

**NON-MYELOABLATIVE T-CELL DEPLETED ALLOGENEIC STEM CELL
TRANSPLANTATION FOLLOWED BY DONOR LYMPHOCYTE INFUSIONS IN
PATIENTS WITH MALIGNANT AND NON-MALIGNANT DISEASES**

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SUMMARY

Myeloablative conditioning followed by allogeneic stem cell transplantation (allo-SCT) is an accepted treatment for patients with hematological malignancies. Although active as anti-leukemic treatment, allo-SCT is associated with severe immediate and late complications, leading to a procedural mortality rate of 10-15% and an impaired quality of life for many of the survivors. Non-myeloablative conditioning followed by standard allo-SCT decreases the acute toxic effects of the myeloablative regimen, but still is complicated by a high incidence of severe acute and chronic graft versus host disease (GvHD).

During the last 3 years we investigated in 20 patients with hematological malignancies and solid tumors the use of non-myeloablative conditioning followed by T cell depleted allo-SCT using high numbers of G-CSF mobilized stem cells to induce donor tolerance. The conditioning regimen of a non-myeloablative allo-SCT was less toxic than the standard allo-SCT regimen resulting in a shorter granulocytopenic and thrombocytopenic period and less organ damage. Engraftment was rapid in all patients with a very high degree of donor chimerism. GvHD after allo-SCT was minimal. The incidence of opportunistic viral infections was relatively high. Also older patients and otherwise ineligible patients tolerated this form of transplantation. Since a high degree of donor tolerance was achieved in all patients without GVHD, we were able to administer subsequently low dosages of donor lymphocytes in all patients with stable partial or complete chimerism and with persistent or relapsed disease. Particularly in patients with a chronic B-cell malignancy or acute myeloid leukemia, a significant graft versus tumor effect was observed after the donor lymphocyte infusions. However this intervention also induced rather frequent severe acute and chronic GvHD.

The aim of this new study is first to preserve high donor chimerism after allo-SCT after decreasing the immunosuppressive conditioning in order to lower the incidence of viral infections. The second aim is to decrease the incidence and severity of GvHD after DLI while maintaining or even increasing the antitumor activity by infusion of variable numbers of lymphocytes at fixed time points.

1. INTRODUCTION

Allo-SCT using stem cells from an HLA-identical sibling donor after conditioning with intensive chemotherapy (high dose cyclophosphamide) and total body irradiation (TBI) leads to long-term disease-free survival in 50% of the patients with acute myeloid leukemia (AML) in complete remission, acute lymphoblastic leukemia (ALL) in remission, refractory anemia with excess of blasts (in transformation) (RAEB(T)), chronic myeloid leukemia (CML) in chronic phase and multiple myeloma (MM). The myeloablative conditioning schedule is associated with immediate (severe mucositis, infections) and late (organ damage) complications as well as irreversible infertility. Due to the high toxicity, myeloablative allo-SCT is only feasible and ethically justifiable for younger patients and for patients suffering from a disease with a high risk of relapse. At present, at the Leiden University Medical Center the overall transplant related mortality of allo-SCT is approximately 10-15%. Furthermore, in a quality of life study performed by the EORTC Leukemia Group on AML patients in first complete remission treated by either intensive chemotherapy, auto- or allo-SCT, the quality of life of those transplanted with an allo-graft was clearly inferior to those who received only intensive chemotherapy or an auto-graft.

Over the years, clinical observations have strongly indicated that an allogeneic stem cell graft exhibits an antileukemic or anti-lymphoma effect, the "graft versus leukemia/lymphoma" (GvL) reactivity. Since T lymphocyte depletion of the stem cell graft results in an increased risk of relapse after transplantation, GvL reactivity has been attributed to donor T lymphocytes present in the stem cell graft. The importance of the immune reactions between donor T lymphocytes and host derived tumor cells has also been demonstrated by the successful transfusions of unmodified peripheral blood mononuclear cells from the HLA-identical sibling donors to patients with a relapse of their AML or CML following alloSCT. Seventy percent of the patients with a relapse of a CML after allo-SCT enter a complete remission following donor lymphocyte infusions (DLI). Furthermore, approximately 30% of patients with relapsed AML or multiple myeloma can also be successfully treated with DLI. These observations indicate that a major therapeutic component of allo-SCT can be ascribed to the GvL effect rather than to chemotherapy- or irradiation-induced elimination of the tumor cells in the recipient by the myeloablative therapy given prior to the transplantation. Hence, the main goal of allo-SCT may be to induce a state of donor tolerance, giving donor T lymphocytes the opportunity to kill host tumor cells.

Allo-SCT is also an effective treatment for some patients with chronic lymphocytic leukemia (CLL) and low grade lymphoma. These diseases are characterized by a less aggressive clinical course compared to acute leukemia. However, once these patients are chemotherapy resistant, the prognosis of these patients is poor. The GvL effect may also be active against these disorders, as was reported recently. The toxicity of the myeloablative conditioning regimens has

limited the use of allo-SCT in this group of patients which usually involves older patients.

Recently, a new allo-SCT approach has been developed in the therapy of hematological malignancies and solid tumors. This treatment primarily focuses on the use of the GVL effect of donor T cells to eradicate the malignant cells of the host. A non-myeloablative conditioning scheme with the aim of short term intensive immunosuppression is used to facilitate donor engraftment of hematopoietic cells to achieve at least a state of mixed chimerism in the hematopoietic and lymphopoietic compartment. This will allow the administration of donor lymphocyte infusions which may be capable of killing the malignant cells and achieving full donor chimerism.

Several pivotal clinical studies demonstrated that donor engraftment can be achieved after transplantation of a non-T cell depleted stem cell graft from an HLA-identical sibling donor using a non-myeloablative conditioning scheme. Slavin et al transplanted 26 patients with leukemia or lymphoma using a conditioning regimen containing fludarabine, anti-T-lymphocyte globuline and low dose busulphan. This conditioning scheme was extremely well tolerated with no severe procedure related toxicity such as mucositis and infections. Non-T cell depleted G-CSF mobilized stem cell transplantation resulted in stable partial (n=9) or complete (n=17) chimerism. Mild granulocytopenia was observed in 35% of the patients. Severe GvHD (grade 3 and 4) was the single major complication diagnosed in 25% of the patients. Khouri et al transplanted fifteen patients with a chronic B cell malignancy with non T cell depleted stem cells after a non-myeloablative regimen containing fludarabine and cyclophosphamide. Donor engraftment was obtained in 11 of the 15 patients, while the four remaining patients recovered with autologous hematopoiesis. Three patients developed severe GvHD. These observations show the feasibility of allo-SCT with non T cell depleted stem cells after a non-myeloablative conditioning regimen. Subsequent clinical experiences through out the world with these and comparable conditioning regimens endorse this initial reported favorable toxicity pattern and the high incidence of GvHD. The advantages of a less toxic non-myeloablative conditioning regimen are numerous. The granulocytopenic period is shorter, resulting in a reduction of incidence of severe infections. Also older patients and patients with limited organ reserves or organ failure due to previous chemotherapy may be treated by this form of transplantation. Another advantage of a less intensive conditioning therapy is the possibility that patients may preserve their fertility. Furthermore, when no donor engraftment will be obtained, the patient will recover with autologous hematopoiesis. These possible advantages of a non-myeloablative conditioning regimen imply an important reduction in the risks of an allo-SCT, although GvHD may still be a severe problem. The approach of non-myeloablative conditioning regimens followed by allo-SCT has also been explored in patients with solid tumors. A significant anti-tumor response of 30%

has been reported in patients with advanced renal cell cancer.

We recently showed in 20 patients predictable and sustained donor engraftment after a non-myeloablative regimen when high numbers of Campath-1-H T cell depleted stem cells were transplanted. The advantage of removing the T cells from the stem cells in this setting was the easy achievement of donor tolerance with a very low incidence of GvHD. As a consequence of the rigorous T-cell depletion we observed a high incidence of CMV reactivation but no CMV disease. Subsequent administration of low dose DLI resulted, in addition to a distinct anti-tumor effect, in a high incidence of acute and chronic GvHD with considerable morbidity and even mortality in one patient. Particularly in patients with a chronic B-cell malignancy or acute myeloid leukemia, a significant graft versus tumor effect was observed after the donor lymphocyte infusions.

Rationale for this study

Our first aim to achieve sustained engraftment of T cell depleted donor stem cells without GvHD using high numbers of T cell depleted stem cells after non-myeloablative conditioning was successful. Our second aim to treat patients, after achieving a state of mixed or complete chimerism, with low dose donor lymphocyte infusions resulted in a moderately satisfactory anti-tumor response but also in an high degree of acute and chronic GvHD probably due to persisting antigen presenting cells of the patient.

The aim of this novel study is first to preserve high donor chimerism after allo-SCT after slightly decreasing the immunosuppressive conditioning regimen in order to shorten the hospitalization period and to lower the incidence of viral infections. The second aim of this study is to preserve or increase the anti-tumor response by DLI while abolishing the severe GvHD complications.

2. OBJECTIVES OF THE STUDY

- 2.1 To determine donor engraftment after allogeneic T-cell depleted stem cell transplantation using the most efficient and least harmful non-myeloablative conditioning regimen.
- 2.2 To assess hematologic and immunologic recovery and pattern of engraftment after allo-SCT.
- 2.3 To determine anti-tumor effect and incidence and severity of graft versus host disease after the allo-SCT.
- 2.4 To determine incidence and severity of graft versus host disease after the subsequent administration of variable numbers of donor T lymphocytes.
- 2.5 To determine donor chimerism and anti-tumor effect of the subsequent administration of variable numbers of donor T lymphocytes.

3. PATIENT SELECTION

3.1 *Entry criteria*

- 3.1.1
 - Patients with a chronic lymphocytic leukemia (CLL), non-Hodgkin lymphoma, Hodgkin's disease or multiple myeloma with a failure to achieve complete remission or who relapse from a complete remission with conventional chemotherapy and/or an auto-SCT.
 - Patients with acute myeloid or lymphoblastic leukemia (AML, ALL), chronic myeloid leukemia (CML) or myelodysplasia who are not eligible for a standard myeloablative allo-or auto-SCT.
 - Selected patients with advanced solid tumor with no further therapeutic options and a life expectancy of more than 6 months.
 - Selected patients with non-malignant hematological disease, not eligible for standard allo-SCT (such as chronic neutropenia/trombocytopenia, thalassemia, sickle cell anemia, PRCA, PNH, aplastic anemia, HIV).
 - Selected patients with severe autoimmune disease with no further therapeutic options
- 3.1.2 Age under 70 years.
- 3.1.3 Availability of an appropriate stem cell donor (see 4).
- 3.1.4 WHO performance status 0, 1 or 2.
- 3.1.5 Informed consent according to rules and regulations of Leiden University Medical Center.

3.2 *Exclusion criteria*

- 3.2.1 Previous allo-SCT.
- 3.2.2 Life expectation of less than 6 months.
- 3.2.3 Severe limited life expectation due to diseases other than the diseases for which this protocol is written

3.2.4 Patients with a history of severe CNS disturbances and psychiatric problems.

3.2.5 Patients with severe uncontrolled infections.

4. DONOR SELECTION

4.1 An HLA-identical or one HLA-locus mismatched family member or an HLA-matched unrelated donor can be accepted as peripheral blood stem cell donor. In case more than one donor is available, the age, CMV status and sex of the donor will be taken into consideration.

4.2 Informed consent according to the rules and regulations of the participating centers

4.3 *Donor exclusion*

4.3.1 Inability to tolerate the leukapheresis procedure due to psychological, medical or logistic reasons.

4.3.2 Positive pregnancy test for female donors.

4.3.3 HIV-positivity.

5. DESIGN AND CONDUCT OF THE STUDY

5.1 *Study design*

This is a multicenter phase II study to determine donor engraftment after T-cell depleted allo-SCT using the most efficient and least harmful non-myeloablative conditioning regimen and to assess hematologic and immunologic recovery, pattern of engraftment, and incidence and severity of GvHD after the allo-SCT. Furthermore to determine incidence and severity of GvHD and the anti-tumor effect after the administration of variable numbers of donor T lymphocytes at predetermined time points.

5.2 *Donor treatment procedures*

5.2.1. *Donor peripheral blood stem cell mobilization and leukaphereses*

Donors will be treated with rhu G-CSF, at a dose of 10 microgram/kg/day by daily subcutaneous injection for 5 consecutive days. Stem cell harvesting will take place on days 5 and 6. The leukapheresis procedure will start in the presence of detectable numbers of CD34⁺ cells i.e. > 0,2% of the nuclear cell fraction. Prior to leukapheresis the donor will need adequate venous access. Two leukapheresis procedures will be undertaken in the morning of day 5 and at day 6 of G-CSF treatment, using a Baxter Fenwal CS-3000 blood cell separator or any other automated continuous flow blood cell separator. The aim will be to collect a total of 15x10⁶ CD34⁺ cells/kg body weight of the recipient. Aliquots will be saved for progenitor cell assays (CFU-GM), CD34⁺ enumeration, as well as enumeration of T cell, B cell and NK cell numbers. If the aimed cell number of 15x10⁶ CD 34⁺ cells/kg body weight is not achieved with two leukapheresis, further treatment of the patient will be at the discretion of the investigator.

5.3 *T-cell depletion of the blood stem cell graft.*

T-cell depletion of the blood stem cell graft will be performed by an in-vitro incubation of the cells with 20 mgr Campath-1H for 30 minutes at 20°C. Following in vitro incubation, the stem cell graft will be infused into the patient without further manipulation.

5.4 *Recipient treatment procedures*

5.4.1 *Non-myeloablative conditioning regimen.*

The non-myeloablative conditioning regimens will be tested in a maximum of four cohorts of ten patients. The first ten patients will be treated with our current non-myeloablative conditioning regimen containing oral fludarabine (**scheme A**). Donor engraftment will be determined as described in section 8.3. Sustained positive donor engraftment is defined as the presence of more than 75% donor cells in unfractionated bone marrow samples.

If sustained donor engraftment is achieved in at least nine of ten patients, the following cohort of ten patients will be treated with **scheme B**, which is less immunosuppressive since it includes only 2 infusions of ATG. Donor engraftment will again be evaluated after treatment of ten patients.

If sustained donor engraftment is again achieved in at least nine of ten patients, the next cohort of ten patients will be treated by **scheme C**, containing only 1 day of ATG. If sustained donor engraftment is achieved in at least nine of ten patients, scheme C will be considered standard for the next patients.

If sustained donor engraftment is **not** achieved in patients treated with scheme B or C, the following cohort of ten patients will be treated with **scheme D**, containing 4 days of ATG but with a lower dose fludarabine (4 days instead of 6 days). If donor engraftment is again achieved, scheme D will be considered standard for the next patients. For patients with a solid tumor, cyclophosphamide (750 mg/m² for two days) or a similar cytostatic drug will be added to the conditioning regimen for additional anti-tumor control.

<p><i>Scheme A</i></p> <ul style="list-style-type: none">- fludarabine 50 mg/m²/day orally on days -10 to -5- anti-T lymphocyte globulin (horse) 10 mg/kg/day i.v. on days -4 to -1- busulphan 3.2 mg/kg/day i.v. or 4 mg/kg/day orally on days -6 to -5

<p><i>Scheme B</i></p> <ul style="list-style-type: none">- fludarabine 50 mg/m²/day orally on days -10 to -5- anti-T lymphocyte globulin (horse) 10 mg/kg/day i.v. on days -4 to -3- busulphan 3.2 mg/kg/day i.v. or 4 mg/kg/day orally on days -6 to -5

Scheme C

- fludarabine 50 mg/m²/day orally on days -10 to -5
- anti-T lymphocyte globulin (horse)10 mg/kg/day i.v. on days -4
- busulphan 3.2 mg/kg/day i.v. or 4 mg/kg/day orally on days -6 to -5

Scheme D

- fludarabine 50 mg/m²/day orally on days -8 to -5
- anti-T lymphocyte globulin (horse)10 mg/kg/day i.v. on days -4 to -1
- busulphan 3.2 mg/kg/day i.v. or 4 mg/kg/day orally on days -6 to -5

5.4.2 *Graft versus host prophylaxis*

All stem cell grafts will be T-cell depleted (see 5.3). No further GvHD prophylaxis will be given.

Note: No ciclosporin will be administered after unrelated donor transplantations

5.4.3 *Concomitant medication*

Throughout the study investigators may prescribe any concomitant medication that is considered necessary to provide adequate supportive care (anti-emetics, prophylactic antibiotic and antimycotic drugs). Hematological supportive care will involve prophylactic platelet transfusions when counts are < 10x10⁹/l and leukocyte-free red blood cell transfusions as clinically indicated.

5.4.4 *Criteria for initiation of donor leukocyte infusions*

When no donor chimerism is achieved, patients will not be treated with donor leukocyte infusions. Patients will go off study. Further therapy will be at the discretion of the treating physician.

Patients with **non-malignant diseases**: donor leukocyte infusions will be administered when loss of sustained donor chimerism (< 75%) is detected or disease progression more than six months after allo-SCT occurs. When no effect is observed after the first dose of donor lymphocytes, a higher dose may be given 3-6 months after the first dose (see appendix VI).

Patients with malignant diseases (see appendix VI):

Patients with stable mixed chimerism but no detectable disease at 9 months after allo-SCT will be treated with low dose donor leukocyte infusions. A second dose may be administered six months later. In case of persistent disease or relapse after remission after allo-SCT patients will be treated earlier after alloSCT with low dose donor leukocyte infusions. The dose is depending on the disease. A second dose of DLI may be given 3-6 months after the first dose. Importantly, patients with GvHD \geq grade II will not be treated with donor lymphocyte infusions until GvHD has resolved. The exact schedule and dosage of DLI for the specific conditions (type of disease and chimerism status) is documented in appendix VI.

Interferon-2a (Roferon) will be administered in patients with a relapse of a rapidly progressive disease (usually acute leukemia and solid tumors). The projected dose is 3×10^6 U/day subcutaneously. Treatment with Roferon will be continued throughout the infusions up to at least six months after the final infusion of cells. In case of GVHD grade 2 or more or a decrease in white blood cells ($< 2 \times 10^9/l$) or platelets ($< 75 \times 10^9/l$) Roferon will be stopped.

5.4.5. *Collection of donor lymphocytes and cryopreservation*

Donor leukocytes will be collected by leukapheresis using citrate-phosphate-dextrose (CPD) as anticoagulant. 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. Several doses may be harvested in one session. The first dose will be administered immediately to the patients. The other doses will be cryopreserved in RPMI supplemented with 1% human albumin and 10% DMSO to allow 50% loss of the cells after thawing.

5.4.6 *Treatment of GvHD*

Treatment of GvHD will be performed according to standard practice:

- GvHD grade 1

Short-term treatment with methylprednisolone (MP) may be started at a dose of 1 mg/kg body weight. The dose MP will be reduced by 50% every 24-48 hours, depending on clinical signs of GvHD.

- GvHD grade 2
Acute GvHD, grade 2, will be treated by the administration of methylprednisolone (MP) 5-10 mg/kg body weight/12 hr i.v. The dose MP will be reduced by 50% every 48 hr, depending on clinical symptoms of GvHD. In case GvHD persists ciclosporin A will be added. No further dose escalation of donor leukocytes will be performed.
- GvHD grade 3 or 4
Acute GvHD, grade 3 or 4, will be treated with MP at a dose of 5-10 mg/kg body weight/12 hr i.v., reduced every 48 hrs by 50% and ciclosporin A at a dose of 3 mg/kg i.v. In case of persistent severe acute GvHD grade 3 or 4, treatment may be given by Campath-1H 5 mg/day i.v. during 5 days.

6. PRETREATMENT INVESTIGATIONS

6.1 *Pretreatment observations recipient*

1. History and physical examination, including height and weight; the following data will be recorded specifically: performance status, time of diagnosis, history of previous chemo- and/or radiotherapy and previous partial or complete remissions, remission duration.
2. Blood cell counts, differential, quantitative platelet count.
3. Immunophenotypic analysis of the malignant cells, including the determination of 2 markers characteristic for the malignant cells and expression of CD80, CD86, class I, class II, CD11a, CD54, CD58 and CD52.
4. Bone marrow aspirates from pelvis for cytology, cytogenetics, molecular diagnostics and cryopreservation for immunological monitoring; a bone marrow biopsy for pathology will also be collected.
5. Other staging procedures as appropriate, related to previous sites of malignancy.
6. Hepatitis B, Hepatitis C, HIV, CMV, EBV, TPHA, and toxoplasma serology.
7. Blood group typing.
8. Bilirubin, alkaline phosphatase, SGOT, SGPT, SLDH, γ GT.
9. Albumin and protein electrophoresis.
10. Serum urea, creatinine, Na, K, uric acid, Ca, glucose.
11. Routine urine analysis.
12. ECG (pretransplant).
13. Chest X-ray (pretransplant).
14. Dental status (including X-OPG) (pretransplant).
15. Lung function tests, including DCO (CO diffusion measurement).
16. Screening for sensitization: allo antibodies against lymphocytes.
17. Sperm analysis and cryopreservation, if feasible.
18. Gonadal/hormonal function (FSH, LH, oestradiol, progesteron, testosteron, T4 and TSH).

6.2 *Pretreatment observations donor*

1. Medical history and physical examination will be performed.
2. Full blood cell counts, differential and platelet count
3. Serum creatinine, glucose, bilirubin, alkaline phosphatase, ALAT, ASAT, SLDH, γ GT.
4. Hepatitis B, Hepatitis C, HIV, CMV, EBV, TPHA, and toxoplasma serology.

5. Blood group typing.
6. Bone marrow aspirates from pelvis for cytology and cryopreservation of cells for chimerism studies and immunological monitoring.
7. Chest X-ray
8. ECG

Unrelated donors will be approached by Eurodonor Foundations according to the usual rules and regulations of unrelated donors.

7.0 STUDY PARAMETERS

7.1 *Donor study parameters*

This will be performed according to standard practice. All adverse events occurring during or after G-CSF administration and the leukapheresis procedure will be documented. The donor is asked for her or his well-being at 4 weeks and at 9 months after alloSCT.

7.2 *Recipient study parameters (Appendix V)*

7.2.1 Daily interim history and physical examination while hospitalized; thereafter at least weekly until three months after allo-SCT.

7.2.2 Blood cell count, differential, reticulocytes, platelet count, three times a week when hospitalized; thereafter once a week until three months after stem cell transplantation.

7.2.3 Creatinine, Urea, Na, K, Cl, Uric acid, Ca, glucose ASAT, ALAT, alkaline phosphatase, gammaGTP, bilirubine, SLDH, total protein, albumin, three times weekly while hospitalized and once every week until three months after stem cell transplantation.

7.2.4 Surveillance cultures according to bacteriology guidelines.

7.2.5 At evaluation days (see appendix V) 100 ml (or a leukapheresis if WBC are below $1.0 \times 10^9/l$) of peripheral blood will be drawn for:

- a) Chimerism analysis.
- b) Cytogenetic analysis.
- c) Monitoring of GvL activity using cellular immunological studies.
- d) Monitoring of immunological recovery.

7.2.6 At evaluation days (see appendix V) bone marrow aspirates will be taken for evaluation of morphology, phenotyping, cytogenetic analysis, and chimerism.

7.2.7 At evaluation days (see appendix V), all other staging procedures as appropriate, related to previous sites of malignancy will be performed.

7.2.8 Gonadal/hormonal function (FSH, LH, oestradiol, progesteron, testosteron, T4, TSH and spermogram) at 1, 2 and 5 years after allo-SCT.

8. CRITERIA OF EVALUATION

8.1 *Diagnostic criteria*

- 8.1.1. *Acute leukemias* and *myelodysplasia* will be classified according to the new WHO classification
- 8.1.2 *Chronic myeloid leukemia* in chronic phase is confirmed by bone marrow examination. Percentage blasts in bone marrow should be less than 10%. Accelerated or blastic phase in remission implies < 5% blast cells.
- 8.1.3 *Multiple myeloma* is classified according to Durie and Salmon.
- 8.1.4 *Non Hodgkin lymphoma (low, intermediate or high grade), Hodgkin's disease, chronic lymphocytic leukemia* is confirmed by histological lymph node and bone marrow biopsy assessment.
- 8.1.5 *Aplastic anemia* is classified according to the International Aplastic Anemia Study Group.
- 8.1.6 *Solid tumors*: the diagnosis is confirmed by histological biopsy assessments. The disease status is defined by CT-scan.

8.2 *Toxicity criteria*

- 8.2.1 General toxicity will be measured using the WHO criteria (appendix IV). In case of grade 3 or 4 toxicity during treatment, the treatment will be stopped. In addition the Leiden mucositis scoring list will be routinely used by the nursing staff at the clinical ward to monitor the severity of mucositis after the treatment.
- 8.2.2 Acute and chronic GvHD will be staged and graded according to the established criteria (see appendix II and III). Biopsies of involved organs are mandatory if considered necessary for differential diagnosis.
- 8.2.3 Definition of major infection:
The diagnosis of a major infection requires one or more of the following

criteria:

1. Systemic treatment of fungal infection
2. Pneumonia documented by X-ray
3. Positive blood cultures + fever + i.v. antibiotics
4. Intravenous antibiotics + soft tissue or CNS infection

8.3 *Definition of engraftment.*

Donor engraftment will be measured in unfractionated bone marrow samples by interphase FISH in case of sex-mismatched transplants and in case of sex-matched transplants by analysis of short tandem repeat markers (STR). Sustained donor chimerism is characterized as more than 75% donor cells measured at two time points with an interval of two weeks. Chimerism will be investigated in the myeloid and in the lymphatic cells separately.

In parallel, hematopoietic recovery will be determined as a platelet count of $> 20 \times 10^9/l$ independent of platelet transfusions and a neutrophil count of $> 0.5 \times 10^9/l$ on two consecutive measurements.

Samples will be collected and frozen to monitor T- and B-cell reconstitution.

8.4 *Criteria of response after alloSCT with or without DLI*

8.4.1 Complete hematological response: normalization of blood counts and morphology in peripheral blood *and* bone marrow without further treatment, and disappearance of paraprotein in MM.

8.4.2 Complete cytogenetic response: normalization of counts in bone marrow *and* peripheral blood, absence of cytogenetic abnormalities in bone marrow (> 30 metaphases counted).

8.4.3 Complete response in (non) Hodgkin lymphoma: disappearance of all localizations.

8.4.4 Partial response: reduction of more than 90% of malignant cells from bone marrow or peripheral blood in AML, CML, ALL, NHL or CLL. Reduction of $> 50\%$ of tumor diameter in lymphoma. Reduction of $> 50\%$ of paraprotein in MM.

8.4.5 Criteria of relapse:

- a) Relapse of AML or ALL or RAEB(T) is defined as an increase in the bone marrow of blast cells to > 10% or blasts + promyelocytes to > 20% (on 2 bone marrows at 2 weeks interval in case of borderline values) or > 5% blasts in peripheral blood, or reappearance of increasing percentages of cells in bone marrow or peripheral blood containing the original chromosomal translocation, or extramedullary relapse based on tissue biopsies.
- b) Relapse of CML after SCT is defined by the reappearance of the bcr-abl transcript, or of Ph¹ chromosome in > 10% of bone marrow metaphases or an increase of Ph¹ chromosomes at least two occasions, or by the reappearance of the characteristic hematological features of the disease.
- c) Relapse of NHL, CLL or Hodgkin's disease after SCT is defined by reappearance of malignant cells in bone marrow (> 5%) or peripheral blood or reappearance of lymphoma at any other localization, proven by tissue biopsy.
- d) Relapse of MM after SCT is defined by increasing percentage of MM cells (at least 20%) in bone marrow aspirate or biopsy, increase of > 10 gr/l of paraprotein in serum or 10 gr/24 hours in urine in at least two occasions or reappearance of histologically proven extramedullary localizations.

8.4.6 Criteria of tumor response for solid tumors after alloSCT with or without donor lymphocyte infusion (bidimensionally or unidimensionally measurable disease).

8.4.6.1 Complete response (CR): The disappearance of all known disease, determined by 2 observations not less than 4 weeks apart.

8.4.6.2 Partial response (PR): In case of bidimensionally measurable disease, a decrease by at least 50% of the sum of the products of the largest perpendicular diameters of all measurable lesions as determined by 2 observations not less than 4 weeks apart. For unidimensionally measurable disease, decrease by at least 50% in the sum of the largest diameters of all lesions as determined by 2 observations not less than 4 weeks apart. It is not necessary for all

lesions to have regressed to qualify for partial response, but no lesions should have progressed and no new lesions should appear. Serial evidence of appreciable change documented by radiography must be obtained.

8.4.6.3 Stable Disease (SD): for bidimensionally measurable disease < 50% decrease and < 25% increase in the sum of the products of the largest perpendicular diameters of all measurable lesions. For unidimensionally measurable disease, < 50% decrease and < 25% increase in the sum of the largest diameter of all lesions. No new lesions should appear.

8.4.6.4 Progressive Disease (PD): > 25% increase in the size of at least one bidimensionally or unidimensionally measurable lesion or appearance of a new lesion. The occurrence of pleural effusion or ascites is also considered as progressive disease if this is substantiated by positive cytology. Assignment to the progression category is done after 6 weeks from entry into the study.

8.5 *Treatment evaluation*

- This includes
- Response to alloSCT
 - Response to DLI
 - Disease-free survival: time from CR to the date of death or relapse whichever occurs first.
 - Occurrence of GvHD
 - Occurrence of severe infections
 - Overall survival
 - Gonadal and hormonal status

9. INVESTIGATOR AUTHORIZATION PROCEDURE

Investigators will be authorized to enter patients in this trial only when a letter of acceptance of the protocol by the local or national (whichever is applicable) Medical Ethical Committee (CME) has been received.

10. FORMS AND PROCEDURES FOR COLLECTING DATA

10.1 Case report forms

The EBMT case report forms for allogeneic stem cell transplantation will be used.

11. STATISTICAL CONSIDERATIONS

11.1 Sample size

The non-myeloablative conditioning regimens will be tested in a maximum of four cohorts of each ten patients.

The first ten patients will be treated with our current non-myeloablative conditioning regimen containing oral fludarabine (**scheme A**). If sustained engraftment is not achieved in more than one patient, patients will not be tested in the following cohorts.

If sustained donor engraftment is achieved in at least nine of ten patients, the following cohort of ten patients will be treated with **scheme B**, which is less immunosuppressive since it includes only 2 infusions of ATG.

If sustained donor engraftment is again achieved in at least nine of ten patients, the next cohort of ten patients will be treated by **scheme C**, containing only 1 day of ATG. If sustained donor engraftment is achieved in at least nine of ten patients, scheme C will be considered standard for the next patients.

If sustained donor engraftment is **not** achieved in more than one patient treated with scheme B or C, the following cohort of ten patients will be treated with **scheme D**, containing 4 days of ATG but with a lower dose fludarabine (4 days instead of 6 days). If donor engraftment is again achieved, scheme D will be considered standard for the next patients.

By this sequence of protocols we will determine the conditioning regimen with the highest engraftment and the least immunosuppressive effects.

11.2 Analysis

All eligible patients who started the treatment will be included in the analyses. The actuarial curves will be computed using the Kaplan-Meier technique and the standard errors (SE) of the estimates will be obtained via the Greenwood formula.

12. QUALITY OF LIFE ASSESSMENT

Quality of life will not be assessed in this study.

13. ECONOMIC EVALUATION

Health-economic evaluation will not be performed in this study.

14. PHARMACOKINETICS

Pharmacokinetic evaluation will not be performed in this study.

15. ETHICAL CONSIDERATIONS

Patients will be extensively informed about the status of their disease, the expected natural course, and possible other treatment modalities available. All possible complications will be discussed. The patient is free to withdraw at any time from the study during donor cell treatment. An independent physician is available for further information (Dr. F.J.M. van der Meer for the LUMC patients).

16. ADMINISTRATIVE RESPONSIBILITIES

The study Coordinator will be responsible for reviewing all case report forms and documenting his/her review on evaluation forms, discussing the contents of the reports with the Data Manager and for publishing the study results. He/she will also generally be responsible for answering all clinical questions concerning eligibility, treatment and the evaluation of patients.

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17. TRIAL SPONSORSHIP/FINANCING

The LUMC is the sponsor of the trial.

18. TRIAL INSURANCE

The LUMC insurance program covers all patients entered in the LUMC.

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APPENDIX I: KARNOFSKY PERFORMANCE - WHO PERFORMANCE STATUS

	<u>Karnofsky</u>	<u>WHO</u>
Normal; no complaints; no evidence of disease.	100%	
Able to carry on normal activity; minor signs or symptoms of disease	90%	0
Normal activity with effort; some signs or symptoms of disease.	80%	
Cares for self. Unable to carry on normal activity or to do active work.	70%	1
Requires occasional assistance but is able to care for most of his needs.	60%	
Requires considerable assistance and frequent medical care	50%	2
Disabled; requires special care and assistance.	40%	
Severely disabled; hospitalization is indicated although death is not imminent.	30%	3
Very sick; hospitalization necessary; active supportive treatment necessary.	20%	
Moribund; fatal processes progressing rapidly.	10%	4
Death		5

APPENDIX II: CLINICAL CLASSIFICATION OF ACUTE GvHD (GLUCKSBERG)

A. Staging of acute GvHD

	Skin	Liver	Gastrointestinal
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 bullae 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: CLINICAL CLASSIFICATION OF CHRONIC GvHD (SHULMAN)

Limited chronic GvHD Either or both:	Extensive chronic GvHD Either:
1. Localized skin involvement	1. Generalized skin involvement: or
2. Hepatic dysfunction due to chronic GvHD	2. Localized skin involvement and/or hepatic dysfunction due to chronic GvHD, plus:
	a. Liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or
	b. Involvement of eye: Schirmer's test with less than 5 mm wetting; or
	c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
	d. Involvement of any other target organ.

APPENDIX IV: MODIFIED WHO LIST AND GRADE OF TOXICITY						
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
GASTROINTESTINAL						
Bilirubin	< 1,25 x N	1.26 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	> 10 x N	
SGOT, SGPT	< 1,25 x N	1.26 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	> 10 x N	
Alkaline phosphatase	< 1,25 x N	1.26 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	> 10 x N	
Oral	no change	soreness, erythema	erythema, ulcers, can eat solids	ulcers, requires liquid diet only	alimentation not possible	
Nausea/Vomiting	none	nausea	transient	vomiting requires therapy	intractable vomiting	
Diarrhea	none	transient < 2 days	tolerable but > 2 days	intolerable requiring therapy	hemorrhagic dehydration	
Constipation	none	mild	moderate	abdominal distention	distention and vomiting	
RENAL						
BUN or blood urea	< 1,25 x N	1.26 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	> 10 x N	
Creatinine	< 1,25 x N	1.26 - 2.5 x N	2.6 - 5 x N	5.1 - 10 x N	> 10 x N	
Proteinuria	none	1 + < 3 g/l	2 - 3 + 3 - 10 g/l	4 + > 10 g/l	nephrotic syndrome	
Hematuria	none	microscopic	gross	gross-clots	obstructive uropathy	
CARDIAC						
Arrhythmia	none	sinus tachycardia > 110 at rest	unifocal PVC atrial arrhythmia	multifocal PVC	ventricular tachycardia	
Function	none	asymptomatic but	transient	symptomatic	symptomatic	

APPENDIX IV: MODIFIED WHO LIST AND GRADE OF TOXICITY						
	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
		abnormal cardiac sign	symptomatic dysfunction, no therapy required	dysfunction responsive to therapy	dysfunction non-responsive to therapy	
Pericarditis	none	asymptomatic changes	symptomatic no tap required	tamponade tap required	tamponade surgery required	
NEUROTOXICITY						
State of consciousness	alert	transient lethargy	somnolence < 50 of waking hours	somnolence > 50 of waking hours	Coma	
Peripheral	none	paresthesia and/or decreased tendon reflexes	severe paresthesia and/or mild weakness	intolerable paresthesia and/or marked motor loss	paralysis	
PULMONARY	none	mild symptom	exertional dyspnea	dyspnea at rest	complete bed rest required	
OTHERS						
Fever	none	fever < 38°C	fever 38 - 40°C	fever > 40°C	fever with hypotension	
Headache	none	very mild	mild	moderate	severe	
Flu-like syndrome	none	very mild	mild	moderate	severe	
Flushing	none	very mild	mild	moderate	severe	
Vasculitis	none	restricted cutaneous	generalized cutaneous	hemorrhagic	systemic	
Allergic	no change	edema	bronchospasm	bronchospasm parenteral	anaphylaxis	
Cutaneous	no change	erythema	dry desquamation	moist desquamation	exfoliative	

APPENDIX IV: MODIFIED WHO LIST AND GRADE OF TOXICITY

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
			pruritus vesiculation	ulceration	dermatitis necrosis requiring surgical intervention	
Pain#	none	mild	moderate	severe	intractable	

APPENDIX V: RECIPIENT STUDY PROCEDURE

	WEEKS											YEARS		
	Pre	0	2	4	6	8	10	12	18	26	38	1	2	5
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Biochemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BM cytol.	X			X		X		X	X	X	X	X	X	X
Peripheral Blood (100cc)	X	X		X		X		X	X	X	X	X	X	X
Tumor evaluation	X	X		X		X		X	X	X	X	X	X	X
Hormone	X											X	X	X
Sperm														

In case patient receives DLI: evaluation for chimerism, immunologic recovery and tumor response will take place after 6 weeks, 3 months, 6 months, 9 months and 12 months.

APPENDIX VI: DONOR LEUKOCYTE TREATMENT (CD3⁺ CELLS/KG)

1. Treatment mixed chimerism after alloSCT in patients with malignant disease:

No disease and molecular disease (bcr-abl)	Stable disease	Progressive disease*
↓ 9 mo 5 x 10 ⁶	↓ 6 mo 5 x 10 ⁶	↓ 3–6 mo 5 x 10 ⁶
↓ 6 mo 1.5 x 10 ⁷	↓ 6 mo 1.5 x 10 ⁷	↓ 3 mo 1.5 x 10 ⁷
↓ 6 mo 5 x 10 ⁷	↓ 6 mo 5 x 10 ⁷	↓ 3 mo 5 x 10 ⁷

* First tumor-reduction preferentially with monoclonal antibodies (Mylotarg, Rituximab).

2. Treatment mixed chimerism after alloSCT in patients with non-malignant disease:

- When loss of sustained donor chimerism is detected or progression of the disease more than six months after alloSCT: 3x10⁶ CD3⁺/kg will be administered. A second (higher) dose may be given 9 months later.