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

Principal Investigator: J.J. Cornelissen

Study coordinator : J. A. E. Somers

Statistician : B. van der Holt

Registration : Study coordinator
Erasmus MC – Daniel den Hoed
Room G-350
P.O. Box 2040
3000 CA Rotterdam
The Netherlands
tel. +31.10.7041367
fax +31.10.7041004

First version : December 2006

Final version : May 2008

Date of activation : 11 August 2008

Approved : CCMO NL18416.078.07 : 15 May 2008
METC Erasmus MC MEC-2007-332: 29 July 2008

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

2 Table of contents

1. Scheme of study 2

2. Table of contents 3

3. Synopsis 6

4 Investigators and study administrative structure 7

5 Introduction 8

5.1 Allogeneic stem cell transplantation 8

5.2 Umbilical cord blood transplantation 8

5.2.1 Single umbilical cord blood transplantation in adults 9

5.2.2. Double umbilical cord blood transplantation in adults 10

5.3 Selection of umbilical cord blood units 11

5.3.1 HLA-matching 12

5.3.2 Cell dose versus HLA-match 12

5.4 Conclusion 13

6 Study objectives 14

7 Study design 15

8 Study population 16

8.1 Patient selection 16

8.1.1 Eligibility criteria for registration 16

8.1.2 Exclusion criteria 17

8.2 Cord blood selection 17

9 Treatments 19

9.1 Allogeneic transplantation 19

9.1.1. Conditioning regimen 19

9.1.2 GVHD prophylaxis 19

9.1.3 Conditioning regimen and immune suppressive schedule 20

9.1.4 Special management orders during hospitalisation 20

9.2 Cord blood processing 21

9.3 Treatment of GVHD 21

9.3.1 Treatment of acute GVHD 21

9.3.2 Treatment of chronic GVHD 21

9.4 Treatment of viral reactivation 22

9.4.1 Treatment of CMV-reactivation/ CMV disease 22

9.4.2. Treatment of EBV-reactivation/ EBV-LPD 22

9.5 Infection prophylaxis 22

10	**End of protocol treatment**	24
11	**Required clinical evaluations**	25
	11.1 Pre-transplant evaluation at entry	25
	11.2 Post-transplant evaluation	28
	11.3 Evaluation of CBU's	30
12	**Toxicities**	32
	12.1 Umbilical cord blood transplant	32
	12.2 Conditioning regimen	32
	12.3 Immune suppressive therapy	32
	12.4 Graft-versus-host disease	33
13	**Serious adverse events**	34
	13.1 Definitions	34
	13.2 Reporting serious adverse events	34
14	**End points**	36
15	**Data collection**	37
16	**Registration**	38
17	**Statistical considerations**	39
	17.1 Patient number and power considerations	39
	17.2 Statistical analysis	39
	17.2.1 Efficacy analysis	39
	17.2.2 Toxicity analysis	39
	17.2.3 Additional analyses	40
	17.3 Interim analysis	40
18	**Ethics**	41
19	**Trial insurance**	42
20	**Patient information and consent**	43
21	**Glossary of abbreviations**	44
22	**References**	47

Appendices

A	**Prognostic score for AML in first relapse**	51
B	**ZUBROD-ECOG-WHO Performance Status Scale**	52
C	**NYHA* scoring list**	53
D	**Definitions of recovery, engraftment and chimerism**	54
E	**Grading of GVHD**	55
F	**Toxicity criteria**	57
G	**Biological studies**	58
H	**Patient information and informed consent form**	64
I	**Information letter for general practitioner**	74

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

Responsibility	**Name**	**Affiliation/Address**
Principal investigator	J.J. Cornelissen	Erasmus MC, Rotterdam
Study Coordinator	J. A. E. Somers	Sanquin Blood Bank South West Region, Leiden
Writing Committee	J. J. Cornelissen	Erasmus MC, Rotterdam
	J. A .E. Somers	
	M. Jongen-Lavrencic	
	E. Meijer	
	E. Braakman	
	B. Löwenberg	
	A. Brand	Leiden University Medical Center / Sanquin Blood Bank South West Region, Leiden
	Y. van Hensbergen	Sanquin Blood Bank South West Region, Leiden
	K. Sintnicolaas	Sanquin Blood Bank South West Region, Dordrecht
	L. F. Verdonck	University Medical Center Utrecht
	E. Petersen	
	M. Oudshoorn	Europdonor Leiden
	J. Lie	
Statistician	B. van der Holt	HOVON Data Center, Rotterdam
Datamanagement	Local Datamanagement	
Serious Adverse Events (SAEs) notification	J.A.E. Somers	Fax +31.10.7041004

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, EV1-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 ≤ 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 1 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. When recipient anti-A/anti-B titer is > 1:500, major incompatible CBU's disqualify.

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 untill 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	
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

Depending on the existence of major ABO-incompatibility between CBU and recipient and number of prefreeze RBC CBU's will undergo a careful washing procedure after thawing or will be infused immediately after a direct-thaw procedure at the bedside.
Major ABO-incompatible CBU's will undergo a post-thaw washing procedure if prefreeze RBC count exceeds 150 x 10^9 per bag. In all other cases CBU´s will be infused immediately after a direct-thaw procedure. Grafts will be infused with at least a two hours time interval in between.

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	
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	
Bone marrow examination	Incl. markers of MRD
Evaluation of disease status	
Check availability of autologous back-up transplant	
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
- Anti-HLA-antibodies
- 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: 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 (EDTA) 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/DAT/antiA/B/haptoglobin	X	X	X	X	X	X
6.	HbF, HbA	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

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, DAT, anti-A/ anti-B, haptoglobin.
6. HbF, HbA weekly, starting at day 0 (before SCT).
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).

9. Heparinized peripheral blood to perform CD3 selection followed by chimerism analysis by DNA (VNTR).
10. Bone marrow aspirate for morphology; heparinized bone marrow to perform chimerism analysis by DNA (VNTR).
11. (see appendix G)
    - For chimerism studies: weekly, starting at day 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, 9, 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
8.	**Total CD19+ cell number (B-cells)**			X
9.	**Total CD3- CD16/56+ cell number (NK cells)**			X
10.	**CFU-GM**			X
11.	**BFUe**			X
12.	**CMV**	X		
13.	**VNTR**			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	

* without and with washing

1. Pre-selection confirmation of HLA-typing (A,B serological split-level and DRB1 at 4-digit level) will be performed by the HLA-laboratory of Sanquin Blood Bank South West region Also HLA 4-digit typing for A, B, DQB1 and Cw will be performed if sufficient DNA is available. After thawing, HLA confirmatory typing (A, B, serological split level and DRB1 at 4-digit level) will be performed. Also, HLA 4-digit typing for A, B, DQB1 and Cw will be performed if not already done and if sufficient DNA is available.

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 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. Availability of an autologous back-up transplant is strongly recommended.

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.

13.2 Reporting serious adverse events

During protocol treatment all deaths, all SAE's that are life threatening and any unexpected SAE must be reported to the study coordinator by fax within 48 hours of the initial observation of the event. All details should be documented on the Serious Adverse Event and Death Report. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 14 calendar days and sent to the study coordinator. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.
At any time after the completion of protocol treatment, unexpected Serious Adverse Events that are considered to be possibly related to protocol treatment and any death (regardless the cause)

must also be reported to the study coordinator using the same procedure, within 48 hours after the SAE or death was known to the investigator.
The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. 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.

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 Data collection

15.1 CRF's

1. Registration
2. Concomitant diseases at baseline
3. EBMT - Cord blood transplant
4. EBMT - Med B Disease set
5. EBMT - allograft
6. EMBT - Histocompatibility
7. EBMT - Med B Follow up
8. Chimerism studies
9. SAE form

15.2 CRF's and schedule for completion

Form	**1, 2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Registration	X							
Post-transplant		X	X	X	X	X	X	(X)
Follow up						X	X	(X)

16 Registration

Eligible patients should be registered before start of the conditioning regimen. The following information will be requested at registration

- Patient's initials or code
- Patient's hospital record number
- Sex
- Date of birth
- Eligibility criteria

All eligibility criteria will be checked with a checklist.

Registration forms will be sent to the study coordinator by fax: +31.10.7041004

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 regiment, 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 (version 2000, Edinburgh, Scotland) and the ICH-GCP Guidelines of 17 January 1997.

19 Trial insurance

Risk insurance for patients enrolled in this trial will be provided by each of the participating centres.

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 (appendix H).

21 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
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'

22 References

1. Baron F, Storb R. Allogeneic hematopoietic cell transplantation as treatment for hematological malignancies: a review. Springer Semin Immunopathol.2004 nov; 26(1-2): 71-94.
2. Schattenberg A, Dolstra H. Cellular adoptive immunotherapy after allogeneic stem cell transplantation. Curr Opin Oncol. 2005;17(6):617-21.
3. Cornelissen J, Van der Holt B, Petersen E, Vindelov L, Russel C, Höglund M, Maertens J, Schouten H, Braakman E, Steijaert M, Zijlmans M, Slaper-Cortenbach I, Boogaerts M, Löwenberg B, Verdonck L. A randomized multicenter comparison of CD34+-selected progenitor cells from blood vs from bone marrow in recipients of HLA-identical allogeneic transplants for hematological malignancies. Exp Hematol. 2003;31(10):855-64.
4. Baron F, Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders. Mol Ther. 2006;13(1):26-41.
5. Niederwieser D, Maris M, Shizuru J, Petersdorf E, Hegenbart U, Sandmaier B, Maloney D, Storer B, Lange T, Chauncey T, Deininger M, Pönisch W, Anasetti C, Woolfrey A, Little M, Blume K, McSweeney P, Storb R. Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with haematological diseases. Blood 2003;101(4):1620-1629.
6. Heemskerk M, Van Walraven S, Cornelissen J, Barge R, Bredius R, Egeler R, Lie J, Revesz T, Sintnicolaas K, Wulffraat N, Donker A, Hoogerbrugge P, van Rood J, Claas F, Oudshoorn M. How to improve the search for an unrelated haematopoietic stem cell donor. Faster is better than more! Bone Marrow Transplant. 2005;35:645-652.
7. Barker J, Krepski T, DeFor T, Davies S, Wagner J, Weisdorf D. Searching for unrelated hematopoietic stem cells: availability and speed of umbilical cord blood versus bone marrow. Biol Blood Marrow Transplant. 2002;8(5):257-260.
8. Gluckman E, Broxmeyer HE, Auerbach AD, Friedman HS, Douglas GW, Devergie A, Esperou H, Thierry D, Socie G, Lehn P et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA-identical sibling. N Engl J Med. 1989;321(17):1174-8.
9. Brunstein C, Wagner J. Umbilical cord blood transplanting and banking. Annu Rev Med. 2006;57:403-17.

10. Eapen M. Unrelated donor hematopoietic stem cell transplantation in children with acute leukemia:risks and benefits of umbilical cord blood versus bone marrow. Biol Blood Marr Transpl 2006;12:1225(a).
11. Wagner J, Champlin R, Petz D. Symposium summary fourth annual international umbilical cord blood transplantation symposium, Los Angeles, California, May 19-20,2006. Biol Blood Marr Transpl 2006;12;1206-1217.
12. Rocha V, Labopin M, Sanz G, Arcese W, Schwerdtfeger R, Bosi A, Jacobsen N, Ruutu T, de Lima M, Finke J, Frassoni F, Gluckman E, Acute leukaemia working party of European Blood and Marrow Transplant Group and the Eurocord-Netcord registry. Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukaemia. N Engl J Med. 2004;351(22):2276-85.
13. Laughlin M, Eapen M, Rubinstein P, Wagner J, Zhang M, Champlin R, Stevens C, Barker J, Gale R, Lazarus H, Marks D, van Rood J, Scaradavou A, Horowitz M. Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukaemia. N Engl J Med. 2004;351(22):2265-75.
14. Takahashi S, Ooi J, Tomonari A, Konuma T, Tsukada N, Oiwa-Monna M, Fukuno K, Uchiyama M, Takasugi K, Iseki T, Tojo A, Yamaguchi T, Asano S. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem-cell transplants from related donors in adult patients with hematologic malignancies after myeloablative conditioning regimen. Blood. 2007;109 (3):1322-30.
15. Angelopoulou M, Novelli E, Grove J, Rinder h, Civin C, Cheng L, Krause D. Cotransplantation of human mesenchymal stem cells enhances human myelopoiesis and megakaryocytopoiesis in NOD/SCID mice. Exp Hematol. 2003;31(5):413-420.
16. Noort W, Kruisselbrink A, in ‘t Anker P, Kruger M, Van Bezooijen R, De Paus R, Heemskerk M, Löwik C, Falkenburg F, Willemze R, Fibbe W. Mesenchymal stem cells promote engraftment of human umbilical cord blood-derived CD34+ cells in NOD/SCID mice. Exp Hematol. 2002(8): 870-878.
17. Fernandez M, Regidor C, Cabrera R, Garcia-Marco J, Fores R, Sanjuan I,Gayoso J, Gil S, Ruiz E, Little A, McWhinnie A, Madrigal A. Unrelated umbilical cord blood transplants in adults: Early recovery of neutrophils by supportive co-transplantation of a low number of highly purified peripheral blood CD34+ cells from an HLA-haploidentical donor. Exp Hematol. 2003;31(6):535-544.
18. Peled T, Landau E, Mandel J, Glukhman E, Goudsmid N, Nagler A, Fibach E. Linear polyamine copper chelator tetraethylenenpentamine augments long-term ex vivo expansion

of cord-blood derived CD34+ cells and increases their engraftment potential in NOD/SCID mice. Exp Hematol. 2004;32(6):547-555.

19. Van Hensbergen Y, Schipper l, Brand A, Slot M, Welling M, Nauta A, Fibbe W. Ex vivo culture of human CD34+ cord blood cells with thrombopoietin (TPO) accelerates platelet engraftment in a NOD/SCID mouse model. Exp Hematol. 2006;34(7):943-950.
20. Barker J, Weisdorf D, DeFor T, Blazar B, McGlave P, Miller J, Verfaillie C, Wagner J. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005;105(3):1343-1347.
21. Verneris M, Brunstein C, DeFor T, Barker J, Weisdorf D, Blazar B, Miller J, Wagner J. Risk of relapse after umbilical cord blood transplantation in patients with acute leukemia: marked reduction in recipients of two units. Blood 2005(11);106:305(a).
22. Barker J, Scaradavou A, Stevens C, Rubinstein P. Analysis of 608 umbilical cord blood (UCB) transplants: HLA-match is a critical determinant of transplant-related mortality (TRM) in the post-engraftment period even in the absence of acute graft-vs-host disease (aGVHD). Blood 2005(11);106:303(a)
23. Nauta A, Kruisselbrink B, Lurvink E, Mulder A, Claas F, Noort W, Willemze R, Fibbe W. Enhanced engraftment of umbilical cord blood-derived stem cells in NOD/SCID mice by cotransplantation of a second unrelated cord blood unit. Exp Hematol. 2005;33(10):1249-1256.
24. Majhail N, Brunstein C, Wagner J. Double umbilical cord blood transplantation. Curr Opin Immunol. 2006;18(5):571-575.
25. Kai S, Misawa M, Iseki T, Takahashi S, Kishi K, Hiraoka A, Kato S, Hara H. Double-unit cord blood transplantation in Japan. Blood 2004(11);104:5166(a).
26. Wooford J, Regan D, Alonso M, Creer M. Myeloablative single cord transplants (SCT) versus dual cord transplants (DCT) in adults with acute leukaemia. Biol Blood Marr Transpl 2006;12:1219-1220(a)
27. Barker J, Weisdorf D, DeFor T, Blazar B, Miller J, Wagner J. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. Blood. 2003; 102(5):1915-1919.
28. Ballen K, Spitzer T, Yeap B, Steve M, Dey B, Attar E, Alyea E, Cutler C, Ho V, Lee S, Soiffer R, Antin J. Excellent disease free survival after double cord blood transplantation using a reduced intensity chemotherapy only conditioning regimen in a diverse adult population. Blood 2005(11);106:2048(a).
29. Miyakoshi S, Yuji K, Kami M, Kusumi E, Kishi Y, Kobayashi K, Murashige N, Hamaki T, Kim S-W, Ueyama J, Mori S, Morinaga S, Muto Y, Masuo S, Kanemaru M, Hayashi T, Takaue

Y, Taniguchi S. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. Clin Cancer Res. 2004;10(11):3586-3592.

30. Brunstein C, Barker J, Weisdorf D, DeFor T, Miller J, Blazar B, McGlave P, Wagner J. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplant outcomes in 110 adults with hematological disease. Blood. 2007 Jun 14 (Epub ahead of print).
31. Kang H, Kho S, Jang M, Lee S, Shin H, Ahn H. Early engraftment kinetics of two units cord blood transplantation. Bone Marrow Transplant. 2006;38(3):197-201.
32. Liu M, Reese J, Jaroscak J, Gerson S. Progressive emergence of a dominant unit during dual umbilical cord blood (UCB) culture. Blood 2005(11);106:2191(a).
33. Ruggeri L, Mancusi A, Burchielli E, Aversa F, Martelli M, Velardi A. Natural killer cell alloreactivity in allogeneic hematopoietic transplantation. Curr Opin Oncol. 2007;19(2):142-147.
34. Wagner E, Duval M, Dalle J-H, Moin H, Bizier S, Champagne J, Champagne M. Assessment of cord blood unit characteristics on the day of transplant : comparison with data issued by cord blood banks. Transfusion. 2006;46(7):1190-1198.
35. Rubinstein P, Carrier C, Scaradavou A, Kurtzberg J, Adamson J, Migliaccio A, Berkowitz R, Cabbad M, Dobrila N, Taylor P, Rosenfield R, Stevens C. Outcomes among 562 recipients of placental-blood transplants from unrelated donors. N Engl J Med. 1998;339(22):1565-1577.
36. Gluckman E, Rocha V, Arcese W, Michel G, Sanz G, Chan K-W, Takahashi T, Ortega J, Filipovich A, Locatelli F, Asano S, Fagioli F, Vowels M, Sirvent A, Laporte J-P, Tiedemann K, Amadori S, Abecassis M, Bordigoni P, Diez B, Shaw P, Vora A, Caniglia M, Garnier F, Ionescu I, Garcia J, Kögler G, Rebulla P, Chevret S;Eurocord Group. Factors associated with outcomes of unrelated cord blood transplant: guidelines for donor choice. Exp Hematol. 2004;32(4):397-407.
37. Simon, R. Optimal two-stage designs for phase II clinical trials. Controlled clinical trials 1989;10:1-10.

Appendix 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

Appendix B

ZUBROD-ECOG-WHO Performance Status Scale

1. Normal activity
2. Symptoms, but nearly ambulatory
3. Some bed time, but to be in bed less than 50% of normal daytime
4. Needs to be in bed more than 50% of normal daytime
5. Unable to get out of bed

Appendix 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

Appendix D

Definitions of recovery, engraftment and chimerism

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:

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:

Loss of peripheral blood counts after initial engraftment without detection of donor markers

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

Appendix 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 abdorminal pian with of 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

Appendix 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://ctep.info.nih.gov/reporting/ctc.html

Appendix 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 at day 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. 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.

References

1. Nauta A, Kruisselbrink B, Lurvink E, Mulder A, Claas F, Noort W, Willemze R, Fibbe W. Enhanced engraftment of umbilical cord blood-derived stem cells in NOD/SCID mice by cotransplantation of a second unrelated cord blood unit. Exp Hematol. 2005;33(10):1249-1256.
2. Kim D, Chung Y, Kim T, Kim Y, Oh I. Cotransplantation of third-party mesenchymal stromal cells can alleviate single-donor predominance and increase engraftment from double cord transplantation. Blood 2004;103(5):1941-1948.
3. Chaudhury S, Barker J, de Baisre A, O'Reilly R, Moore M. An animal model that predicts unit engraftment in double unit cord blood transplant recipients. Blood 2007(11);110:176(a).
4. Barker J, Weisdorf D, DeFor T, Blazar B, McGlave P, Miller J, Verfaillie C, Wagner J. Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy. Blood. 2005;105(3):1343-1347.
5. Scaradavou A, Smith K, Hawke R, Lee S, Schaible A, Abboud M, Collins N, Kernan N, van den Brink M, Barker J. CD34+ viability is a critical determinant of the engraftment potential of umbilical cord blood (UCB) in double unit transplantation. Blood 2007(11)110:2015(a).
6. Yoo K, Lee S, Kim H,Sung K, Jung H, Cho E, Park H, Kim H, Koo H. The impact of post-thaw colony-forming units-granulocyte/macrophage on engraftment following unrelated cord blood transplantation in pediatric recipients. Bone Marrow Transplant. 2007;39(9):515-21.
7. Kang H, Kho S, Jang M, Lee S, Shin H, Ahn H. Early engraftment kinetics of two units cord blood transplantation. Bone Marrow Transplant. 2006;38(3):197-201.
8. Brunstein C, Barker J, Weisdorf D, DeFor T, Miller J, Blazar B, McGlave P, Wagner J. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplant outcomes in 110 adults with hematological disease. Blood 2007;110(8):3064-3070.
9. Haspel R, Kao G, Yeap B, Cutler C, Soiffer R, Alyea E, Ho V, Koreth J, Dey B, McAfee S, Attar E, Spitzer T, Antin J, Ballen K. Preinfusion variables predict the predominant unit in the setting of reduced-intensity double cord blood transplantation. Bone Marrow Transplant. 2007 nov 26 (Epub ahead of print).
10. Ballen K, Spitzer T, Yeap B, McAfee S, Dey B, Attar E Haspel R, Kao G,Liney D, Alyea E, Lee S, Cutler C, Ho V, Soiffer R, Antin J. Double unrelated reduced-intensity umbilical cord blood transplantation in adults. Biol Blood Marr Transpl 2007;13:82-89.

Appendix H

Patiënteninformatie behorend bij de studie

"Een fase II studie naar herstel van bloedaanmaak en mechanismen van herstel van bloedaanmaak na dubbele navelstrengbloed transplantatie voorafgegaan door voorbehandeling met verminderde intensiteit bij patiënten die in aanmerking komen voor een allogene stamceltransplantatie maar voor wie geen onverwante stamceldonor beschikbaar is".

Geachte heer, mevrouw,

Uw behandelend arts heeft u voorgesteld aan het hierboven genoemde onderzoek deel te nemen en heeft u reeds het een en ander uitgelegd. Uw deelname moet u kunnen baseren op goede informatie onzerzijds. Daarom ontvangt u deze schriftelijke informatie, die u rustig kunt doorlezen en in eigen kring bespreken. Ook daarna kunt u altijd nog vragen voorleggen aan uw behandelend arts of aan een van de artsen die aan het einde van deze informatie vermeld staan.

Uw medische situatie en de bestaande mogelijkheden tot behandeling

Zoals bekend heeft u *een kwaadaardige hematologische ziekte, namelijk …….. waarvoor u met goed resultaat bent behandeld met chemotherapie, of *een aplastische anemie die niet of slechts tijdelijk op behandeling heeft gereageerd. De kans dat uw ziekte in de toekomst weer terugkomt wordt als zeer groot ingeschat. Een allogene stamceltransplantatie (transplantatie met stamcellen van een gezonde donor) is voor u nu de beste behandeling om de kans op terugkeer van ziekte te verkleinen. Bij een allogene stamceltransplantatie wordt bij voorkeur gebruik gemaakt van stamcellen (cellen die uitgroeien tot bloedcellen) die afkomstig zijn van een HLA-identieke (weefsel-identieke) familiedonor, of van een goed passende donor uit de wereldwijde donorbank. In uw geval is een familiedonor of passende onverwante donor niet beschikbaar. Daarom komt u in aanmerking voor een transplantatie met navelstrengbloed.

Achtergrond en doel van het onderzoek

Om navelstrengbloed te verkrijgen, wordt direkt bij de bevalling, na het doorknippen van de navelstreng, de inhoud uit de navelstreng en moederkoek opgevangen. Het navelstrengbloed wordt diepgevroren bewaard in daartoe gespecialiseerde donorcentra. Navelstrengbloed blijkt rijk te zijn aan bloedvormende stamcellen. Het gebruik van navelstrengbloed voor een allogene stamceltransplantatie is tamelijk nieuw. Bij kinderen is hier inmiddels veel ervaring mee opgedaan. Bij kinderen met acute leukemie zijn de resultaten van een transplantatie met navelstrengbloed (een zgn. cordblood transplantatie) minstens zo goed als van transplantaties met stamcellen van een volwassen onverwante donor. Bij volwassenen is de ervaring met navelstrengbloed transplantaties nog beperkt. Dit komt met name doordat navelstrengbloed relatief weinig stamcellen bevat. Daardoor is er bij volwassenen een grotere kans dat het transplantaat niet aanslaat. Eén geschikte eenheid navelstrengbloed met voldoende stamcellen is niet voor elke volwassen patiënt beschikbaar. Een manier om het aantal stamcellen vergroten, is het geven van twee eenheden navelstrengbloed (afkomstig uit twee verschillende navelstrengen) ofwel een dubbel cordblood transplantatie. Hiermee treedt een beter herstel van bloedaanmaak op. Het bijzondere hierbij is dat er meestal uiteindelijk slechts één van beide eenheden overleeft. Tot op heden is het niet bekend welke faktoren bepalen welke eenheid overleeft, maar een sneller en beter herstel van de bloedaanmaak in vergelijking tot transplantatie met een enkele eenheid is inmiddels aannemelijk gemaakt.

Een voordeel van navelstrengbloed is dat voor een transplantatie het weefseltype van het navelstrengbloed en de ontvanger niet precies identiek hoeft te zijn. Dit betekent dat er voor bijna iedereen voor wie geen volwassen stamceldonor beschikbaar is, er toch geschikte eenheden navelstrengbloed gevonden kunnen worden.
Een nadeel van een transplantatie met navelstrengbloed, zelfs bij gebruik van twee eenheden, blijft het lage aantal stamcellen, waardoor de tijd tot herstel van voldoende bloedaanmaak langer is dan na een transplantatie met een familiedonor of onverwante donor. Ook is de kans op afstoting van het transplantaat door de ontvanger wat groter.

In het voorgestelde onderzoek willen we nagaan hoe goed transplantaties met twee eenheden navelstrengbloed aanslaan en of er daarbij minder complicaties ontstaan. Bovendien willen we door middel van laboratoriumonderzoek aan bloed en beenmerg onderzoeken welke factoren bepalen welke eenheid uiteindelijk de bloedaanmaak gaat overnemen.

Het behandelplan

Voorafgaand aan de stamceltransplantatie krijgt u een standaard combinatie van lage dosis chemotherapie (cyclofosfamide en fludarabine) en lage dosis radiotherapie (totale lichaamsbestraling). Deze middelen worden ook bij een transplantatie met stamcellen van een familielid gegeven. De medische term voor deze combinatie van lage dosis chemotherapie met lage dosis radiotherapie is “conditionering met gereduceerde intensiteit”. De algemene informatie hierover vindt U in de bijgevoegde folder van het KWF. Chemotherapie wordt gedurende 5 dagen gegeven via een infuus. De totale lichaamsbestraling wordt over de daaropvolgende twee dagen verdeeld. De bestralingsarts zal u nog apart informatie geven over de totale lichaamsbestraling.
De dag na de tweede bestraling vindt de toediening van de twee eenheden navelstrengbloed plaats. Soms is het noodzakelijk de transplantaatinfusie te verdelen over 2 dagen. Het navelstrengbloed wordt ontdooid en direct daarna toegediend. Van elke eenheid navelstrengbloed wordt voor transplantatie een zeer kleine hoeveelheid afgenomen voor laboratoriumonderzoek. De transplantatie is vergelijkbaar met een bloedtransfusie. Als bijwerking van de transplantaatinfusie kan een zogenaamde transfusiereaktie ontstaan, waarbij koorts en rillingen kunnen optreden. Deze symptomen zijn van voorbijgaande aard.
De behandeling na de transplantatie is nagenoeg hetzelfde als na een transplantatie met stamcellen van een volwassen donor. Ter voorkoming van omgekeerde afstoting (zie verder) krijgt u een tweetal medicijnen, ciclosporine en cellcept. Ciclosporine wordt in eerste instantie in een infuus gegeven en later in tabletvorm; cellcept wordt in tabletvorm gegeven. Zo mogelijk wordt cellcept een maand na transplantatie gestaakt; ciclosporine wordt na 3 maanden afgebouwd. De belangrijkste bijwerkingen van cellcept zijn misselijkheid, buikklachten, diarrhee en daling van de bloedwaarden. De belangrijkste bijwerkingen van ciclosporine zijn hoge bloeddruk, trillingen van de handen, misselijkheid en nierfunktiestoornissen. Deze bijwerkingen verdwijnen doorgaans na verlagen van de dosering of staken van de medicatie.

Risico’s en bijwerkingen van de transplantatie.

De bijwerkingen en complicaties na een transplantatie met navelstrengbloed zijn dezelfde als na een transplantatie met stamcellen van een volwassen donor en worden in detail in bijgevoegde KWF folder beschreven.
De belangrijkste bijwerkingen van de chemotherapie voorafgaande aan de transplantatie zijn moeheid, misselijkheid, diarrhee en beschadiging van slijmvliezen van met name het maagdarmstelsel. De kans op bijwerkingen ten gevolge van de totale lichaamsbestraling is klein.

De belangrijkste bijwerkingen die eventueel kortdurend kunnen optreden zijn misselijkheid, branderigheid van de huid en pijnlijke speekselklieren. Er bestaat een zeer kleine kans op het optreden van longschade door de bestraling.
Als gevolg van de chemotherapie en bestraling zal uw eigen bloedaanmaak geleidelijk afnemen om vervolgens plaats te maken voor bloedaanmaak vanuit de gegeven navelstrengbloed stamcellen. Tijdens de periode van tekortschietende bloedaanmaak krijgt u zo nodig transfusies met rode bloedcellen en bloedplaatjes. In deze periode bent u extra vatbaar voor infekties. Indien de donorcellen niet aanslaan, nemen uw eigen stamcellen de bloedaanmaak weer geleidelijk over. In het zeldzame geval dat de donorcellen niet aanslaan en uw eigen bloedaanmaak zich onvoldoende snel herstelt, is de kans op ernstige infekties groot.

De belangrijkste bijwerkingen en risico's van de stamceltransplantatie zijn in het algemeen een verhoogd risico op infekties, het optreden van een afstotingsreaktie ofwel het niet aanslaan van de donorcellen en het optreden van een omgekeerde afstotingsreaktie ofwel graft-versus-host ziekte (GVHD). Doordat de weerstand na transplantatie sterk verminderd is, is de kans op het optreden van infekties verhoogd. Ter voorkoming hiervan krijgt u gedurende minimaal 1 jaar na transplantatie antibiotica en medicatie tegen bepaalde virussen voorgeschreven. Als er zich infekties voordoen, zijn deze soms moeilijk te behandelen.
Graft-versus-host ziekte ontstaat doordat de afweercellen in het transplantaat uw lichaam als "vreemd" kunnen herkennen en een afweerreaktie kunnen gaan maken tegen uw weefsels. Deze graft-versus-host ziekte uit zich met name als een reaktie tegen uw huid (roodheid, branderigheid, jeuk, schilfering), maagdarmstelsel (braken, diarrhee, buikpijn) en/of lever (geelzucht) maar kan ook tegen andere organen gericht zijn. Ter voorkoming van graft-versus-host ziekte krijgt u de eerder genoemde ciclosporine en cellcept. Als ondanks deze medicatie toch graft-versus-host ziekte optreedt, wordt dit meestal behandeld met extra medicatie, met name prednison.
Hoewel de stamceltransplantatie u de beste kans op genezing biedt, bestaat er helaas nog altijd een kans dat uw ziekte na transplantatie toch terugkomt. Het risico daarop wordt vooral door de onderliggende ziektestatus bepaald.

Controle en follow-up

Na transplantatie wordt zeer regelmatig bloed- en beenmerg onderzoek gedaan om het beloop van de behandeling goed te monitoren. Naast dit "routine" onderzoek zullen in het kader van het onderzoek op gezette tijden extra buisjes bloed worden afgenomen voor laboratoriumonderzoek. Dit wordt zo veel mogelijk gekoppeld aan een medisch noodzakelijke bloedafname. Bij "routine"

beenmergonderzoeken wordt zo mogelijk een extra buisje beenmerg afgenomen voor laboratoriumonderzoek.

Extra belasting door deelname aan het onderzoek

Naast het “routine” bloedonderzoek zullen op gezette tijden extra buisjes bloed worden afgenomen voor laboratoriumonderzoek. Dit wordt zo veel mogelijk gekoppeld aan een medisch noodzakelijke bloedafname. Echter in sommige gevallen betekent het dat er in het kader van het onderzoek een extra bloedafname moet plaatsvinden waarvoor u geprikt moet worden. Uiteraard wordt geprobeerd dit zo veel mogelijk te beperken. Bij “routine” beenmergonderzoeken wordt zo mogelijk een extra buisje beenmerg afgenomen voor laboratoriumonderzoek.

Vertrouwelijkheid

Tot uw persoon herleidbare onderzoeksgegevens kunnen slechts met uw toestemming door daartoe bevoegde personen worden ingezien. Deze personen zijn medewerkers van het onderzoeksteam, medewerkers van de Inspectie voor de Gezondheidszorg of bevoegde inspecteurs van een buitenlandse overheid, en leden van de Medisch Ethische Toetsings Commissie Erasmus MC. Inzage kan nodig zijn om de betrouwbaarheid en kwaliteit van het onderzoek na te gaan. Onderzoeksgegevens zullen worden gehanteerd met inachtneming van de Wet bescherming persoonsgegevens en het privacyreglement van het Erasmus MC.
Persoonsgegevens die tijdens deze studie worden verzameld, zullen worden vervangen door een codenummer. Alleen dat nummer zal gebruikt worden voor studiedocumentatie, in rapporten of publicaties over dit onderzoek. Slechts degene, die de sleutel van de code heeft (de onderzoeker en de behandelend arts) weet wie de persoon achter het codenummer is.

Opslag van bloed en beenmerg

Als u er geen bezwaar tegen heeft, zal het bloed en beenmerg dat eventueel overblijft na het uitvoeren van de benodigde onderzoeken, ingevroren bewaard blijven. Dit wordt gedaan om later eventueel nog aanvullend onderzoek te verrichten naar het mechanisme van overleving van de transplantaten. Als u hier wel bezwaar tegen heeft, wordt het overblijvende materiaal vernietigd. U kunt dit op het toestemmingsformulier aangeven. Lichaamsmaterialen die tijdens deze studie worden verzameld, worden tot de persoon herleidbaar opgeslagen. Na afloop van de studie worden de opgeslagen lichaamsmaterialen vernietigd of, indien u daarvoor toestemming geeft, gedurende maximaal 15 jaar na afloop van de studie bewaard.

Huisarts

Uw huisarts zal schriftelijk worden ingelicht over uw deelname aan dit onderzoek. Dit is in het belang van uw eigen veiligheid.

Weigeren voor en tijdens het onderzoek

Het staat u uiteraard volledig vrij aan het onderzoek mee te doen. Als u niet wilt deelnemen, hoeft u daarvoor geen reden te geven. Indien u afziet van transplantatie met twee eenheden navelstrengbloed, bestaat er een kleine kans dat u in aanmerking komt voor een stamceltransplantatie met een enkele eenheid navelstrengbloed. Dit is afhankelijk van de beschikbaarheid van een eenheid navelstrengbloed met voldoende stamcellen om herstel van bloedaanmaak in uw geval aannemelijk te maken. Transplantatie met een enkele eenheid navelstrengbloed wordt op dit moment gezien als de standaardbehandeling. Ook indien u nu toestemming geeft, kunt u die te allen tijde zonder opgave van redenen weer intrekken. Uw besluit zal geen invloed hebben op uw verdere behandeling. Wat u ook besluit, het zal geen consequenties hebben voor de verzorging en begeleiding van uzelf en uw familie.

Verzekering

Voor de deelnemers aan dit onderzoek is een verzekering afgesloten. Deze verzekering dekt schade door dood of letsel die het gevolg is van deelname aan het onderzoek, en die zich gedurende de deelname aan het onderzoek openbaart, of binnen vier jaar na beëindiging van de deelname aan het onderzoek. De schade wordt geacht zich te hebben geopenbaard wanneer deze bij de verzekeraar is gemeld. Als u van mening bent dat u door of tijdens het onderzoek schade hebt opgelopen, adviseren wij u zo snel mogelijk contact op te nemen met de hieronder genoemde verzekeraar of schaderegelaar*. U dient in dat geval de verzekeraar of schaderegelaar* alle benodigde informatie te verschaffen. Het niet nakomen van deze verplichtingen kan leiden tot het niet vergoeden van de schade.

De verzekeraar van het onderzoek is:
Naam:
Adres:
Telefoonnummer:
Contactpersoon:

De verzekering biedt een maximum dekking van …….. per proefpersoon, met een maximumbedrag van ……. voor het gehele onderzoek. Indien de opdrachtgever van dit onderzoek meerdere onderzoeken heeft lopen, geldt een maximumbedrag van ……. euro per verzekeringsjaar voor álle onderzoeken. De dekking van specifieke schades en kosten is verder tot bepaalde bedragen beperkt. Dit is opgenomen in het 'Besluit verplichte verzekering bij medisch-wetenschappelijk onderzoek met mensen'. Informatie hierover kunt u vinden op de website van de Centrale Commissie Mensgebonden Onderzoek: www.ccmo.nl. U kunt ook aan de onderzoeker hierover vragen stellen.

Voor deze verzekering gelden voorts een aantal uitsluitingen. De verzekering dekt niet:

- schade waarvan op grond van de aard van het onderzoek zeker of nagenoeg zeker was dat deze zich zou voordoen
- schade aan de gezondheid die ook zou zijn ontstaan indien u niet aan het onderzoek had deelgenomen;
- schade die het gevolg is van het niet of niet volledig nakomen van aanwijzingen of instructies;
- schade aan nakomelingen, als gevolg van een nadelige inwerking van het onderzoek op u of uw nakomeling;
- bij onderzoek naar bestaande behandelmethoden: schade die het gevolg is van één van deze behandelmethoden;
- bij onderzoek naar de behandeling van specifieke gezondheidsproblemen: schade die het gevolg is van het niet verbeteren of van het verslechteren van deze gezondheidsproblemen.

Goedkeuring

Voor dit onderzoek is goedkeuring verkregen van de Raad van Bestuur na een positief oordeel van de Centrale Commissie Mensgebonden Onderzoek (CCMO). De voor dit onderzoek geldende internationale richtlijnen zullen nauwkeurig in acht worden genomen.

Nadere informatie

Indien u vragen heeft over het onderzoek of over wel of geen deelname aan het onderzoek dan kunt u die voorleggen aan uw behandelend specialist of aan één van onderstaande artsen:

……..
……..
……..

Indien u twijfelt over deelname kunt u een onafhankelijke arts raadplegen, die zelf niet bij het onderzoek betrokken is, maar die wel deskundig is op het gebied van dit onderzoek en uw ziekte:

……..

Ook als u voor of tijdens de studie vragen heeft die u liever niet aan de onderzoekers stelt, kunt u contact opnemen met de onafhankelijke arts.

Als u niet tevreden bent over het onderzoek of de behandeling kunt u terecht bij de onafhankelijke klachtencommissie van het ……
De klachtencommissie is te bereiken op telefoonnummer ……..

Bijlagen
Folder wetenschappelijk onderzoek bij patiënten met kanker
Folder “stamceltransplantatie” (KWF)

Om er zeker van te zijn dat u deze patiënteninformatie heeft ontvangen en dat deze met u besproken is, verzoeken wij u bijgaand formulier gedateerd en getekend aan uw specialist te geven. Na ondertekening ontvangt u een kopie van het toestemmingsformulier.

TOESTEMMINGSVERKLARING

voor deelname aan het wetenschappelijk onderzoek:

Een fase II studie naar herstel van bloedaanmaak en mechanismen van herstel van bloedaanmaak na dubbele navelstrengbloedtransplantatie voorafgegaan door voorbehandeling met verminderde intensiteit bij patiënten die in aanmerking komen voor een allogene stamceltransplantatie maar voor wie geen onverwante stamceldonor beschikbaar is.

Ik ben naar tevredenheid over het onderzoek geïnformeerd. Ik heb de schriftelijke informatie (versie 1.3 dd 21-7-08) goed gelezen en begrepen. Ik ben in de gelegenheid gesteld om vragen te stellen over het onderzoek. Mijn vragen zijn naar tevredenheid beantwoord. Ik heb goed over deelname aan het onderzoek kunnen nadenken.

Ik heb het recht mijn toestemming op ieder moment weer in te trekken zonder dat ik daarvoor een reden behoef te geven.

Ik stem vrijwillig toe met deelname aan het onderzoek.

Ik geef toestemming om de gegevens te verwerken voor de doeleinden zoals beschreven in de patiënteninformatie.

Ik geef toestemming om mijn huisarts in te lichten over mijn deelname aan het onderzoek

Ik geef toestemming om mijn gegevens gedurende maximaal 15 jaar na afloop van de studie te bewaren

Ik geef wel / geen* toestemming om lichaamsmateriaal gedurende maximaal 15 jaar na afloop van de studie te bewaren om dit in de toekomst eventueel te gebruiken voor onderzoek met een zelfde onderzoeksdoel.

Ik geef toestemming voor deelname aan bovengenoemd onderzoek.

Naam : .....
Adres : .....
Woonplaats : .....
Geboortedatum : .....

Handtekening : ..... Datum: .....

Ondergetekende verklaart, dat de hierboven genoemde persoon zowel schriftelijk als mondeling over het bovenvermelde onderzoek geïnformeerd is.

Naam : .....
Functie : .....
Handtekening :

Appendix I

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…….