THE USE OF MISMATCHED FAMILY DONORS OR MATCHED UNRELATED DONORS IN MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANTATION

LUMC 2003-02

Follow up of AZL 95-03 protocol

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TABLE OF CONTENTS

1. INTRODUCTION AND BACKGROUND

1.1 Allogeneic stem cell transplantation (SCT) after intensive chemotherapy and total body irradiation (TBI) leads to long term disease-free survival in 40-50% of the patients with acute myelogenous leukemia or acute lymphoblastic leukemia transplanted in first remission. Similar results have been obtained in patients with chronic myelogenous leukemia transplanted in first chronic phase or in patients with the myelodysplastic syndrome.

1.2 Of the potential candidates for allogeneic stem cell transplantation only 30% has an HLA-identical family donor available for transplantation, whereas another 10% has a family member with only one mismatch on the non-shared haplotype. For the remaining patients the use of matched unrelated donors represents the only possibility for an allogeneic transplantation.

Although already at the end of the sixties transplantations using unrelated donors have been performed in several centers (including Leiden), the first successful transplantation with bone marrow of an unrelated donor has been published in 1980 in Seattle.

Over the past years, the number of patients transplanted using an unrelated donor have been increased progressively. Results of unrelated marrow transplantation have benefitted from the improved typing methodologies as well as the increased size of the unrelated donor pool. Patients who receive transplants from HLA non-identical family members or from HLA matched unrelated donors are at a significantly higher risk for acute GVHD than recipients of HLA genotypically matched grafts. Therefore in most centers T cell depletion has been used to prevent acute GVHD. Campath-1 antibodies have also been used in this setting, in particular in transplants from parents for immunodeficiencies and other inborn errors. T cell depletion in-vitro gave good control of GVHD with only 25 cases of severe acute GVHD out of 180 patients evaluable (14%). However, the incidence of graft failure was substantial in patients who did not receive additional therapy with Campath-1-G in-vivo. This is despite the fact that many of them received various other treatments designed to reduce the risk of graft rejection, such as additional irradiation, Cyclosporin-A or other antibodies including LFA-1 and CD2. Patients who received in-vitro T cell depletion combined with in-vivo treatment with Campath-1 G had a significantly lower incidence of graft failure (24%), although it was still a substantial problem. The use of in-vivo antibody alone combined with

conventional GVHD profylaxis using Methotrexate and Cyclosporin resulted in a similar rate of graft failure and GVHD.

The largest relatively homogeneous subset treated with Campath antibodies includes patients transplanted for CML in chronic phase from unrelated donors. They are of particular interest in view of the high incidence of relapse which has been seen following T cell depletion in HLA-matched sibling transplants in these patients. Thirty patients received in-vivo Campath-1 G combined with in-vitro T cell depletion and 60 received only in-vivo Campath-1 G treatment with conventional GVHD prophylaxis. There were no significant differences between the incidence of graft failure and GVHD in the two groups although the trend was towards less GVHD. More graft failure was observed with in-vitro T cell depletion alone. However, in both cases the incidence of relapse seemed to be much lower than in comparible transplants from HLA matched sibling donors. The 2 years survival rate was significantly better in the group who received only in-vivo antibody treatment and the leukemia-free survival in this group approached 50% which is almost as good as in matched sibling transplants.

The development of lymphoproliferative disorders, in particular related to the Epstein-Barr virus represents a problem that appears to be associated with matched unrelated transplants. Out of 462 transplants, 5 cases of lymphoproliferative syndrome or secondary lymphoma have been reported, representing an actuarial risk at 5 years of 2.3%. Campath-1 antibodies recognize B cells as well as T cells and it is possible that bone marrow purging with Campath antibody helps to reduce the potential target for EBV driven proliferation of B cells in the early post-transplant period. This may lead to fatal malignant lymphoma. In other centers, using post-transplant immune suppression with antibodies, an even higher incidence up to 25% has been reported. Therefore, the use of antibody treatment prior to transplantation to prevent graft rejection seems to be preferable over post-transplant use.

1.3 Although both the incidence and the severity of acute graft versus host disease are decreased after in vitro removal of the T cells, early and late graft failure or graft rejection is a major problem in patients receiving T-cell depleted stem cell grafts. Even in HLA-identical sibling combinations the incidence of this complication has been reported to rise as high as 10-30%. Mechanisms involved in graft failure have not been elucidated yet. However, several theories have been put forward, such as lack of immune suppression mediated by T cells in the graft, the lack of T-helper function necessary for engraftment, the concurrent depletion of pluripotent hematopoietic stem

cells, or the concurrent depletion of accessory or bone marrow stromal cells. Most of the current evidence, however, indicates that acute graft failure is mediated by cytotoxic T cells of host origin, surviving the conditioning regimen. In order to abolish these residual T cells, more intensive immunosuppressive treatment of the recipient is necessary. Various approaches have been used for this purpose, such as addition of cytosine-arabinoside (Ara-C) busulphan, antithymocyte globulin (ATG) or anti-T-cell monoclonal antibodies (like Campath) to the conditioning regimen, and of an increase in the dose of irradiation. In dogs various TBI schedules have been evaluated for their effect to permit engraftment of mismatched marrow. A regimen of two large fractions of TBI of 6 Gy each has been suggested to allow these grafts to take.

1.4 Since 1995 it is also possible (depending on the residence of the donor) to obtain peripheral stem cells from unrelated donors, the advantage of transplantation with peripheral stem cells compared to bone marrow stem cells is the faster recovery of granulocytes and platelets after transplantation.

1.5 From 1995-2001, we have transplanted 37 patients with an unrelated donor. Increasing numbers of peripheral stem cell donors are used (Table 1a). Indications for the transplants are listed in Table 1b. All grafts were T-cell depleted using Campath-antibodies. The overall survival of this cohort of patients is significant higher than the group of patients transplanted between 1988 and 1994 (45% versus 25%).

Table 1a Number of MUD transplantations per year in the LUMC

	BM	**PSCT**	**Total**
1995	2	0	2
1996	7	0	7
1997	6	1	7
1998	3	0	3
1999	1	1	2
2000	5	2	7
2001	4	5	9
			37

Table 1b Indication MUD transplantations

CML	17
AML/ALL	15
MDS	2
NHL	2

Other	1
	37

1.6 The aim of this study is to further investigate the feasibility with respect to engraftment and GVHD of transplantation of stem cells of mismatched family donors or matched unrelated donors, using the described intensive conditioning regimen and T cell depleted stem cells.

References

1. Zwaan FE, Hermans J, Barrett AJ, Speck B. Bone marrow transplantation for acute non-lymphoblastic leukaemia: a survey of the European Group for Bone Marrow Transplantation (E.G.B.T.M.) Br J Haematol 1984; 56: 645-653.
2. Zwaan FE, Hermans J. Barret AJ, Speck B. Bone marrow transplantation for acute lymphoblastic leukaemia: a survey of the European Group for Bone Marrow Transplantation (E.G.B.M. T .1. Br J Haematol 1984; 58: 33-42.
3. Hale G, Waldmann H, for CAMPATH users. CAMPATH-1 monoclonal antibodies in bone marrow transplantation. CAMPATH-Users Report 1993.
4. Cullis JO, Szydlo TM, Cross NC, Marks DI, Schwarer AP, Hughes AP, MacKinnon S, Hale G, Waldmann H, Hows JM et al. Matched unrelated donor bone marrow transplantation for chronic myeloid leukaemia in chronic phase: comparison of ex vivo and in vivo T cell depletion. Bone Marrow Transplantation 1993; 11 (S 1): 107-111.
5. Fischer A, Blanche S, Le-Bidois J, Bordigoni P, Garnier JL, Niaudet P, Morinet F, Le Deist F, Fischer AM, Griscelli C, et al. Anti B-cell monoclonal antibodies in the treatment of severe B cell lymphoproliferative syndrome following bone marrow and organ transplantation. New.Engl.J.Med. 1991; 324: 1451-1456.
6. Hale G, Waldmann H for Campath users. Control of graft-versus-host disease and graft rejection by T cell depletion of donor and recipient with Campath-1 antibodies. Results of matched sibling transplants for malignant diseases. Bone Marrow Transplantation 1994; 13: 597-611.
7. Willemze R, Richel DJ, Falkenburg JHF, Hale G, Waldmann H, Zwaan FE, Fibbe WE. In vivo use of Campath 1 G to prevent graft-versus-host disease and graft rejection after bone marrow transplantation. Bone marrow transplantation 1992; 9: 255-261.
8. Jacobs P, Wood L, Fullard L, Waldmann H, Hale G. T cell depletion by exposure to Campath-1 G in vivo prevents graft-versus-host disease. Bone Marrow Transplantation 1994; 13: 763-769.
9. Champlin RE, Schmitz N, Horowitz MM, Chapuis B, Chopra R, Cornelissen JJ, Gale RP, Goldman JM, Loberiza Jr FR, Hertenstein B, Klein JP, Monsterrat E, Zhang MJ, Ringden O, Tomany SC, Rowlings PA, van Hoef ME, Gratwohl A. Blood stem cells compared with bone marrow as a source of hematopoietic cells for allogeneic transplantation. IBMTR

Histocompatibility ans Stem Cell Sources Working Committee and the European Group for Blood and Marrow Transplantation (EBMT). Blood 2000; 95: 3702-3709.

10. Hale G, Zhang MJ, Bunjes D, Prentice HG, Spence D, Horowitz MM, Barrett AJ, Waldmann H. Improving the outcome of bone marrow transplantation by using CD52 monoclonal antibodies to prevent graft-versus-host disease and graft rejection. Blood 1998; 92: 4581-4590.
11. Hale G, Jacobs P, Wood L, Fibbe WE, Barge R, Novitzky N, Toit C, Abrahams L, Thomas V, Bunjes D, Duncker C, Wiesneth M, Selleslag D, Hidajat M, Starobinski M, Bird P, Waldmann H. CD52 antibodies for prevention of graft-versus-host disease and graft rejection following transplantation of allogeneic peripheral blood stem cells. Bone marrow transplantation 2000; 26: 69-76.
12. Rebello P, Cwynarsky K, Varughese M, Eades A, Apperley JF, Hale G. Pharmacokinetics of Campath-1H in bone marrow transplant patients. Cytotherapy 2001; 3: 261-267.
13. Sehn LH, Alyea EP, Weller E, Canning C, Lee S, Ritz J, Antin JH, Soiffer RJ. Comparative outcomes of T-cell-depleted and non-T-cell-depleted allogeneic bone marrow transplantation for chronic myelogenous leukemia: impact of donor lymphocyte infusion. Journal of Clinical Oncology 1999; 17: 561-568.

2. OBJECTIVES OF THE STUDY

2.1 To determine whether an intensified immunosuppressive conditioning regimen is able to prevent graft rejection following allogeneic SCT, using family donors other than HLA-identical siblings, and unrelated donors.

2.2 To determine the incidence and severity of acute and chronic graft versus host disease.

2.3 To assess hematologic and immunologic recovery.

2.4 To determine survival and leukemia-free survival.

3. PATIENTS SELECTION

3.1 Entry criteria

3.1.1

- Patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL) in remission: **a)** AML in second remission **b)** secondary AML or high-risk AML in first or subsequent remission **c)** standard risk ALL in second remission **d)** high risk ALL in first remission.
- Patients with the following myelodysplastic syndromes: refractory anaemia with excess of blasts (RAEB) with life threatening pancytopenia, and RAEB in transformation.
- Patients with chronic myelogenous leukemia (CML) in accelerated phase or in second chronic phase.
- Patients with lymphoblastic lymphoma in second or subsequent remission.
- Patients with myeloma stage III.

Patients are eligible regardless of previous chemotherapy or radiotherapy. AII subtypes of AML (according to the FAB classification) are eligible to the study. Diagnoses should be confirmed by the Dutch “Leukemie preparaten commissie ".

3.1.2 Age under 50.

3.1.3 Availability of an appropriate donor: (see 4).

3.1.4 Informed consent according to rules and regulations of the participating hospital.

3.1.5 Adequate renal (serum creatinine < 150 μmol/l (1), liver (bilirubin < 20 μmol/l (1), cardiac and pulmonary function.

3.1.6 WHO performance status of 0, 1 or 2 (see appendix IV).

3.2 Exclusion criteria

3.2.1 Life expectancy severely limited by diseases other than leukemia.

3.2.2 The availability of a genotypically HLA-matched donor.

3.2.3 Severe psychological disturbances.

3.2.4 Inability to tolerate the conditioning regimens due to medical reasons.

4. DONOR SELECTION

4.1 The following donor categories are included:

HLA-MISMATCHED RELATED DONORS

The most suitable mismatched members of the family will be used as the donor. These include the family members who are phenotypically identical to the recipient or who have only 1 locus-mismatch on the unshared haplotype. In case of more mismatched only those will be selected where the mismatch involves gross reacting groups or splits.

HLA-MATCHED UNRELATED DONORS

A suitable donor is selected in collaboration with Europdonor Foundation. In case more than one donor is available, CMV status, blood group, age and sex of the donors will be taken into consideration.

4.2 Donor exclusion

4.2.1 Inability to tolerate the stem cell harvesting procedure due to psychological or medical reasons.

5. TRIAL DESIGN

This is a prospective non-randomized phase II study designed to test the feasibility, toxicity and anti-tumor activity of myeloablative allogeneic stem cell transplantation using mismatched family donors or matched unrelated donors in patients with hematological malignancies.

5.1 Pretransplant observations recipient:

1. History and physical examination, including height and weight; the following data will be recorded specifically: performance status, time of diagnosis, onset of complete remission (for patients with acute leukemia); and also history of previous chemo- and/or radiotherapy and previous transfusions.
2. Blood cell counts, differential, quantitative platelet count.
3. Bilirubin (direct and indirect), alkaline phosphatase, SGOT, SGPT, SLDH.
4. Albumin and protein electrophoresis.
5. Serum urea, creatinine, Na, K, Cl, uric acid, Ca, Mg, glucose.
6. Routine urine analysis.
7. EKG.
8. Chest X-ray.
9. X-sinus.
10. Dental status (including X-OPG), consultation dentist (if indicated).
11. Bone marrow aspirates from pelvis for cytology, cytogenetics and molecular analysis; bone marrow biopsy for pathology (if indicated).
12. Other staging procedures as appropriate, related to previous sites of leukemia.
13. Hepatitis B screening.
14. Mantoux skin testing (if indicated).
15. Lung function tests, including DCO (if indicated).
16. Radiotherapy planning (Iocalization).
17. Screening for sensitization: allo antibodies against lymphocytes.
18. Blood typing and cross match; immunoglobulin allotyping; red and white cell iso-enzyme determination.
19. Sperm cryopreservation, if feasible.

5.2. Pretransplant observation donor:

Performed in separate donor centers. Before start of conditioning regimen, Europdonor confirms the availability of the donor by letter. In case a family member is donor, the pretransplant observations are performed conform protocol LUMC 2003-01.

5.3 *Conditioning regimen*

Stem cell transplant recipients will be treated with either intravenous Campath-1H (15 mg/daily dose) on days -6, and -4, TBI on 2 consecutive days and Cyclophosphamide (60 mg/kg bodyweight) on days -6 and -5 or intravenous Campath-1H (15 mg/daily) on days -6, and -4, Busulphan intravenously (0.8 mg/kg x 4) on day -8, -7, -6, and -5 and intravenous Cyclophosphamide (50 mg/kg bodyweight) on days -6, -5, -4 and -3. Alternatively, Cyclophosphamide may be administered on days - 4 and -3 at a dose of 60 mg/kg bodyweight. Stem cells will be infused on day 0. The conditioning regimen without TBI will be used in those patients where TBI is considered not appropriate.

- TBI will be delivered with 2 horizontal beams (AP and PA with the patient on either side) with a linear accelerator, and an average dose rate of 22 cGy/min. The dose will be 6 Gy on two consecutive days, resulting in a total dose of 12 Gy to the midline of the body. Lung shielding will be applied resulting in a cumulative lung dose of 6 Gy. In addition, partial eye shielding may be applied, resulting in a dose of 5 Gy to the eyes.
- Cyclophosphamide will be infused in 500 ml of glucose 5% water over 2 hr. MESNUM (20 mg/kg) will be administered at -10 min, + 3 hr. and + 7 hr. following cyclophosphamide infusion. Patients will be hydrated with dextrose 5% water and normal saline (NaCI 0.9%), beginning 2 hr. before the first cyclophosphamide dose. KCL will be further supplemented as needed. Additional bicarbonate ($NaHCO_3$ 8.4%) will be given to keep urinary pH > 6.0. A urinary flow of at least 100 ml/hr will be maintained during 48 hr following the beginning of the cyclophosphamide infusion. Furosemide will be added during this period depending on fluid-in and output status. Patients will be medicated with antiemetics as needed.
- Busulphan i.v. 3.2 mg/kg/day divided into every 6 hours. Since administration of high-dose busulphan has been temporarily associated with the development of generalized seizures, prophylactic administration of phenytoin (5 mg/kg/dose p.o. q 6 hrs beginning 2 days before the first dose of busulphan, then 5 mg/kg/day p.o. daily through day -1 is recommended. Intravenous administration of phenytoin may be required if the patient is unable to tolerate oral medications

or if a therapeutic level needs to be attained. Anticonvulsant levels should be monitored and the doses adjusted to maintain levels in the therapeutic range.

- Campath-1H monoclonal antibody will be dissolved in 500 ml glucose 5% and infused in at least 4 hours under hydrocortisone cover. Adverse reactions are most likely to occur during the first two days and may include fever, chills, rigour and changes in pulse or blood pressure. Pethidine may be used to alleviate these symptoms. The fluid balance should be carefully controlled.

5.4 Additional treatment:

5.4.1 Cyclosporin-A (CyA) will be started on day -1. Initially, CyA is given as an i.v. infusion of 3 mg/kg b.w./24 hr in 500 ml of dextrose 5% water as a continuous infusion. After 14 days CyA may be given orally at a dose of 9 mg/kg b.w. adapted according to levels in the plasma and/or serum creatinine concentration. Administration will be continued until 3 months after transplantation. Thereafter the dosage will be gradually tapered in 3-6 months. When signs of GVHD emerge following discontinuation, CyA will be reinstituted.

5.4.2. Treatment of acute GVHD

The presence of acute GVHD will be determined by established criteria (appendix I). These will include clinical evaluation of the skin, liver and gastro-intestinal tract. Skin biopsies or rectal mucosal biopsies will be performed to confirm GVHD. Acute GVHD, grade I-II, will be treated with low dose prednison or by increasing the dose of CyA. In case of insufficient response methylprednisolone (MP) 10 mg/kg b.w./24 hr i.v. will be given. The dose MP will be reduced by 50% every 48 hr, depending on clinical symptoms of GVHD. Progression of GVHD during this treatment or severe acute GVHD (grade III-IV) will be treated with horse ATG (20 mg/kg b.w.) or Campath-1H during 5 days.

5.4.3 Antiemetics, including methylprednisolone, may be prescribed as needed.

5.4.4 Chronic graft versus host disease

The presence of chronic GVHD will be determined by established criteria (Appendix II). Chronic GVHD will be treated according to the discretion of the responsible physician.

5.4.5 Non engraftment

Patients in whom a neutrophil count at day 28 after transplantation of $0.1x10^9$/l, a platelet count of $20x10^9$/l (without transfusions) and a reticulocyte count of 1% is not reached will be considered as non-engrafted.

5.4.6 Prior to treatment a central venous catheter will be placed.

5.4.7 Partial antibiotic decontamination of the digestive tract and oral cavity will be applied, according to local protocols. Patient will be nursed in a laminar down flow room until the granulocyte counts will have risen to a minimum of $0.1x10^9$/l.

5.4.7.1 Female patients will be started on anovulatory drugs (Iynesterol, 5-15 mg daily).

5.4.8 Hematological supportive care will involve prophylactic platelet transfusions when counts are < $10x10^9$/l and leucocyte-free red blood cell transfusions as clinically indicated. All blood products will be irradiated with 15 Gy.

6. STEM CELL COLLECTION AND PROCUREMENT

6.1 Bone marrow collection

Bone marrow will be aspirated under general anaesthesia from the iliac crests by multiple 2-4 ml aspirates and will be collected in sterile bottles containing Hanks' balanced salt solution (HBSS) with preservative-free heparin. A total of at least $2x10^8$ nucleated cells/kg b.w. will be collected. A bone marrow sample will be checked for $CD34^+$ determination and colony culture in vitro.

6.2 Peripheral stem cell collection

Donors will be treated with rhu G-CSF, at a dose of 10 μg/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 $7.5x10^6$ $CD34^+$ 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.

6.3. In-vitro depletion of T cells from the stem cell graft

6.3.1 Monoclonal anti-T-cell antibodies: (Campath-1H)

The stem cell graft will be incubated with Campath-1H (20 mg) for 30 minutes at room temperature (20°C). Following in-vitro incubation, the stem cell graft will be infused in the patients without any further manipulation. Prior to and after in-vitro incubation with antibody, a sample will be taken to assess the number of hematopoietic progenitor cells and the number of T cells and NK cells.

7. CLINICAL EVALUATION AND FOLLOW UP

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) a maximum of 100 ml (or a leukapheresis if WBC are below $1.0x10^9$/l) of peripheral blood will be drawn for regular patient care (a en b) and additional research (c and d):

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 are classified according to the new WHO classification.

8.1.2 Chronic myelogenous leukemia in chronic phase is defined by sustained leukocytes > $20x10^9$/l in peripheral blood, in combination with the presence of myeloid precursor cells. Basophilia (> 2%) should be present. The percentage of blast cells in blood or bone marrow should be < 10%. Cytogenetic studies of bone marrow should reveal the Philadelphia chromosome.

8.1.3 Accelerated phase of CML is defined by the presence of inappropriate splenomegaly, with blast cells in excess of 10% in the blood or bone marrow, or the presence of cytogenetic changes in addition to the Ph^1 chromosome.

8.1.4 Myeloma, stage III is classified according to Durie and Salmon.

8.2 Criteria of relapse

8.2.1 Relapse of AML: relapse following complete remission is defined as reappearance of blasts in the blood or the finding of more than 5% blasts in the BM, not attributable to another cause (e.g. BM regeration).

8.2.2 Relapse of ALL or lymphoblastic lymphoma in the bone marrow is defined as an increase of blast cells to > 5%. Relapse can be suspected in case of unexpected cytopenia, or overt reappearance of circulating blast cells.

8.2.3 Relapse of CML is defined by the reappearance of the Ph^+ chromosome or bcr-abl fusion-gene. Relapse may be present in the absence of the characteristic hematologic features of the disease.

8.2.4 Relapse of myeloma is defined by the reappearance of the monoclonal spike in the plasma or urine.

8.3 Engraftment

Engraftment will be determined by a number of parameters. These wilI include reconstitution of peripheral blood counts following Iethal chemoradiation preparation. T-cell reconstitution will be monitored by FACS-analysis with several monoclonal antibodies (CD1, CD2, CD3, CD4, CD8). Chimerism will be assessed using STR-PCR based methods or sex-chromosome analysis.

8.4 Graft-versus-host disease (GVHD)

The severity of acute GVHD and the occurrence of chronic GVHD will be correlated with the efficacy of the monoclonal antibody treatment of the marrow and the peripheral blood T-cell phenotype at the time of onset of acute GVHD.

8.5 Survival and disease-free survival

Using life-table analysis techniques survival and disease-free-survival probabilities will be determined at regular intervals.

9. FORMS AND PROCEDURES FOR COLLECTING DATA

9.1 Case report forms

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

10. STATISTICAL CONSIDERATIONS

10.1 AII relevant patient data will be anonimized and collected in the EBMT leukaemia Registry. The data will be analyzed at regular intervals and will be compared with the result of other transplant centres in Europe.

10.2 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.

11. QUALITY OF LIFE ASSESSMENT

Quality of life will not be assessed in this study.

12. ECONOMIC EVALUATION

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

13. PHARMACOKINETICS

Pharmacokinetic evaluation will not be performed in this study.

14. ETHICAL CONSIDERATIONS

14.1 Declaration of Helsinki

The investigator will ensure that the study is conducted in full accordance with the Declaration of Helsinki.

14.2 Informed consent

It is the responsibility of the investigator to obtain witnessed oral or written informed consent from recipient and the donor after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. The name of the witness and the date that informed consent was obtained will be reported in the patient's hospital notes.

At each individual center the approval of the Ethical Committee must be obtained before the study may be started.

14.3 Patient confidentiality

The investigator will ensure that the patient's anonymity is maintained. On the CRF's patients will be identified by their initials and patient's study number.

15. 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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16. TRIAL SPONSORSHIP/FINANCING

The LUMC is the sponsor of the trial.

17. TRIAL INSURANCE

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

APPENDIX I

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 (103-255 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

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 II

CLINICAL CLASSIFICATION OF CHRONIC GVHD (SHULMAN)

Limited chronic GvHD	**Extensive chronic GvHD**
Either or both:	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 5mm wetting; or
	c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
	d. Involvement of any other target organ.

APPENDIX III

KARNOFSKY PERFORMANCE - WHO PERFORMANCE STATUS

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

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	torerable but > 2 days	intorerable 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						
Arythmia	none	sinus tachyardia > 110 at rest	unifocal PVC atrial arrhythmia	multifocal PVC	ventricular tachycardia	
Function	none	asymptomatic but abnormal cardiac sign	transient symptomatic dysfunction, no therapy required	symptomatic dysfunction responsive to therapy	symptomatic dysfunction non-responsive to therapy	
Pericarditis	none	asymptomatic changes	symptomatic no tap required	tamponade tap required	tamponade surgery required	
NEUROTOXICITY						
State of consiousness	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 pruritus vesiculation	moist desquamation ulceration	exfoliative 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 Sperm	X											X	X	X

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.