/GOT
ATG	Anti Thymocyte Globulin
BM	Bone Marrow
BMT	Bone Marrow Transplant
BUN	Blood Urea Nitrogen
Ca	Calcium
CBC	Complete Blood Count
CBU	Cord Blood Unit
CIBMTR	Center for International Blood and Marrow Transplant Research
CML	Chronic Myeloid Leukemia
CMV	Cytomegalo Virus
CR	Complete Remission
CRF	Case Report Form
CsA	Cyclosporine A
CTC	Common Toxicity Criteria
DAT	Direct Antiglobulin Test
DCO	Diffusion Capacity
EBMT	European Group for Blood and Marrow Transplantation
EBV	Epstein-Barr Virus
ECG	Electrocardiogram
EFS	Event Free Survival
EORTC	European Organization for Research and Treatment of Cancer
FEV1	Forced Expiratory Volume in one second
γ-GT	Gamma Glutamyl Transferase
GCP	Good Clinical Practice
G-CSF	Granulocyte Colony-Stimulating Factor

GI	Gastro Intestinal
GVHD	Graft Versus Host Disease
HB	Hemoglobin
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte histocompatibility Antigen
HSV	Herpes Simplex Virus
HLTV	Human T-cell Lymphotropic Virus
IBMTR	International Bone Marrow Transplantation Registry
IRB	Institutional Review Board
IU	International Units
IV	Intravenous
KIR	Killer-cell Immunoglobulin-like Receptor
LDH	Lactate Dehydrogenase
MCV	Mean Corpuscular Volume
MDS	Myelodysplastic Syndrome
METC	Medical Ethical Review Committee
MMF	Mycophenolate Mofetil
MRD	Minimal Residual Disease
MUD	Matched Unrelated Donor
NHL	Non-Hodgkin Lymphoma
NK	Natural Killer
NYHA	New York Heart Association
OS	Overall Survival
PB	Peripheral Blood
PBSC	Peripheral Blood Stem Cell(s)
PBSCT	Peripheral Blood Stem Cell Transplantation
PCP	Pneumocystis Carinii Pneumonia
PCR	Polymerase Chain Reaction
PFS	Progression-free survival
PO	Per Os
PR	Partial Response
PT	Prothrombin Time
RBC	Red Blood Cell
RIC	Reduced-Intensity Conditioning
SAA	Severe Aplastic Anemia
SAE	Serious Adverse Event

SCT	Stem Cell Transplantation
SD	Selective Decontamination
SGOT	see ASAT
SGPT	see ALAT
STR	Short Tandem Repeat
TBI	Total Body Irradiation
TNC	Total Nucleated Cell Count
TRM	Treatment Related Mortality
UCB	Umbilical Cord Blood
UCBT	Umbilical Cord Blood Transplantation
VC	Vital Capacity
VSAA	Very Severe Aplastic Anemia
VNTR	Variable Number Tandem Repeats
VOD	Veno-occlusive Disease
WBC	White Blood cell Count
WHO	World Health Organization
WMO	`Wet Medisch-Wetenschappelijk Onderzoek met mensen'

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A. Prognostic score for AML in first relapse (age: 15-60 years)

Prognostic factor	**Points**
RFI = Relapse free interval from first CR	
Longer than 19 months	0
7 to 18 months	3
6 months or shorter	5
CYT = cytogenetics at diagnosis	
T(16;16) * of inv(16) *	0
T(8;21) *	3
Other **	5
AGE = age at first relapse	
35 years or younger	0
36 to 45 years	1
Older than 45 years	2
SCT = SCT before first relapse	
No SCT	0
Previous SCT (autologous or allogeneic)	2

CR: complete remission, SCT: stem cell transplantation

* with or without additional cytogenetic abnormalities

** normal, intermediate, unfavourable and unknown cytogenetics

Prognostic score = RFI + CYT + AGE + SCT (range: 0 -14)

Characteristics of the the prognostic groups A-C

667 patients with AML in first relapse	Prognostic score, range	Overall survival, % (se) One-year	Five-year
Favourable risk group A	0 – 6	70 (6)	46 (8)
Intermediate risk group B	7 – 9	49 (4)	18 (4)
Poor risk group C	10 – 14	16 (2)	4 (1)

Reference: Breems D, Van Putten W, Huijgens P, et al. Prognostic index for adult patients with acute myeloid leukemia in first relapse. JCO 2005; 23: 1969-1978

B. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

C. NYHA* scoring list

Grade 1 No breathlessness

Grade 2 Breathlessness on severe exertion

Grade 3 Breathlessness on mild exertion

Grade 4 Breathlessness at rest

The *New York Heart Association functional and therapeutic classification applied to dyspnoea

D. Definitions of recovery, engraftment and chimerism

Recovery

Neutrophil recovery:

First of 2 consecutive days with neutrophils ≥ 0.5 x 10^9/l

Platelet recovery:

First of 2 consecutive days with platelets ≥ 20 x 10^9/l without platelet support for 7 days

Engraftment

Engraftment:

Neutrophil recovery in association with donor hematopoiesis > 10% in bone marrow

Primary graft failure:

Cytopenia and marrow hypoplasia after 60 days with donor hematopoiesis < 10%

Secondary graft failure:

Complete loss of donor hematopoiesis after initial engraftment.

Chimerism

Complete chimerism:

>95% donor hematopoiesis, < 5% recipient hematopoiesis in bone marrow

Mixed chimerism:

10-95% donor hematopoiesis (single or two donors) and >5% recipient hematopoiesis in bone marrow

Autologous reconstitution

>95% recipient hematopoiesis, < 5% donor hematopoiesis in bone marrow

E. Grading of GVHD

Acute GVHD

Severity of organ involvement

Skin	+1	maculopapular eruption involving less than 25% of the body surface
	+2	maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	moderate increase in ASAT (150-170 IU) and bilirubin (20-40 µmol/l)
	+2	bilirubin rise 40-75 µmol/l with or without an increase in ASAT
	+3	bilirubin rise 75-200 µmol/l with or without an increase in ASAT
	+4	bilirubin rise to> 200 µmol/l with or without an increase in ASAT
GI		Diarrhea, nausea and vomiting graded +1 to +4 in severity
		The severity of GI involvement is assigned to the most severe involvement noted
Diarrhea	+1	> 500 ml stool/day
	+2	> 1000 ml stool/day
	+3	> 1500 ml stool/day
	+4	> 2000 ml stool/day and / or severe abdominal pain with or without ileus.

Severity of acute GVHD

Grade I	+1 to +2 skin rash no GI involvement no more than +1 liver involvement no decrease in performance
grade II	+1 to +3 skin rash +1 to +2 GI involvement and/or +1 to +2 liver involvement mild decrease in performance
Grade III	+2 to +4 skin rash and +2 to +4 GI involvement with or without +2 to +4 liver involvement marked decrease in performance with or without fever
Grade IV	pattern and severity of GVHD similar to grade III with extreme constitutional symptoms

Chronic GVHD

Limited	Localized skin involvement and/or liver function abnormalities
Extensive	Generalized skin involvement or localized skin involvement and/or liver function abnormalities + other organ inolvements

F. Toxicity criteria

The grading of toxicity and adverse events will be done using the NCI Common Terminolgy Criteria for Advers Events, CTCAE version 3.0, published December 12, 2003. A complete document (72 pages) may be downloaded from the following site :

http://www.hovon.nl (under ‘Studies > algemene studie-informatie’)

G. Biological studies

Introduction

The mechanism of cord blood unit predominance after double cord blood transplantation (UCBT) is unknown.

Only a few preclinical studies have been done on this subject. Nauta et al. performed studies in a mouse model (1). Nonobese diabetic/ severe combined immunodeficient (NOD/SCID) mice were transplanted with human CD34+ cells derived from one or two cord blood units. Double unit transplantation resulted in enhanced engraftment ability of one unit. Possible explanations given by the authors are presence of unequal amounts of true long-term repopulating cells in each unit or the presence of an undefined graft-versus-graft stimulatory effect. Kim et al. found that transplantation of two lineage-depleted cord blood units in NOD/SCID mice led to alleviation of a single-donor predominance, suggesting a graft –versus-graft reaction in single-donor predominance (2). Chaudhury et al. found that predomination of a certain unit in mice correlated with predomination in patients (3). Engraftment of the winner unit was not improved by addition of the second, losing unit. They suggest that unit predominance is due to an inherent (but not specified) advantage of the winning unit and that the presence of a losing unit does not augment the engraftment of the winner.

The following clinical studies describe detailed chimerism data after double UCBT.

Barker et al. reported 23 patients who underwent double UCBT after a myelo-ablative conditioning regimen (4). 21 patients reached sustained donor neutrophil engraftment and were evaluable for chimerism analysis. Chimerism was measured on bone marrow and/or blood. Blood was separated in neutrophil and mononuclear fractions provided the total white blood cells were more than $1.0x10^9$/l. At day +21 following double UCBT single donor hematopoiesis was observed in 16 patients (76%; median donor chimerism 100%;range 73-100%) and double donor hematopoiesis was observed in 5 patients (24%; median total donor chimerism 91%; range 64-100%). In patients with double donor hematopoiesis always one donor predominated (median 74% vs 20%). Double-unit hematopoiesis was observed in 2 patients at day 60 and in none of the evaluable patients by day 100. In predominating units CD3+ cell dose was found to be significantly higher compared to non-predominating units (median $0.6x10^7$/kg vs $0.4x10^7$/kg, $p< 0.01$).The relative percent viability, infused dose of TNC/kg, CD34+cell dose/kg, donor-recipient HLA-disparity and CFU-GM/kg did not predict unit predomination and neither did blood group, sex match and order of infusion. In a recent report of Scaradavou et al. data of 26 patients were presented (5). Double UCBT had been preceded by a myelo ablative conditioning regimen. At day +21 25 patients (96%) demonstrated

donor engraftment in bone marrow with one unit predominating. All engrafting units were found to have a CD34+ viability (as measured by 7-AAD staining) of > 75%. None of the units with a CD34+ viability below 75% engrafted. In cases where both units had a CD34+ viability of >75% one unit predominated as well. The authors suggested that the % post-thaw CD34+viability could be a predictor for engraftment potential of a certain unit. However this does not elucidate why a particular unit predominates in case of good viability of both units. Yoo et al. described data of 18 children who underwent double UCBT preceded by a myeloablative conditioning regimen (6). Chimerism was evaluated in bone marrow at 1, 3 and 6 months after UCBT. They found a significantly higher CFU-GM among the “winning” units. Kang et al. reported data of 8 children who underwent double UCBT after a myelo ablative conditioning regimen (7). In all patients one unit dominated at day 28. Factors that could influence dominancy could not be identified.

Brunstein et al. describe the largest series of patients transplanted after a reduced-intensity conditioning regimen (8). Of 93 patients transplanted with two units 81 patients had sustained donor chimerism. At day +21 in 43% of patients hematopoiesis was derived from both cord blood units. At day +100 double chimerism existed in 9% of patients and at day +180 in 3% of patients. At one year all patients had single donor hematopoiesis. At day +21 the median percent of donor-derived cells attributable to the dominant unit was 83% (range 8-100%) and beyond day +100 100% (range 34-100%). TNC, CD34+ cells, CD3+ cell dose, HLA match, nucleated cell viability, ABO type, gender match or order of infusion were not predictive for final predomination. Haspel et al. analysed 43 patients who underwent double UCBT preceded by a reduced-intensity conditioning.regimen (9). Chimerism analysis was performed on peripheral blood at day +30, day +60 and day +100. 29% of evaluable patients had single unit hematopoiesis at day +30 and 66% of evaluable patients had single unit hematopoiesis at day +100. At one year follow up, 12% of patients showed double donor chimerism (hematopoiesis of predominant units 66% and 83%) and 88% of patients show single donor chimerism. In multivariate analysis order of infusion, post-thaw CD34+ cell dose and post-thaw TNC dose were all identified as independent predictors of cord predominance. However, it should be noted that these results can be influenced by the fact that in 21 patients the larger unit was given first. Ballen et al. described 21 patients who underwent double UCBT after a reduced-intensity conditioning regimen (10). Chimerism analysis was performed at peripheral blood (unseparated blood; if possible also CD3+ and CD33+ enriched fractions) at weeks 2,4,6,8,10 and 12 and in bone marrow at 3 months posttransplant. 17 patients were evaluable for chimerism analysis. At day +28, a single cord blood unit predominated in 8 patients. In 6 patients hematopoiesis was originating from both units and in 3 patients hematopoiesis of recipient origin in combination with one cord blood unit was detectable. At 12 weeks posttransplant, 13 patients had single donor hematopoiesis or predominantly single donor

hematopoiesis. In 76% of patients the dominating unit at 3 months was the unit that had been infused first (P=0.049). Allele-level HLA-match, ABO match or sex match did not correlate with unit predominance. The authors suggest that the first unit infused may fill the hematopoietic stem cell niche,when the units are infused with several hours in between.

It can be concluded that compared to single unit transplantation, double cord transplantation results in a higher proportion of engraftment, in the NOD/SCID mouse transplant model as well as in patients. Several biological and clinical studies agree that sustained hematopoiesis is usually derived from a single donor after double UCBT. However, preclinical and clinical studies no dot unravel the mechanism of predomination of one particular unit. So far, contributing factors could not be identified consistently. It is suggested that a growth advantage of one of the units or immunological mechanisms or both could play a role.

Aim of biological studies

The aim of the biological studies is:

1. To record detailed and complete information about the composition and growing potential of infused umbilical cord blood units (UCB's)
2. To study engraftment and engraftment kinetics of individual cell lines
3. To study potential interactions between patient and grafts and vice versa
4. To study potential interactions between both grafts

1. Composition of infused UCB's

A complicating factor in studying the composition of UCB's is that only a limited volume per UCB can be used for analysis because the patient has to benefit from as many CD34+ cells as possible. Taking into consideration that our criteria for cord blood selection require a relatively large TNC, it seems safe to withhold a maximum volume of 5% per CBU for additional tests.

The following parameters of the infused UCB's will be examined:

- TNC count, viability of TNC
- Total CD34+ cell number, viability of CD34+ cells
- Total CD3+ cell number
- Total CD19+ cell number
- Total CD3-CD16/56+ cell number
- CFU-GM
- BFUe
- Allele-level HLA-typing including assessment of KIR (mis)match

Absolute cell counts are obtained by single platform flow cytometry Assays of cell counts and cell cultures are performed at the stem cell laboratory of the participating transplant center. Allele-level HLA-typing including assessment of KIR (mis)match is performed at
Sanquin Blood Bank region South West or Leiden University Medical Center

In addition to the above-mentioned tests it is important to have UCB-derived cells at our disposal for future experiments (see par 3.4). Therefore UCB-cells of both UCB's will be expanded and crypreserved for later use. Recipient cells will be cryopreserved as well.

2. Engraftment and engraftment kinetics of individual cell lines (see par 11.2)

Engraftment of individual cell lines will be examined by single platform flow cytometry. With the use of discriminating HLA-specific monoclonal antibodies (HLA-MoAbs) the origin (host, UCB1, UCB2) of CD34+ cells, granulocytes, platelets, B-lymphocytes, T-lymphocytes and NK-cells can be determined. The assay is performed at Sanquin Blood Bank region South West, Leiden. Peripheral blood will be examined weekly, starting between day 11-14, until single chimerism or a stable mixed / double chimerism has been reached. Bone marrow will be examined at 1, 2, 3, 6, 12 and 24 months. Samples of pretransplant peripheral blood and of each thawed UCB are used to test whether discriminating HLA-specific monoclonal antibodies are available for a certain patient-UCB combibation.

Discriminating HLA-MoAbs are under development at the Department of Immunohematology and Blood Bank of the Leiden University Medical Centre in collaboration with the Sanquin Bloodbank Southwest. So far, the number of available HLA-MoAbs is limited. If discriminating antibodies are available for a certain combination of patient and UCB's, the assay will be carried out on freshly obtained patient's peripheral blood and bone marrow. If discriminating antibodies are not (yet) available, peripheral blood and bone marrow are cryopreserved until they become available.
For each assay 10 ml of peripheral blood (EDTA) or 10 ml of bone marrow (EDTA) is taken.

3,4. Interactions between patient and grafts and vice versa; interactions between grafts

Theoretically interaction between patient and grafts and vice versa could be studied with the use of mixed lymphocyte reaction / cell mediated lympholysis /intracellular cytokine production assays. Whether this will be successfull depends on the final pattern of chimerism in a certain patient and the availability and reactivity of preserved/expanded UCB-cells. For this purpose plasma and peripheral blood will be cryopreserved at certain points in time.

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H. Information letter general practitioner

Datum……

Geachte collega……

Uw patient(e)……. zal deelnemen aan het onderzoek “A phase II study to assess engraftment and engraftment kinetics after double cord blood transplantation with a reduced-intensity conditioning regimen in patients eligible for allogeneic stem cell transplantation lacking a matched unrelated donor”.

Omstreeks…. zal patient(e) worden opgenomen voor de double cord blood transplantatie.

U wordt van het verdere beloop op de hoogte gehouden.

Hoogachtend…….