

THE USE OF HAPLOIDENTICAL FAMILY DONORS IN ALLOGENEIC STEM CELL TRANSPLANTATION

LUMC 2003-03

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Final version: October 2003

MEC approval:

Activated:

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SUMMARY

Allogeneic stem cell transplantation is an accepted treatment modality for a variety of hematological disorders. Of the potential candidates for allogeneic stem cell transplantation only 30% has an HLA-identical family donor, whereas an additional 5% has a family member with only one mismatch on the non-shared haplotype. Matched unrelated donors can be found in due time in another 45-50%, leaving approximately 15-20% of the patients without a suitable stem cell donor. The introduction of haploidentical stem cell transplantation has opened up new possibilities for the patients without a suitable donor. The use of allogeneic stem cells from family donors who are only matched for half of their HLA antigens with the recipient potentially increases the regularly available donor pool to almost all candidates for transplantation. During the last 10 years the concept of the use of haploidentical stem cells has been successfully developed in adult patients by Martelli's group at Perugia, and in children by Handgretinger's group at Tuebingen and by O'Reilly's group in New York. The procedure is based on the usage of megadoses of highly purified donor CD34⁺ cells given after immuno- and myelo-ablative conditioning regimens containing total body irradiation plus thiotepa, fludarabine and anti-thymocyte globulin (ATG). Engraftment was achieved in most patients and GvHD was almost fully prevented when using an extensive depletion of T cells. The major drawback of this intensive conditioning and the rigorous T cell depletion was prolonged immune-incompetence resulting in a high opportunistic infection-related mortality rate in the selected group of end stage patients. In spite of these complications the overall survival in the total Perugia experience for all patients was 35% at 7 years. For the least bad risk patients (comprising only one third of the study group) the eventfree survival was approximately 50%, which is not inferior to the transplantation results using stem cells from matched unrelated donors.

The aim of this study is to investigate the feasibility with respect to engraftment, GVHD, immune reconstitution and opportunistic infections of transplantation of stem cells of an haploidentical family donor, using an intensified myelo-ablative conditioning regimen and massive numbers of donor T cell depleted stem cells in patients lacking a HLA-identical family donor or a matched unrelated donor. No additional donor lymphocytes will be administered at a later time point.

1. INTRODUCTION AND BACKGROUND

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.

Of the potential candidates for allogeneic stem cell transplantation only 30% has an HLA-identical family donor, whereas an additional 5% has a family member with only one mismatch on the non-shared haplotype. Matched unrelated donors could be found in another 45-50%, leaving 15-20% of the patients without a suitable stem cell donor.

The introduction of haploidentical stem cell transplantation has opened up new possibilities for the patients without a suitable donor. The use of allogeneic stem cells from family donors who are only matched for half of their HLA antigens with the recipient potentially increases the regularly available donor pool to almost all candidates for transplantation.

During the last 10 years the concept of the use of haploidentical stem cells was successfully developed in adult patients by Martelli's group at Perugia, in children by Handgretinger's group at Tuebingen and by O'Reilly's group in New York. The procedure is based on the usage of megadoses of highly purified CD34⁺ cells given after highly immuno- and myeloablative conditioning regimens containing total body irradiation plus thiotepa, fludarabine and anti-thymocyte globulin (ATG). Engraftment was achieved in most patients and GvHD was almost fully prevented when using an extensive depletion of T cells (to less than 2×10^4 /kg body weight in the final graft). The drawback of this intensive conditioning and the rigorous T cell depletion was a high infection-related mortality rate (40%) in the selected group of end stage patients. In spite of these opportunistic complications the overall survival in the total Perugia experience for all patients was 35% at 7 years. For the least bad risk patients (comprising only one third of the study group) the eventfree survival was approximately 50%, which is not inferior to the transplantation results using stem cells from matched unrelated donors.

The most successful haploidentical stem cell transplantation program started in 1993 in Perugia (It.). Thirty-six patients received high numbers of CD34⁺ cells, depleted from T cells by soybean agglutinin and E-rosetting leading to graft containing a median number of 10.8×10^6 CD34⁺ cells/kg and of 2.2×10^5 CD3⁺ cell/kg. The conditioning regimen consisted of total body irradiation, ATG, thiotepa and cyclophosphamide. Overall engraftment was 92%, acute

GvHD occurred 18% and chronic GvHD in 4% of the cases. From 1995 they used E-rosetting combined with CellPro. In addition they replaced cyclophosphamide by fludarabine in the conditioning regimen. Forty-three end stage patients (ages 4-53 yrs) were treated. Engraftment was successful in 95%. No acute GvHD greater than grade II was observed and chronic GvHD occurred in 1/37. Eventfree survival after 5 years was only approx. 25%, mainly due to relapses and opportunistic infections. From 1999 the CliniMACS device to deplete T cells was introduced leading to a graft that contained a median number of 13.6×10^6 CD34+ cells/kg and 1.2×10^4 CD3+ cells/kg. The conditioning regimen consisted of TBI, fludarabine, thiotepa and ATG. The group of patients (n=86) contained 55 with AML (CR1 n=15, CR>1 n=18, remaining n=22) and 31 with ALL (CR1 n=12, CR>1 n=8 and remaining n=11 patients) with a median age of 38 yrs (for AML) and 26 yrs (for ALL), range 9-64 years. Primary engraftment occurred in 92% (80/86 patients) with PMN $> 1 \times 10^9/l$ on day 12 and trombocytes $> 25 \times 10^9/l$ on day 18 (five out of 6 graft failures engrafted after subsequent transplantation with stem cells of different haploidentical family donors). Acute GvHD greater than grade I was observed in 8/80 and chronic GvHD in 4/61 (9%). Treatment related mortality after 1 year was 37% (32/86). In 21 patients opportunistic infecties (CMV 11, aspergillus, bacterial etc.) was the cause of death and in 11 cases other causes such as interstitial pneumonias, GvHD and MOF. AML and ALL patients transplanted in relapse or with refractory disease did poorly. Their eventfree survival was only 9-16%. For the ALL patients who were transplanted in first or second complete remission the eventfree survival was 42% and for the AML patients who were transplanted in first or second complete remission the eventfree survival was 52%. The treatment related mortality only 15%, suggesting that the high treatment related mortality rate was rather caused by the stage of the disease and performance status of the patient at the time of transplantation than by the transplantation schedule.

Donor selection may now involve not only standard donor criteria, but also a deliberate search for the "perfect" mismatch at certain HLA-loci, that is for the mismatch that drives donor vs recipient NK-cell alloreactivity. Haploidentical donors can be selected for KIR ligand mismatches on the unshared haplotype in the graft vs host direction. Selection for KIR mismatching is based on HLA typing. KIR epitope mismatch in the graft-versus-host direction is associated with a significantly lower proportion of relapses after haploidentical SCT for AML and CML. This is not the case for ALL. KIR epitope mismatch in the graft-versus-host direction also seems to be associated with a lower proportion of graft rejection. This means that KIR epitope mismatch (HLA-C group 1 or 2, Bw4) in the graft-versus-host direction has become a major selection criterion of the donor.

Currently adult patients without a HLA-compatible family donor are treated in the Department of Hematology of the LUMC according to transplantation protocol LUMC 2003-04. This protocol includes an intensified conditioning schedule (comprising of TBI, cyclophosphamide and intravenous Campath) followed by transplantation of a Campath T cell depleted bone marrow or blood stem cell graft. In addition, considerable experience with the harvest and transplantation of large numbers of (by Campath) T cell depleted stem cells is obtained with our non-myeloablative conditioning (comprising of fludarabine, busulphan and ATG) protocol (updated LUMC 2003-01).

Since the experience with both protocols is satisfactory, it is logical to proceed along these lines with the development of an haploidentical transplantation protocol for adult patients. To reach an acceptable level for the treatment related mortality we will select the study group by avoiding endstage and otherwise poorly performing patients. Furthermore, we intend to compare the treatment related complications of haploidentical donors that are either mismatched for maternal or paternal antigens or KIR epitopes.

The aim of this study is to investigate the feasibility with respect to engraftment, GVHD, donor immune reconstitution and opportunistic infections of transplantation of stem cells of an haploidentical family donor, using an intensive conditioning regimen and massive numbers of T cell depleted stem cells in patients lacking an HLA-identical family donor or a matched unrelated donor. No additional donor lymphocytes will be administered at a later time point.

2 OBJECTIVES OF THE STUDY

- 2.1 To determine donor engraftment after allo-SCT with stem cells from haploidentical family donors using an intensified myeloablative and immunosuppressive conditioning regimen.
- 2.2 To assess hematologic and immunologic recovery.
- 2.3 To determine the incidence and severity of acute and chronic graft versus host disease.
- 2.4 To determine the incidence and severity of opportunistic infections
- 2.5 To determine anti-tumor effect after allo-SCT (relapse rate).
- 2.6 DFS and survival.

2.6 3. PATIENTS SELECTION

3.1 *Entry criteria*

3.1.1

- Patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL) in complete (or partial) remission: **a)** high risk AML in first remission **b)** AML in second remission **c)** secondary AML in first or subsequent remission **d)** standard risk ALL in second remission **e)** very 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): refractory for imatinib or in accelerated phase or in second chronic phase.

3.1.2. Age limits 18 - 50 years

3.1.3 Inavailability of an appropriate HLA-matched donor.

3.1.4 If possible, availability of a sufficient number of frozen autologous stem cells (to be used as rescue stem cells)

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). Informed consent according to rules and regulations of the Leiden University Medical Center.

3.2 *Exclusion criteria*

3.2.1 Life expectancy severely limited by diseases other than the hemato-oncological disorder.

3.2.2 Evidence of active infection.

3.2.3 HIV positivity.

3.2.4 The availability of a HLA-matched family or unrelated donor.

3.2.5 Severe psychological disturbances.

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

3.2.7 Previous allo-SCT.

4 DONOR SELECTION

4.1 Donor selection:

The two most suitable haploidentical members of the family (18-65 years) will be selected as potential donor. Preferably a brother or sister mismatched for a KIR (based on HLA typing) epitope in the GvHD direction will be used. In case more donor are available, the age, CMV status and sex of the donor will also be taken into consideration.

4.2 Donor inclusion

See protocol 2003-01 and 2003-04.

4.3 Donor exclusion

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

4.2.2 Positive pregnancy test/lactating for female donors.

4.2.3 Recipient CMV positive and potential donor CMV negative.

4.2.4 HIV-positivity.

5 DESIGN AND CONDUCT OF THE STUDY

5.1. *Study design*

This is a phase II study to determine donor engraftment, GvHD, infectious complications, immunological recovery and anti-tumor effect after allo-SCT using stem cells from a haplo-identical family donor.

5.2. *Donor treatment procedures*

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

Donors in accordance with the guidelines for mobilization for HLA-identical family donors (LUMC 2003-01/2003-04), 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 10-15 x 10⁶ CD34⁺ cells/kg body weight of the recipient. No more than 2 cycles of leukaphereses will be performed. 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. The harvested cells will be infused in the patient even if the aimed cell number of 10-15 x 10⁶ CD34⁺ cells/kg body weight is not achieved with two leukapheresis. In case of subsequent non-engraftment autologous stem cells or the other already selected haplo-identical donor will be used.

Note: Sufficient numbers of patient's stem cells (autologous) will be harvested before (according their original treatment protocols or according above described mobilization and harvesting protocol) to be served as rescue in case of non-engraftment after transplantation of the haplo-identical stem cells.

5.2.2. *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 mg 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.

5.3 Recipient treatment procedures

5.3.1. Intensive chemo- and radiotherapy conditioning (according to Perugia)

The conditioning regimen of the stem cell transplant recipients will consist of:

- TBI 9 Gy on day -9

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 9 Gy. Lung shielding will be applied resulting in a cumulative lung dose of 6 Gy. In addition, partial eye shielding may be applied.

- Thiotepa 5 mg/kg body weight per day intravenously on days -8 and -7 as four-hours infusion

- Fludarabine 40 mg/m² per day intravenously on days -6 to -3 as daily 30 minutes infusion

- ATG (horse) ATG 10 mg/kg on days -6 to -3 as a eight-hours infusion

Stem cells will be infused on day 0.

5.3.2. Graft versus host prophylaxis

All allogeneic stem cell grafts will be T-cell depleted (see 5.2.2.). No further GvHD prophylaxis will be given.

5.3.3. Concomitant medication

Throughout this study investigators may describe any concomitant medication that is considered necessary to provide adequate supportive care. Antiemetics, including methylprednisolone, may be prescribed as needed. Prior to treatment a central venous catheter will be placed.

Partial antibiotic decontamination of the digestive tract and oral cavity will be applied, according to the LUMC protocols.

Patient will be nursed in a laminar down flow room until the granulocyte counts will have risen to a minimum of 0.1 x 10⁹/l.

- Anti-fungal prophylaxis will include itraconazole oral solution from day -10 to +120.

- Twice weekly CMV antigenemia is determined by CMV-DNA PCR in blood samples. If CMV PCR positivity develops, patients will be treated with ganciclovir foscarnet until resolution or valganciclovir in case of recurrent CMV positivity.
- Oral cotrimoxazol 480 mg/daily as anti-streptococcal and anti-pneumocystis prophylaxis, will be given during the first year after transplantation.

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

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

5.3.4. 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.

5.3.5 Chronic graft versus host disease

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

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. Full blood cell counts, differential, 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, HSV, VZV and toxoplasma serology.
7. Complete red blood group typing.
8. Bilirubin, alkaline phosphatase, SGOT, SGPT, SLDH, γ GT.
9. Albumin and protein electrophoresis; quantitative immunoglobulins.
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 (according to protocol LUMC 2003-04)*

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, HSV, VZV and toxoplasma serology.
5. Red 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.

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) a maximum of 100 ml (or a leukapheresis if WBC are below $1 \times 10^9/l$) of peripheral blood will be drawn for regular patient care (a and 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 reconstitution

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.

8.2 *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% circulating 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*

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

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 Partial response: reduction of more than 90% of malignant cells from bone marrow or peripheral blood in AML, CML, ALL.

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.

8.5 *Treatment evaluation*

This includes:

- Response to alloSCT
- 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

All patients up to the age of 50 years without an available HLA compatible family or unrelated donor will be offered an haploidentical stem cell transplantation.

11.1 Sample size

A total number of 32 patients are planned for this phase II feasibility study. With the entry rate of 4 pts/year, the approximate duration of this study will be of approximately 8 yrs.

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.

11.3 All relevant patient data will be collected in the EBMT Leukemia Registry. The data will be analyzed at regular intervals and will be compared with the result of other transplant centres in Europe.

12. QUALITY OF LIFE ASSESSMENT

Quality of life studies will not be performed 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. QUALITY ASSURANCE

16. ETHICAL CONSIDERATIONS

16.1 Declaration of Helsinki

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

16.2 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.

16.3 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.

17. 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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18. TRIAL SPONSORSHIP/FINANCING

The LUMC is the sponsor of the trial.

19. 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. 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 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	

APPENDIX IV: MODIFIED WHO LIST AND GRADE OF TOXICITY

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
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 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	

APPENDIX IV: MODIFIED WHO LIST AND GRADE OF TOXICITY

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
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	X											X	X	X
Sperm														