

## **Prophylactic infusion of CD4 positive donor lymphocytes early after T-cell depleted stem cell transplantation**

A randomized phase II study

### **LUMC 2007-01**

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## LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ALL	Acute lymphoblastic leukaemia
Allo-SCT	Allogeneic stem cell transplantation
AML	Acute Myeloblastic Leukemia
CLL	Chronic lymphocytic leukaemia
CME	Medical research ethics committee (MREC); in Dutch: commissie medisch ethiek (CME)
CML	Chronic Myeloid leukemia
CMV	Cytomegalovirus
DLI	Donor lymphocytes infusion
EBV	Epstein Barr Virus
GVHD	Graft versus host disease
HLA	Human leukocyte antigen
MDS	Myelodysplastic Syndrome
MM	Multiple Myeloma
MUD	Matched unrelated donor
NMA	Non-myeloablative
PCR	Polymerase Chain Reaction
(S)AE	(Serious) Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCR	T Cell Receptor
VZV	Varicella Zoster Virus
WHO	World Health Organisation
WMO	Medical Research Involving Human Subjects Act (Wet Medisch-wetenschappelijk Onderzoek met Mensen)

**SUMMARY**

<b>Title of the study</b>	<p align="center"><b>Prophylactic infusion of CD4 positive donor lymphocytes early after T-cell depleted stem cell transplantation</b></p> <p align="center">A randomized phase II study</p>
<b>Objective</b>	<p>The main objectives of this open label randomized phase II study are</p> <ol style="list-style-type: none"> <li>1. to evaluate whether CD4<sup>+</sup> lymphocytes infusion given three months after T-cell depleted allogeneic SCT improves immunological recovery (number of circulating CD4<sup>+</sup> lymphocytes) with an incidence of GvHD requiring systemic treatment not exceeding 30% of the patients.</li> <li>2. to evaluate whether CD4<sup>+</sup> lymphocytes infusion three months after T-cell depleted allogeneic SCT influences chimerism, disease status (minimal residual disease), number of virus specific T cells and incidence of viral infections.</li> </ol>
<b>Methodology</b>	<p>This is a randomized open label single centre study comparing the effects of CD4<sup>+</sup> lymphocytes infusion given three months after T-cell depleted allogeneic SCT (intervention group) to no infusion (control group)</p>
<b>Number of patients</b>	<p>A maximum number of 70 patients will enter the study. The first 20 randomized patients will be restricted to patients with a HLA identical family donor. If the incidence of GVHD requiring systemic treatment in the first ten patients receiving CD4<sup>+</sup> lymphocytes infusion, is not higher than 30%, patients with stem cell transplantation from unrelated HLA matched donors will be included as well.</p>
<b>Main criteria for Inclusion and registration (week 0)</b>	<ul style="list-style-type: none"> <li>• Patients with AML, myelodysplasia (MDS), ALL, CML in accelerated phase or blastic transformation, CLL, MM or aggressive lymphoma, who are scheduled to receive an allogeneic transplantation without major class I HLA mismatch between patient and donor</li> <li>• Absence of any concomitant disease preventing the safe administration of donor lymphocytes</li> <li>• WHO performance status of 0, 1 or 2 (see appendix)</li> <li>• No pregnancy, not breast feeding</li> <li>• Life expectation of &gt; 3 months</li> <li>• No severe psychological disturbances</li> <li>• No HIV positivity (tested at time of alloSCT)</li> </ul>

	<ul style="list-style-type: none"> <li>Informed consent according to the rules and regulations of the LUMC</li> </ul>
<b>Main criteria for randomization (week 10)</b>	<p>Evaluated ten weeks after allogeneic SCT:</p> <ul style="list-style-type: none"> <li>No systemic immunosuppressive treatment</li> <li>No progressive GVHD</li> <li>No GVHD of the skin &gt; grade 2 (see appendix)</li> <li>No progressive disease needing cytoreductive treatment</li> </ul>
<b>Treatment arm</b>	<p>At thirteen weeks after allogeneic SCT: Infusion of <math>1 \times 10^6</math> CD4<sup>+</sup> cells/kg</p>
<b>Control arm</b>	<p>No infusion of CD4<sup>+</sup> cells</p>
<b>Criteria of evaluation</b>	<p>Primary endpoint is the number of CD4<sup>+</sup> T cells in the blood at 6 months after stem cell transplantation.</p> <p>Secondary endpoints are chimerism, disease status (including minimal residual disease), number of virus specific T cells and incidence of viral infections.</p> <p>Patients will be monitored for toxicity, graft versus host disease (GVHD), immunological recovery, changing chimerism, disease status (minimal residual disease), and incidence of viral infections.</p> <p>At least every two weeks evaluation of all patients for toxicity, GVHD and viral infections will take place. Besides, blood will be drawn for measurement of numbers and phenotype of circulating lymphocytes and the appearance of virus specific T cells.</p> <p>At week 13 (before CD4<sup>+</sup> lymphocytes infusion): Bone marrow investigation for chimerism and minimal residual disease.</p> <p>At week 19: Bone marrow investigation for chimerism and minimal residual disease.</p> <p>At week 25: Bone marrow investigation for chimerism and minimal residual disease.</p>
<b>Statistical methods</b>	<p>Final evaluation of effects of CD4<sup>+</sup> infusion will be performed after a maximum of 60 patients has been randomized. Two-sample t-test will be used to detect a possible effect of the CD4<sup>+</sup> cells infusion on concentration of CD4<sup>+</sup> lymphocytes in the blood of the patient 6 months after transplantation. Results will be compared between patients who were randomized for CD4<sup>+</sup> infusion and control patients who were randomized for getting standard care.</p>



	<p>A safety analysis will be performed when 20 patients with HLA identical family donors have entered the study. If severe GvHD (overall grade II or more) occurs in less than 3 out of the 10 patients, who received CD4<sup>+</sup> cells, patients with HLA compatible non related donors will also be included. If severe GvHD is seen in 5 or more patients, the dose of CD4<sup>+</sup> lymphocytes will be decreased. If severe GVHD is seen in 3 or 4 patients, another 5 patients will be treated with 1.0*10<sup>6</sup> cells/kg. If in more than 5 patients (out of 15) severe GVHD is seen, the dose of CD4<sup>+</sup> lymphocytes will be decreased.</p>
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**SUMMARY**

**Rationale:** Allogeneic hematopoietic stem-cell transplantation (allo-SCT) regimens using the CD52 antibody alemtuzumab for T cell depletion demonstrate efficient engraftment and reduced graft-versus-host disease (GVHD)<sup>1-2</sup>. However, alemtuzumab-containing regimens result in decreased post-transplant anti-infection immunity<sup>2-4</sup>. Due to poor T cell immune reconstitution, particularly of the CD4<sup>+</sup> T-cell subset<sup>5</sup>, T cell dependent anti-tumor effects are also impaired, requiring the administration of donor lymphocyte infusions (DLI) early after transplantation. Although unmanipulated DLI can induce considerable anti-tumor responses, morbidity and mortality due to GVHD occur frequently<sup>6</sup>.

Several studies have shown the capacity of CD8 depleted DLI to improve immune reconstitution<sup>7-8</sup>. In a small randomized trial, infusion of CD8 depleted DLI six months after T-cell depleted SCT was associated with considerably less severe GVHD than infusion of unmanipulated DLI with no difference in relapse rates<sup>9</sup>. However, CD8 depletion appears not to be able to completely eliminate GVHD<sup>10</sup>, possibly due to residual low numbers of CD8<sup>+</sup> cells. DLI based on selection of CD4<sup>+</sup> positive donor cells may be more effective in preventing GVHD<sup>11</sup> and, in addition, may improve immune reconstitution<sup>15</sup>.

**Objective:** In this phase II study, the toxicity and treatment effects of early donor derived CD4<sup>+</sup> lymphocyte infusion, three months after SCT, will be evaluated.

**Study design:** Randomized open label single centre intervention study.

**Study population:** Patients treated with an allo-SCT for acute myeloid leukemia (AML), high-risk myelodysplastic syndromes (MDS-HR), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) in accelerated or blastic phase, chronic lymphocytic leukemia (CLL), multiple myeloma (MM) and aggressive lymphoma will be included in this study in the absence of a class 1 HLA mismatch between patient and donor. In the absence of severe GVHD and irrespective of chimerism status, patients will be randomized to receive either CD4<sup>+</sup> lymphocytes infusion or not, three months after transplantation.

**Intervention:** The intervention is the infusion of a subset of donor lymphocytes (the CD4<sup>+</sup> cells), three months after stem cell transplantation.

**Main study parameters/endpoints:** To evaluate whether CD4<sup>+</sup> lymphocytes infusion given three months after T-cell depleted allo-SCT improves immunological

recovery i.e. recovery of circulating CD4<sup>+</sup> T cells with an incidence of GvHD requiring systemic treatment not exceeding 30% (primary endpoint).

To evaluate whether CD4<sup>+</sup> lymphocytes infusion given three months after T-cell depleted allo-SCT influences chimerism, disease status as measured by minimal residual disease and incidence of viral infections (secondary endpoint).

**Nature and extent of the burden and risks associated with participation, benefit and group relatedness:** Participating patients will visit the outpatient clinic once every two weeks for physical examination and blood sampling, which is at this moment the standard care for patients during the first six months after allogeneic stem cell transplantation at our institution. The total amount of blood which will be taken for study purposes will be maximally 250 cc in a three months period. One extra bone marrow examination will be performed (six weeks after CD4<sup>+</sup> infusion). Theoretically, the risk of CD4<sup>+</sup> donor lymphocyte infusion is acute GVHD, as is seen in patients receiving total donor lymphocyte infusion after allogeneic stem cell transplantation.

## 1. INTRODUCTION AND RATIONALE

Allo-SCT provides potentially curative therapy for patients with a variety of hematologic malignancies. However, acute GVHD and its treatment are major causes of transplant-related morbidity and mortality. The most efficient method for prevention of GVHD consists of T-cell depletion of the graft. Allo-SCT regimens using the CD52 antibody alemtuzumab for T cell depletion demonstrate efficient engraftment and reduced acute GVHD<sup>1-2</sup>. However, these protocols substantially impair post-transplant antiviral and antitumor immunity<sup>2-4</sup>. Patients show a poor immune reconstitution, particularly with slow recovery of the CD4<sup>+</sup> T-cell subset<sup>5</sup>. Due to the lower anti-tumor effect of T-cell depleted grafts, donor lymphocyte infusions (DLI) are frequently needed for treatment of mixed chimerism or of persistent disease, leading to increased incidence of acute GVHD.

CD8<sup>+</sup> lymphocytes have been implicated to play a major role in the development of acute GVHD<sup>13</sup>. Moreover, decreased levels of CD4<sup>+</sup>CD25<sup>+</sup> cells are associated with increased risk of acute GVHD<sup>14</sup>. After allo-SCT, levels of circulating CD4<sup>+</sup> lymphocytes are strongly correlated to overall survival and to a lower incidence of opportunistic infections<sup>15</sup>. Depletion of CD8<sup>+</sup> T cells from DLI may decrease DLI - induced GVHD. Several studies have shown the capacity of CD8 depleted DLI to improve immune reconstitution and increase donor chimerism after allo-SCT<sup>7-8</sup>. Nine patients with multiple myeloma received  $3 \times 10^7$  CD4<sup>+</sup> T-cells, six months after myeloablative T cell depleted (using anti CD6 antibodies) SCT. This resulted in a higher increase in CD20<sup>+</sup> lymphocytes and TCR repertoire complexity score compared to patients not receiving CD4<sup>+</sup> T-cells. In addition, more patients in the intervention group converted to full donor chimerism compared to controls<sup>7</sup>.

In a small randomized trial, infusion of CD8 depleted DLI six months after myeloablative T-cell depleted SCT (using anti CD6 for T-cell depletion) was associated with considerable less acute GVHD than infusion of unmanipulated DLI<sup>9</sup>. Six out of 9 patients receiving unmanipulated DLI developed acute GVHD (4 patients with overall grade III or more) and none of the nine patients receiving CD8<sup>+</sup> depleted DLI experienced acute GVHD. In this small study, no differences in relapse rate were observed<sup>9</sup>.

Recently, effects of CD8<sup>+</sup> depleted T-cell infusion two or four months after non-myeloablative (NMA) T cell depleted SCT have been described<sup>8</sup>. Alemtuzumab was used for T cell depletion of the graft. Twenty-three patients received an allo-SCT

(three sibling donors, thirteen matched unrelated donors and seven unrelated donors with one HLA mismatch) for hematologic malignancy. In the absence of acute GVHD, CD8<sup>+</sup> lymphocytes depleted DLI was given two months after NMA SCT with a matched sibling donor and four months after NMA SCT with non-related donor. In the absence of GVHD, escalating dose infusions of CD8<sup>+</sup> depleted T-cells were repeated with three months intervals (see Table1). Eleven patients received a total of 21 infusions. These infusions resulted in increased circulating T-cell numbers as well as enhanced frequencies of CMV-specific CD4 and CD8 T cells. Several patients developed a complete reversion of declining hematopoietic donor chimerism. Five patients experienced acute GVHD overall grade I. Two patients, both with a HLA mismatched unrelated donor, suffered acute GVHD overall grade II or III.

**Table 1.** Infusion scheme of CD8 T cell depleted DLI as described by Meyer et al<sup>8</sup>.

<b>Time after SCT:</b>	<b>Related donor</b>	<b>Unrelated Donor</b>
2 months	1x10 <sup>6</sup> CD4 <sup>+</sup> T cells/kg	
4 months		1x10 <sup>6</sup> CD4 <sup>+</sup> T cells/kg
4-5 months	3x10 <sup>6</sup> CD4 <sup>+</sup> T cells/kg	
6-7 months		3x10 <sup>6</sup> CD4 <sup>+</sup> T cells/kg
6-8 months	1x10 <sup>7</sup> CD4 <sup>+</sup> T cells/kg	
8-10 months		1x10 <sup>7</sup> CD4 <sup>+</sup> T cells/kg

In conclusion, CD8<sup>+</sup>T cell depleted DLI can be given at an early stage after allo-SCT with an acceptable risk of acute GVHD in patients who had been transplanted with stem cells from matched unrelated or related donors, irrespectively from chimerism status at time of DLI. CD8 depleted DLI improves immune reconstitution and may provide anti tumor effects early after SCT. Most GVHD induced by CD8 depleted DLI is mild and responsive to immunosuppressive therapy.

DLI based on selection of CD4<sup>+</sup> positive donor cells may be even more effective in preventing GVHD and inducing immune reconstitution because of improved selection of CD4<sup>+</sup> lymphocytes<sup>11</sup>. Therefore, we intend to study the feasibility and the effects of infusion of CD4<sup>+</sup> selected donor lymphocytes three months after allo-SCT.

## 2. OBJECTIVES

**Primary Objective:** To evaluate whether CD4<sup>+</sup> lymphocytes infusion given three months after T-cell depleted allo-SCT improves immunological recovery, i.e. recovery of circulating CD4<sup>+</sup> T cells with an incidence of GvHD requiring systemic treatment not exceeding 30%

**Secondary Objective(s):** To evaluate whether CD4<sup>+</sup> lymphocytes infusion given three months after T-cell depleted allo-SCT influences chimerism, disease status as measured by minimal residual disease, appearance of virus specific T lymphocytes, and incidence of viral infections

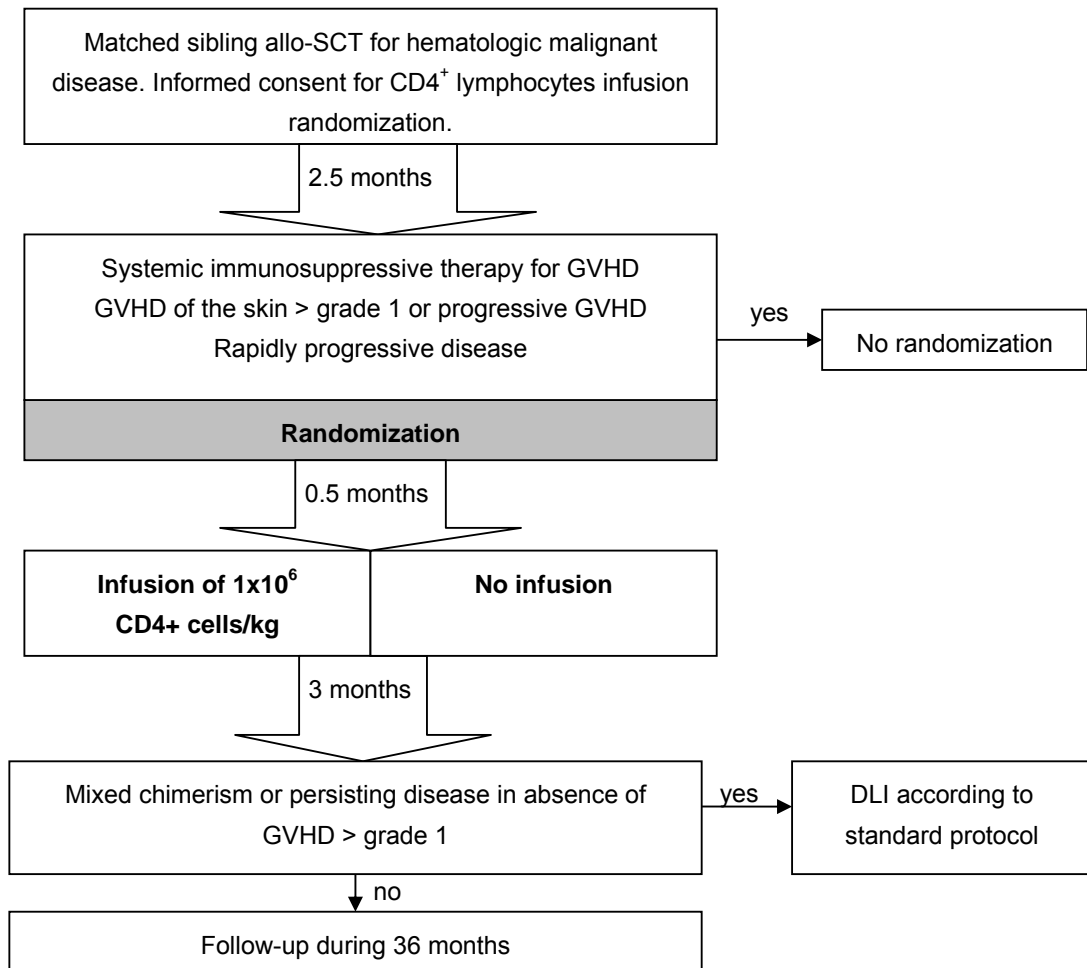
### 3. STUDY DESIGN

This is an open label single centre randomized phase II study in which immunologic effects of infusion of donor derived CD4<sup>+</sup> T-cells three months after T-cell depleted allo-SCT for hematological malignant disease (AML, ALL, MM, CLL, malignant lymphoma, MDS or CML in accelerated or blastic phase) will be evaluated. Patients will be asked for written informed consent at the time of allo-SCT. Ten weeks after allo-SCT, acute GVHD and disease status will be evaluated. In case of progressive malignant disease needing cytoreductive treatment, in case of worsening GVHD, in case of the need for systemic immunosuppressive treatment, or in case of the presence of GVHD of the skin > grade 2, patients will not be eligible for randomization. The informed consent which had been given at the time of the transplantation will be discussed again with the patient. In the absence of conditions as described above, patients will be randomized for CD4<sup>+</sup>lymphocytes infusion or no infusion. In case of randomization for CD4<sup>+</sup> infusion, patients will receive 1x10<sup>6</sup> CD4<sup>+</sup> donor derived T-cells/kg (in case of a matched unrelated donor, 0.5x10<sup>6</sup> CD4<sup>+</sup> T cells/kg). The first twenty randomizations will be done only for patients transplanted with stem cells of a sibling family donor (Figure 1A). If the incidence of GVHD more than grade I in this group does not exceed 20%, patients with MUD donors will be randomized as well (Figure 1B). If the incidence of severe GVHD after CD4<sup>+</sup> T cell infusion exceeds 40% in the first ten patients, the dose of the CD4<sup>+</sup> infusion will be decreased to 0.3x10<sup>6</sup> CD4<sup>+</sup> T cells/kg. The next 10 patients with related donors will all receive this dose without randomization (Figure 2). If again significant GVHD occurs (in >30% GVHD of overall > grade I), the trial will be terminated; if not, 40 additional patients, including patients with MUD donors, will be randomized for receiving CD4<sup>+</sup> infusion. In that case, patients with related donors will receive 0.3x10<sup>6</sup> CD4<sup>+</sup> T cells/kg and patients with MUD donors will receive 0.15x10<sup>6</sup> cells/kg. If 3 or 4 out of the first ten treated patients develop GVHD > grade I (30 or 40%), five more patients will be treated with 1x10<sup>6</sup> CD4<sup>+</sup> T cells/kg. Based on the incidence of GVHD > grade I in those 15 patients, the decision will be made whether to proceed to randomize patients with MUD donors and whether to change the dose of CD4<sup>+</sup> cells (see Figure 2).

All randomized patients will be monitored for toxicity, immune response and clinical response before and after the time of infusion of donor derived CD4<sup>+</sup> T cells. In case

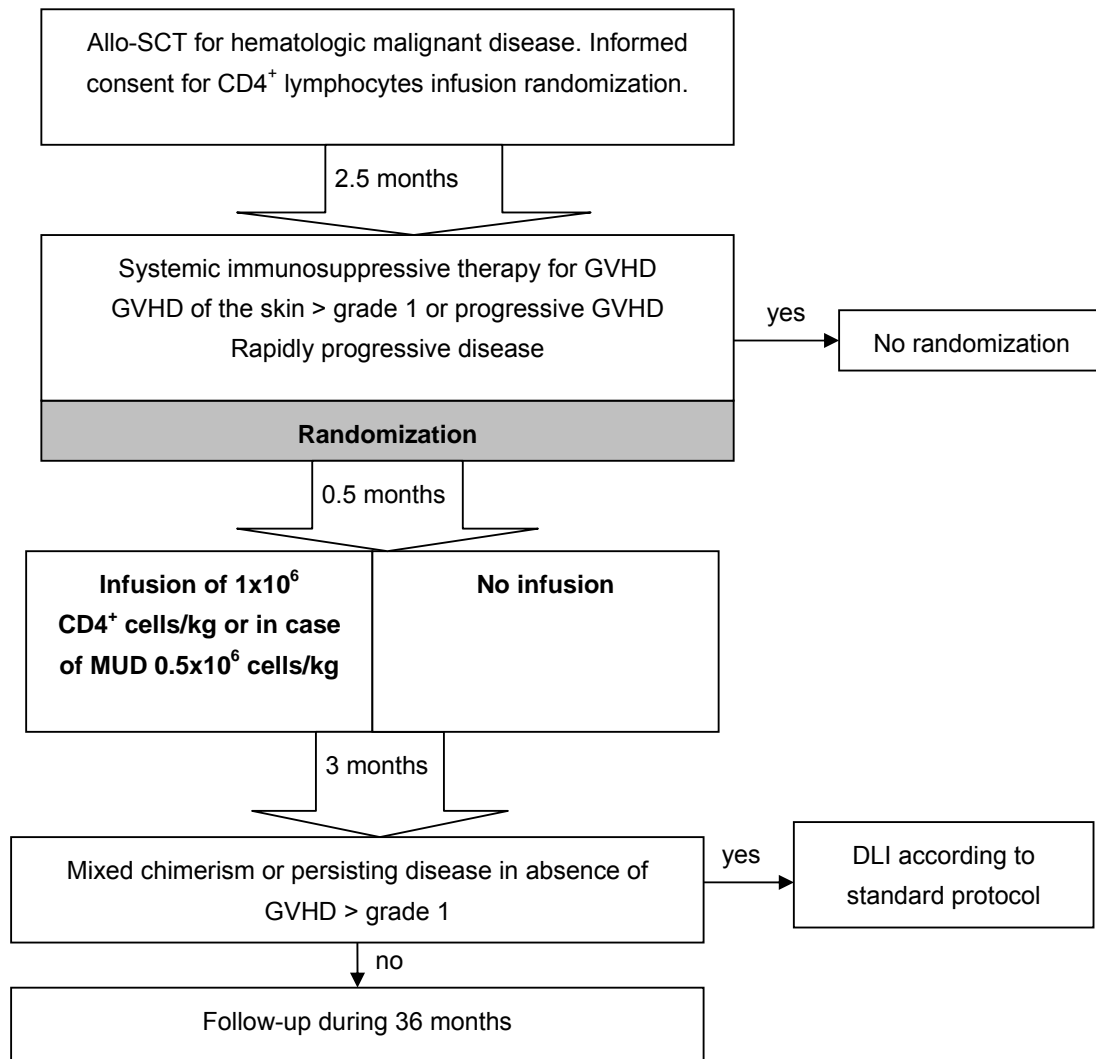
of rapidly progressive malignant disease needing cytoreductive therapy, patients will start cytoreductive treatment.

Six months after allo-SCT, patients may be eligible for unmanipulated donor lymphocytes infusion in case of mixed chimerism and/or persisting/recurrent disease, and lack of GvHD, according to our standard DLI protocol. In the absence of mixed chimerism or residual disease, immune responses and chimerism will be monitored for three years.

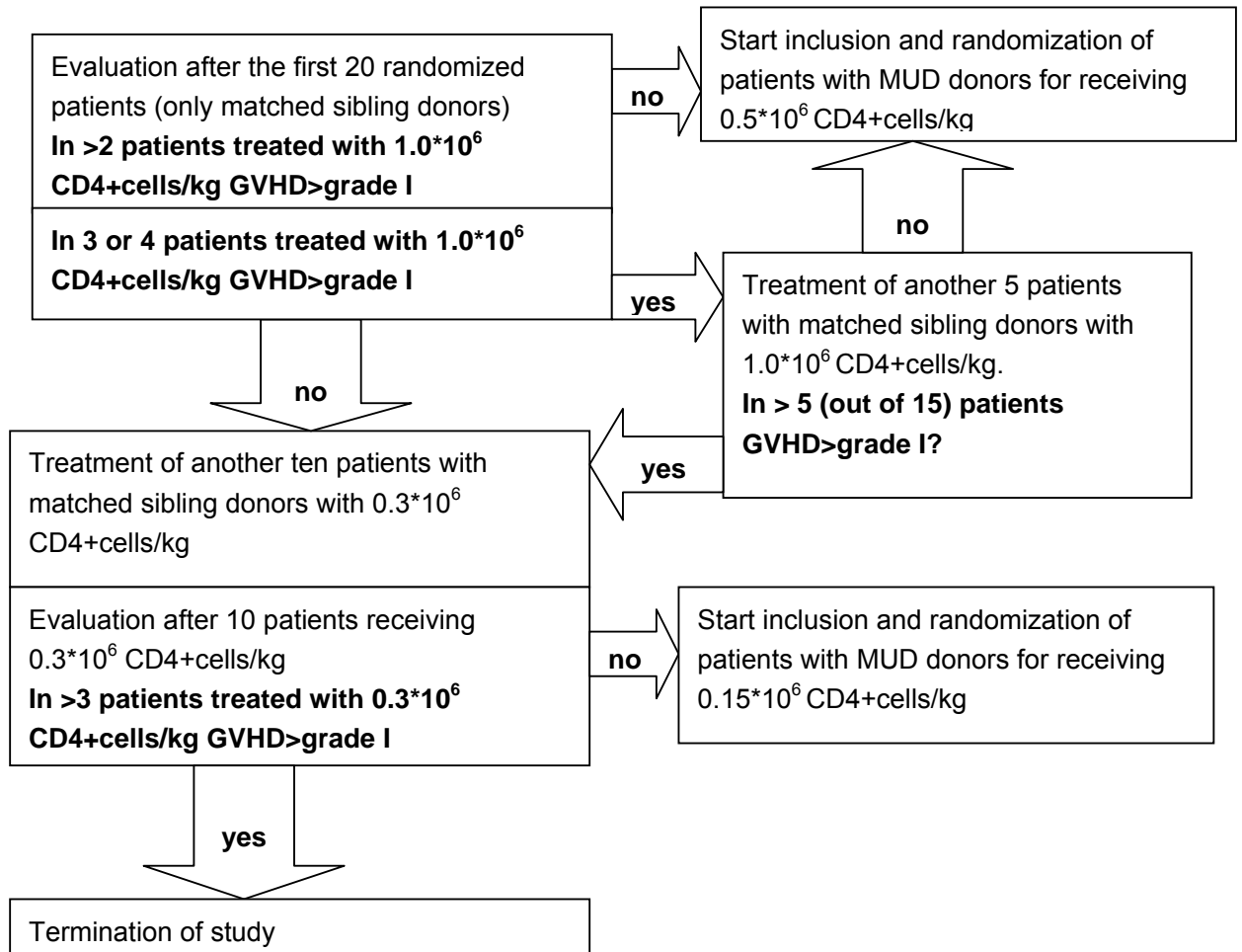


**Figure 1A** Study design in the first twenty randomized patients





**Figure 1B** Study design if in the first ten treated patients (with related donors only) the incidence of GVHD needing systemic treatment does not exceed 30%

**Figure 2:** Flow chart for interim safety analysis for severe GVHD

Hematological supportive care will involve prophylactic platelet transfusions when counts are  $< 10 \times 10^9/l$  and leukocyte-free red blood cell transfusions as clinically indicated. All blood products will be irradiated with 25 Gy.

In case of rapid progressive disease for which cytoreductive therapy (chemotherapy or clonal antibody therapy) is necessary according to the treating physician, the patient will go off protocol.

#### 4. STUDY POPULATION

##### 4.1 Population (base)

In the LUMC, T-cell depleted allo-SCT is performed according to standard procedures, at present based on protocols LUMC 2003-1 (non-myeloablative conditioning) and LUMC 2003-4 (myelo-ablative conditioning). After allo-SCT bone marrow aspiration is performed for morphology, immunophenotyping, cytogenetic analysis and chimerism assessment as clinically indicated but at least 1.5, 3, 6, 9, 12, 15, 18, 21, 24, 30 and 36 months after allo-SCT. If mixed chimerism or persisting/recurrent disease is present six months after allo-SCT, patients are treated with donor lymphocyte infusion ( $3 \times 10^6$  T cells/kg if a family donor has been used or  $1.5 \times 10^6$  T cells/kg in case of a matched unrelated donor).

In this protocol, all patients who do not suffer from acute GVHD three months after allo-SCT, will be considered for randomization for an early infusion with low dose donor derived CD4<sup>+</sup> T-cells in order to improve immune reconstitution. This CD4<sup>+</sup> lymphocyte infusion will be done irrespectively of chimerism status.

##### 4.2 Inclusion criteria (at time of inclusion)

- Patients with AML, myelodysplasia, ALL, CML in accelerated phase or blastic transformation, CLL, MM or (non) Hodgkin lymphoma, who are definitely planned to undergo an allogeneic stem cell transplantation. No Class I HLA mismatch between patient and donor is allowed.
- Life expectation of > 3 months
- Absence of any concomitant disease preventing the safe administration of donor lymphocytes
- WHO performance status of 0, 1 or 2 (see appendix)
- No severe psychological disturbances
- No severely limited life expectation due to diseases other than the malignancy
- No rapidly progressive disease preceding allo-SCT for which already unselected DLI is planned to be given 3 months after allo-SCT (aggressive lymphoma or refractory acute leukemia)
- Written informed consent according to the rules and regulations of the Leiden University Medical Center
- Age > 18 years

### **4.3 Exclusion criteria (at time of randomization)**

- Use of systemic immunosuppressive treatment (due to GvHD), acute GVHD of the skin > grade 2 or progressive acute GVHD.
- Progressive disease needing cytoreductive treatment.
- Any concomitant disease preventing the safe administration of donor lymphocytes.

### **4.4 Sample size calculation**

All patients will be registered for this study at the moment that they are admitted for an allogeneic stem cell transplantation. Only patients who did develop no or only limited GvHD of the skin or in whom GvHD was easily controlled by the transient use of immunosuppressive drugs, and fulfilling the remaining entry criteria will be eligible for randomization at 2.5 months after allo-SCT. The primary endpoint is the number of CD4<sup>+</sup> T cells at 6 months after transplantation. In order to be able to show a significant difference in circulating CD4<sup>+</sup> lymphocytes at six months after allo-SCT between the intervention and the control group, one should be able to evaluate circulating CD4<sup>+</sup> lymphocytes six months after allo-SCT in 48 patients (24 in each arm, see statistical analysis). With an expected entry rate of 20 randomized patients per year, the study will be open for patient entry for 3 years, in order to randomize a maximum of 60 patients. The final analysis of the study will be performed 6 months after 48 patients have been randomized. If at that moment less than 48 patients are evaluable with respect to the primary outcome, the analysis is postponed until 48 patients are evaluable. The underlying assumption is that the drop out rate is at most 20% which implies that among 60 randomized patients, at most 12 patients are not evaluable. In case of GVHD > overall grade I in the first ten treated patients, ten more patients will be treated with a lower dose (see Study design). In that case, in total 70 patients will enter the protocol.

## **5. TREATMENT OF SUBJECTS**

Patients randomized for DLI with a family donor will receive  $1.0 \cdot 10^6$  donor CD4+ cells/kg three months after allogeneic SCT and are candidate for receiving non selected donor lymphocyte infusion six months after alloSCT in case of mixed chimerism (according to current protocol). In case of a matched unrelated donor, dose of CD4+ cells will be  $0.5 \cdot 10^6$  donor CD4+ cells/kg. Control patients will receive standard care and are candidates for receiving non selected donor lymphocyte infusion six months after alloSCT in case of mixed chimerism (according to the current standard protocol).

### **5.1 Investigational treatment**

Patients randomized for treatment will receive CD4<sup>+</sup> donor lymphocytes, three months after allogeneic SCT.

#### **5.1.1 Collection of donor leukocytes**

Donor leukocytes will be collected by leukapheresis using ACD-A as anticoagulant 3 months after allo-SCT. The leukapheresis procedure per mononuclear cell dose will be restricted to maximally 6 hours per session and maximally 12 hours in total with maximal volume of blood to be processed of 20 liters per session. Donor leukocytes that are not used for CD4 selection can be cryopreserved for future usage as unselected DLI 6 months after allo-SCT according to protocols LUMC 2003-1 (non-myeloablative conditioning) and LUMC 2003-4 (myelo-ablative conditioning).

#### **5.1.2 Selection of CD4<sup>+</sup> lymphocytes from donor leukocytes**

Mononuclear cells will be purified with CD4 monoclonal antibody (mAb)-coated magnetic beads (Milteniy Biotech) using a clinical grade Clinimax method (see appendixes III and IV for information). CD4 monoclonal antibodies are CE marked. CD4 purity will be considered sufficient if <5% of lymphocytes are CD4 negative. Several test-runs in our institute have shown that more than 98% of selected lymphocytes is CD4 positive.

### **5.2 Expected toxicity of treatment**

In a recent study using CD8 depleted lymphocyte infusion after allo-SCT no severe GVHD was seen in patients who were transplanted using a HLA-matched donor. After CD4+ lymphocytes infusion, acute GVHD might occur. In our experience, 10% of patients develop GVHD after allo-SCT, and an additional 20% of patients develop GVHD after DLI infusion. Therefore, in this study we have set the limit for the

occurrence of GVHD requiring systemic treatment at 30%. Mild GVHD (only requiring local therapy) is associated with Graft versus leukemia effects and is therefore an anticipated and accepted side effect in this study.

## 6. METHODS

### 6.1 Study parameters

#### 6.1.1 Main study parameter

The number of circulating CD4+ lymphocytes six months after allogeneic stem cell transplantation.

#### 6.1.2 Secondary study parameters

The incidence of GVHD requiring systemic disease

Chimerism status six months after alloSCT

The incidence of viral infections between three and six months after allo SCT

### 6.2 Randomization and treatment allocation

Patients will be randomized using the donor type as stratification factor (related versus unrelated)

### 6.3 Study procedures

#### 6.3.1 Pretreatment investigations prior to the administration of CD4+ positive donor T-cells

1. History and physical examination
2. Complete blood cell counts and differential white cell count.
3. Percentage CMV, VZV and EBV-tetramer+ CD8+ T cells (if applicable: only patients for whom tetramers are available).
4. Bone marrow aspirates for cytology and cytogenetics
5. Measurement of lymphocyte subsets in peripheral blood: CD4 count, CD8 count, B-cell count (CD19+), NK cell count (CD3-/CD16/56+). Additional markers will be used to differentiate between the several CD4+ and CD8+ subsets naïve, memory and regulatory T cells (CD25, CD27, CD28, CD45RA, CCR7, HLA-DR, FOXP3).
6. Chimerism analysis using short tandem repeat (STR)-PCR to determine the percentage of residual recipient cells
7. Other staging procedures as appropriate, related to sites of malignancy.
8. Bilirubin (direct and indirect), alkaline phosphatase, ASAT, ALAT, LDH,  $\gamma$ GT.
9. Albumin and protein electrophoresis.

10. Serum urea, creatinine, Na, K, uric acid, glucose.
11. Viral-DNA loads (PCR), and specific IgG and IgM titers for CMV, EBV, VZV and adenovirus

### 6.3.2 Evaluation during treatment in first three months

- Interim history and physical examination will be performed at intervals of 2 weeks.
- Blood cell count, differential, quantitative platelet counts, creatinine, Urea, Na, K, Uric acid, glucose, ALAT, ASAT, alkaline phosphatase, bilirubin, LDH, gammaGT, total protein, albumin, once every two weeks after infusion.
- Treatment will be evaluated at following time points (see figure 3.):  
Every two weeks measurement of lymphocyte subsets in peripheral blood: CD4 count, CD8 count, B-cell count (CD19+), NK cell count (CD3-/CD16/56+). Additional markers will be used to differentiate between the several CD4+ and CD8+ subsets naïve, memory and regulatory T cells (CD25, CD27, CD28, CD45RA, CCR7, HLA-DR, FOXP3)

**Figure 3:** evaluation after CD4<sup>+</sup> donor T cell infusion

Weeks after SCT:

13	14	15	16	17	18	19	20	21	22	23	24	25	26
X		X		X		X		X		X		X	
B						B						B	
R						R						R	
<b>CD4</b>													

X: Venous blood sampling for evaluation of immune response (see above)

B: bone marrow aspiration for chimerism and disease status (see above)

R: Radiological evaluation in case of malignant lymphoma

**CD4:** infusion of CD4<sup>+</sup> lymphocytes

### 6.3.3 Evaluation starting three months after CD4<sup>+</sup> infusion

If a patient does not receive unmanipulated DLI six months after SCT, follow up will take place according to Figure 4, which is the standard follow-up procedure after allogeneic SCT.



**Figure 4:** evaluation after CD4<sup>+</sup> donor T cell infusion from 3 months after SCT\*

Months after SCT:

6	9	12	15	18	21	24	30	36
X	X	X	X	X	X	X	X	X
B	B	B	B	B	B	B	B	B
R	R	R	R	R	R	R	R	R

X: venapunction for evaluation of immune response (see above)

B: bone marrow aspiration for chimerism and disease status (see above)

R: Radiological evaluation in case of malignant lymphoma

\*in case of non-enrolment in unmanipulated DLI protocol

#### **6.4 Withdrawal of individual subjects**

Subjects can withdraw from the study at any time for any reason without any consequences for standard medical care. The investigator can decide to withdraw a subject from the study for medical reasons.

#### **6.5 Premature termination of the study**

In case of acute GVHD > overall grade I in > 30% of patients with a related donor receiving  $0.3 \times 10^6$  CD4+cells//kg, the study will be terminated (see chapter 3 study design). Thirty percent GVHD > overall grade I will be accepted based on our historic results as GVHD > overall grade I was seen in 10% of patients after allo-SCT and in another 20% of patients after unselected DLI, six months after allo-SCT.

## **7. SAFETY REPORTING**

### **7.1 Section 10 WMO event**

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

### **7.2 Adverse and serious adverse events**

Adverse events are defined as any undesirable experience occurring to a subject during this clinical trial, whether or not considered related to the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded and will be reported to the principal investigator.

A serious adverse event is any untoward medical occurrence or effect that at any dose results in death;

- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, major safety finding from a newly completed animal study, etc.

All SAEs will be reported within 24 hours to the principal investigator.

#### **7.2.1 Annual safety report**

The investigator will submit, once a year throughout the clinical trial, a safety report to the accredited METC. This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

### 7.3 *Suspected unexpected serious adverse reactions (SUSARs)*

All SUSARs during this study will be reported to the CCMO and the competent authority within 15 days. For fatal or life-threatening cases, a preliminary report will be send within 7 days, followed by a definite report within 8 days.

### **7.4 Follow-up of adverse events**

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated.

## 8. STATISTICAL ANALYSIS

### 8.1. Statistical design

This is an open label randomized phase II trial in which we will study the effects of purified CD4<sup>+</sup> cells infusion at 3 months after allo-SCT on the number of CD4<sup>+</sup> cells in the blood of the patient at 6 months after transplantation.

### 8.2. Sample size and analysis

Patients will be included for this study at the moment that they are admitted for receiving an allogeneic stem cell transplantation. Only patients who developed no or only limited GvHD of the skin or in whom the GvHD was easily controlled by the transient use of immunosuppressive drugs, and fulfilling the remaining entry criteria are eligible for randomization at 2.5 months after allo-SCT. Donor type (related versus unrelated) will be used as a stratification factor.

The primary endpoint is the number of CD4<sup>+</sup> T cells at 6 months after transplantation. According to our historic results (obtained between 2004 and 2006) the number of CD4<sup>+</sup> T cells in 40 non-selected patients at 6 months after allogeneic transplantation is 260 (+/- 227) x 10<sup>6</sup>/L (mean +/-SD).

In a recently published study, CD8 depleted lymphocyte infusions were shown to double the number of circulating CD4<sup>+</sup> T-cells in the blood after allogeneic stem cell transplantation<sup>8</sup>. The aim of our study is to detect a comparable increase in the number of circulating CD4<sup>+</sup> T cells at 6 months after allogeneic transplantation, i.e. from 260 to 520 x 10<sup>6</sup>/L. In order to detect such a difference (using a one-sided, two-Sample T-Test, with alpha 0.05, power 0.90, expected standard deviation=270) between the two arms one should follow 48 (24 in each arm), for circulating CD4<sup>+</sup> analysis evaluable, patients until 6 months after transplantation. As we expect a maximum dropout of 10%, we will randomize a maximum of 60 patients. In case of GVHD > overall grade I in the first ten treated patients, ten more patients will be treated with a lower dose (see Study design). In that case, in total 70 patients will enter the protocol.

## **9. ETHICAL CONSIDERATIONS**

### **9.1 Regulation statement**

The study will be conducted according to the principles of the Declaration of Helsinki (version 2004) and in accordance with the Medical Research Involving Human Subjects Act (WMO)

### **9.2 Recruitment and consent**

The treating haematologist will inform potential participants about this study and explain him or her about the informed consent procedure. All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. An example of a patient informed consent statement is given as an appendix to this protocol. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before they are randomized. The patient is given the information letter. During a second visit, information will be repeated if necessary and participation to the study will be considered.

### **9.3 Benefits and risks assessment, group relatedness**

Potential benefits of participation to this study are a faster immune recovery after allo-SCT and a lower incidence of potential life-threatening opportunistic infections. Potential risk of participation to this study is the development of severe potential life-threatening GVHD.

### **9.4 Compensation for injury**

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23rd June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study. As the development of GVHD is an anticipated effect of CD4+ lymphocytes infusion, the insurance does not apply for morbidity and mortality due to GVHD.

## 10. ADMINISTRATIVE ASPECTS AND PUBLICATION

### 10.1 Registration and storage of data and documents

Patients will be included after verification of eligibility by the IKW Data Center. A list of questions to be answered during the inclusion procedure is included in the registration check-list,

Standard questions:

- Eligibility criteria: all inclusion criteria will be checked
- Date of written informed consent

The patient's code will be entered in the CRF. Ten weeks after the inclusion, the treating hematologist will be contacted by the principal investigator in order to check eligibility for randomization. If the patient meets inclusion criteria for randomization, he or she will be randomized. A second check-list should be completed by the responsible investigator before the patient is randomized.

Questions to be asked at randomization:

- Is the patient using systemic immunosuppressive treatment for GVHD?
- Is the patient suffering progressive GVHD, probably needing systemic immunosuppressive treatment within two weeks?
- Is the patient suffering GVHD of the skin > grade 2?
- Is the patient suffering progressive malignant disease needing cytoreductive treatment?
- Which type of donor has been used for the transplantation (related or unrelated)

If the first four questions are answered with no, the treatment will be randomly allocated to the patients, as well as a patient sequential randomization number. This number and the allocated treatment have to be recorded on the randomization check-list, along with the date of randomization. The completed check-list must be signed by the responsible investigator and returned to the data center. The randomization code identifies the patient and must be reported on all case report forms. The IKW Data Center will be responsible for handling and storage of all CRFs.



**10.2 Annual progress report**

The principal investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

**10.3 End of study report**

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last control visit.

In case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

**10.4 Public disclosure and publication policy**

The writing committee will be responsible for the publication of the results of this study.

## 11. REFERENCES

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**APPENDIX I: KARNOFSKY PERFORMANCE - WHO PERFORMANCE STATUS**

	<u>WHO</u>
Normal; no complaints; no evidence of disease. Able to carry on normal activity; minor signs or symptoms of disease	0
Normal activity with effort; some signs or symptoms of disease. Cares for self. Unable to carry on normal activity or to do active work.	1
Requires occasional assistance but is able to care for most of his needs. Requires considerable assistance and frequent medical care	2
Disabled; requires special care and assistance. Severely disabled; hospitalization is indicated although death is not imminent.	3
Very sick; hospitalization necessary; active supportive treatment necessary. Moribund; fatal processes progressing rapidly.	4
Death	5

**APPENDIX II: CLINICAL CLASSIFICATION OF ACUTE GVHD (GLUCKSBERG)****A. Staging of acute GVHD**

	<b>Skin</b>	<b>Liver</b>	<b>Gastrointestinal</b>
0	No rash	Bilirubin < 2 mg / dl ( < 34 umol/L)	Diarrhea < 500 ml/day
1	Maculopapular rash on < 25% of body surface	Bilirubin 2-3 mg/dl (34-50 umol/L)	Diarrhea 500-1000 ml/day
2	Maculopapular rash on 25-50% of body surface	Bilirubin 3-6 mg/dl (51-102 umol/L)	Diarrhea 1000-1500 ml/day
3	Generalized erythroderma	Bilirubin 6-15 mg/dl (103-225 umol/L)	Diarrhea > 1500 ml/day
4	Generalized erythroderma with formation of bullea and desquamation	Bilirubin > 15 mg/dl ( > 225 umol/L)	Severe abdominal pain with or without ileus

**B. Grading of acute GVHD**

Overall grade	Stage		
	Skin	Liver	Gut
I (mild)	1 or 2	0	0
II (moderate)	1-3	1	1
III (severe)	2 or 3	2 or 3	2 or 3
IV (life-threatening)	2-4	2-4	2-4

## **APPENDIX III: Clinimax protocol for purification of CD4+ lymphocytes**

**APPENDIX IV: Data collection sheet for purification of CD4+ lymphocytes**