TREATMENT WITH DONOR PERIPHERAL BLOOD LEUKOCYTE TRANSFUSIONS OF PATIENTS WITH PRIMARY REFRACTORY OR RELAPSED LEUKEMIA, NON-HODGKIN'S LYMPHOMA, REFRACTORY ANEMIA WITH EXCESS BLASTS OR MULTIPLE MYELOMA AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

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SUMMARY

Treatment with donor lymphocyte infusions (DLI) and interferon-α of patients with a relapse of chronic myeloid leukemia after HLA-identical bone marrow or peripheral blood stem cell transplantation has been shown to induce long lasting hematological and cytogenetic remissions. In this study patients with a relapse of acute myeloid leukemia (AML), refractory anemia with excess blasts (in transformation) (RAEB(T)), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), high-grade non-Hodgkin's lymphoma (NHL), or multiple myeloma (MM) after allogeneic HLA-identical bone marrow or peripheral blood stem cell transplantation will be treated with escalating doses of DLI combined with interferon-α. Patients with AML, RAEB-T, ALL, CML, NHL, or MM that have been shown to be refractory to standard high dose chemotherapy regimens will be treated with high dose chemotherapy followed by standard allogeneic HLA-identical bone marrow transplantation or peripheral blood stem cell transplantation. Interferon-α treatment will be initiated as soon as a relapse occurs, followed by infusion of escalating doses of DLI. Treatment will be postponed or stopped in case of severe graft-versus-host-disease (GVHD) or progression of the disease, not responding to debulking chemotherapy, respectively. Concomitant treatment with low-dose chemotherapy will be allowed but patients treated with high dose corticosteroids or immunosuppressive drugs will be excluded from the study. The aim of the study is to determine the anti-leukemic effect and toxicity of α-Interferon with increasing doses of donor-derived lymphocyte infusions in patients with relapsed AML, RAEB(T), ALL, CML, NHL or MM after allogeneic stem cell transplantation. Also, the anti-leukemic effect and toxicity of allogeneic SCT followed by α-Interferon with increasing doses of donor-derived lymphocyte infusions in patients with primary refractory AML, RAEB(T), ALL, CML in blast crise, NHL or MM will be studied. Furthermore, the possible mechanisms of the anti-leukemic effect of this treatment will be assessed.

1.0 INTRODUCTION

Allogeneic stem cell transplantation (SCT) has been successfully applied in the treatment of hematologic malignant diseases. Treatment of patients with acute myeloid leukemia (AML) in first or second complete remission, acute lymphoblastic leukemia (ALL) in remission, refractory anemia with excess blasts (in transformation) (RAEB(T)), chronic myeloid leukemia (CML) in chronic phase, high grade non Hodgkin's lymphoma (NHL), or multiple myeloma (MM) with high dose chemotherapy (usually cyclophosphamide 2x60 mg/kg bodyweight) and total body irradiation (TBI) followed by transplantation with the stem cell graft from an HLA-identical sibling donor, has led to long term disease-free survival of approximately 50% of the patients. 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 appears to lead to an increased risk of relapse after transplantation, GvL reactivity has been attributed to donor T lymphocytes present in the stem cell graft. Furthermore, MHC non-restricted natural killer (NK) cell activity may also contribute to the GvL effect after SCT.

Transfusion of unmodified peripheral blood mononuclear cells from the HLA-identical sibling donors for relapsed AML or CML following allogeneic bone marrow transplantation may lead to strong reduction in the number of leukemic cells. Long term hematologic and molecular remissions have been reported. Recently, it was demonstrated that 70% of the patients with a relapse of a chronic myeloid leukemia after allogeneic SCT entered a complete remission following administration of donor derived peripheral blood leukocytes. Furthermore, approximately 30% of patients with relapsed AML or multiple myeloma could also be successfully treated with donor leukocytes. Although in most studies, only 10-20% of patients with accelerated phase or blast crise of chronic myeloid leukemia after allogeneic SCT was successfully treated with donor leukocytes, recently in our institute 3 of 5 patients with blast crise or accelerated phase CML after allogeneic SCT entered a complete remission on combined therapy of α-Interferon with donor leukocytes. A recent survey from European centers (EBMT) revealed that AML, ALL, and

CML blast crise responded significantly more frequently after infusion of at least 10^8 donor derived peripheral blood leukocytes/kg body weight (Dr. H.-J. Kolb, personal communication). These results illustrate that donor leukocyte transfusions after relapse following allogeneic SCT may be successful in 20-70% of patients.

Although the effectiveness of the treatment is frequently associated with the induction of graft-versus-host disease (GvHD) in these patients, complete remissions have also been observed in the absence of GvHD. In addition, in most cases GvHD has been shown to be manageable. The severity of GvHD following the administration of donor leukocytes after T cell depleted bone marrow transplantation has been less than after transplantation of bone marrow without T cell depletion. It has been hypothesized that this may be due to the fact that many antigen presenting cells after SCT are of donor origin. Furthermore, tissue damage caused by the irradiation and chemotherapy during the conditioning regimen is no longer present several months after the transplantation. Therefore, T cell responses directed against antigens presented during tissue damage are no longer induced, possibly leading to a clinically less severe GvHD than shortly after the conditioning regimen. Several reports have indicated that administration of donor leukocytes shortly after SCT may induce more severe GvHD, and it has been suggested that an interval of at least 6 months after SCT is associated with the lower incidence of severe GvHD using more than 10^6 T lymphocytes/kg.

Probably due to the allo-reactivity of the donor lymphocytes directed against residual hematopoietic cells from the recipient, severe aplasia has been observed after successful treatment of relapsed CML after allogeneic SCT. This aplasia has been shown to last for a long time in several cases. Therefore, it may be necessary to transfuse a second stem cell graft from the same donor to restore hematopoiesis in vivo. In these cases, no conditioning of the recipient is required when the majority of lymphoid cells from the circulation has been shown to be of donor origin. Because of this complication, availability of the same donor for a second stem cell donation in case of persistent aplasia of the recipient is relevant. Since the likelihood of aplasia is probably correlated with the contribution of recipient cells to the hematopoiesis after SCT, this complication is less likely to occur shortly after SCT, or at the onset of relapse.

At present, at the LUMC the direct transplantation related mortality of allogeneic T cell

depleted stem cell transplantation is approximately 15%. Since it has been estimated that the chance of entering a second complete remission after a relapse of the leukemia following SCT using T cell depleted grafts is around 20-40% of the patients with blast crise or accelerated phase of chronic myeloid leukemia, acute myeloid leukemia, acute lymphoblastic leukemia or multiple myeloma, it is hypothesized that in patients primarily refractory to chemotherapy before allogeneic SCT a long term disease-free survival may be projected at 15-35%. Since in these patients with leukemia or high-grade lymphoma refractory to chemotherapy no other curative options are available at present, treatment of donor leukocytes following allogeneic T cell depleted transplants may be a reasonable alternative.

α-Interferon has been applied alone or in combination with donor-leukocytes in the treatment of relapsed chronic myeloid leukemia after SCT. Although following donor-leukocyte transfusions remissions have been observed also in the absence of α-Interferon, the rationale for the addition of α-Interferon has been both the responses to this cytokine prior to SCT, and the observation that α-Interferon may augment the immune response. Recently, we demonstrated that α-Interferon may facilitate the generation of cytotoxic T cell responses in vitro against chronic myeloid leukemia, and possibly Philadelphia-chromosome positive ALL. α-Interferon may be beneficial due to its anti-proliferative effect on chronic myeloid leukemic precursor cells, the upregulation of HLA-class I or class II antigens on immunologically recognizable target cells, or by the upregulation of co-stimulatory molecules necessary for the recognition of malignant cells by T lymphocytes. In multiple myeloma and non-Hodgkin's lymphoma, α-Interferon may also clinically delay progression of the disease and therefore this treatment may add to the GvL effect of leukocyte transfusions.

The objectives of this study are to establish the toxicity and anti-leukemic effects of the administration of donor derived leukocytes in the presence of adjuvant α-Interferon following allogeneic T cell depleted stem cell transplantation in patients with relapsed leukemia, RAEB(T), lymphoma, or multiple myeloma after HLA-identical stem cell transplantation or primary refractory leukemia, RAEB(T), lymphoma, or multiple myeloma.

2.0 OBJECTIVES OF THE STUDY

1. To determine the anti-leukemic effect and toxicity of α-Interferon with increasing doses of stem cell donor-derived mononuclear peripheral blood leukocytes in patients with relapsed AML, RAEB(T), ALL, CML, NHL or MM after allogeneic stem cell transplantation .

2. To determine the anti-leukemic effect and toxicity of allogeneic SCT followed by α-Interferon with increasing doses of stem cell donor-derived mononuclear peripheral blood leukocytes in patients with primary refractory AML, RAEB(T), ALL, CML in blast crise, NHL or MM.

3. To assess the possible mechanisms of the anti-leukemic reactivity by this treatment.

3.0 PATIENT SELECTION

3.1 *Entry criteria*

3.1.1 Patients with a hematologic or cytogenetic relapse of acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) or a clinical relapse of non Hodgkin's lymphoma or multiple myeloma after allogeneic HLA-identical SCT.

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 Ph^1 chromosome in >10% of bone marrow metaphases or an increase of Ph^1 chromosomes at least two occasions, or by the reappearence of the characteristic hematologic features of the disease.

c) Relapse of NHL after SCT is defined by reappearance of malignant cells in bone marrow (> 5%) or peripheral blood or reappearance of any other localization, proven by tissue biopsy.

e) 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.

3.1.2 - Patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL) that have been shown to be refractory to standard high dose chemotherapy or patients with an early relapse prior to allogeneic SCT.

- Patients with refractory anaemia with excess of blasts in transformation (RAEB(T)) and patients with RAEB, both refractory to high dose chemotherapy, or patients

with an early relapse prior to allogeneic SCT.

- Patients with chronic myelogenous leukemia (CML) in accelerated phase or blast crisis refractory to high dose chemotherapy or patients with an early relapse prior to allogeneic SCT.
- Patients with intermediate- or high-grade non-Hogdkin's lymphoma refractory to high dose chemotherapy.
- Patients with multiple myeloma with partial response or refractory to chemotherapy.

3.1.3 Age under 60 years.

3.1.4 Availability of an appropriate stem cell donor: (see 4).

3.1.5 Adequate renal (serum creatinine < 150 µmol/)l, liver (bilirubin < 30 µmol/l and transaminases < twice normal), and adequate cardiac and pulmonary function (> 50% of normal) except when due to the malignancy.

3.1.6 WHO performance status of 0, 1 or 2.

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

3.2 *Exclusion criteria*

3.2.1 Life expectation of < 1 month

3.2.2 Severely limited life expectation due to diseases other than leukemia, lymphoma or multiple myeloma

3.2.3 Severe psychological disturbances

3.2.4 Necessity for treatment with immunosuppressive drugs or high dose corticosteroids for prolonged periods

3.2.5 GvHD ≥ grade 2 at initiation of treatment

3.2.6 HIV positivity

3.2.7 Evidence of graft rejection

4.0 DONOR SELECTION

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

4.2 Only the original stem cell donor will be selected as donor for the leukocyte infusion.

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

4.4 *Donor exclusion*

4.4.1 Inability to tolerate the bone marrow harvesting or leukapheresis procedure due to psychological, medical or logistic reasons. This includes the risk associated with general anaesthesia or epidural anaesthesia.

5.0 STEM CELL TRANSPLANTATION

Stem cell transplantation (SCT) will be performed according to standard procedures, at present according to protocol 95-01 / CME 20/95

5.1 *Patient monitoring for relapse after SCT*

After SCT bone marrow aspiration will be performed for morphology, cytogenetic analysis and chimerism as clinically indicated but at least 3, 6, 9, 12, 18, 24, 30, and 36 months after SCT. In patients with refractory hematological malignancies prior to SCT, additional analysis will be performed at 6 and 18 weeks after SCT.

5.2 *Debulking chemotherapy prior to SCT*

Patients with resistant AML, RAEB(T), ALL, or CML blast crise will be treated prior to conditioning regimen with high dose chemotherapy to reduce total leukemic cell load. The nature of the chemotherapy will depend on the type of leukemia and (partial) responses to previous chemotherapy. Patients must have at least a responding relapse of their malignancy on re-induction chemotherapy. In case of refractory leukemia (AML, RAEB(T), ALL, or blast crise CML) before SCT, the SCT will be performed in the hypoplastic phase following the high dose chemotherapy.

6.0 CHEMOTHERAPY AS PART OF THE TREATMENT OF RELAPSE AFTER SCT

6.1 *Debulking chemotherapy prior to donor leukocyte infusion (DLI)*

In case of rapidly progressing malignancy, debulking chemotherapy may be applied prior to DLI. The nature of the chemotherapy will depend on the type of malignancy and clinical responses prior to transplantation. For ALL and CML in lymphatic blast crisis treatment may consist of a combination of asparaginase, daunorubicin, vincristine, and prednisone according to the local treatment protocols. For AML and CML in myeloid blast crisis treatment may consist of a combination of cytarabin and amsacrin or with another regimen at the discretion of the treating physician.

6.2 *Chemotherapy after donor leukocyte infusion*

Low dose of chemotherapy may be necessary to control the in vivo growth of malignant cells. In CML, AML or ALL hydroxyurea and/or mercaptopurine may be used. In multiple myeloma low dose of cyclophosphamide or vincristine may be used in the absence of high-dose corticosteroids. Chemotherapy administration to control leukocyte counts must be as low as possible to control leukocyte counts.

7.0 CRITERIA FOR INITIATION OF DONOR LEUKOCYTE INFUSION

7.1 *Relapse identification*

Patients will be treated as soon as a relapse is documented after the SCT. Relapse is identified by the inclusion criteria described in paragraph 3.

7.2 *Interval after SCT*

Donor engraftment must be apparent as measured by interphase FISH in case of sex-mismatch transplants, restriction fragment-length polymorphisms (RFLP) or variable numbers of tandem repeats (VNTR).

8.0 SCHEDULE OF DONOR LEUKOCYTE TREATMENT

8.1 *Start of interferon therapy*

Immunosuppressive therapy will be stopped. Interferon-α 2a (Roferon) will be administered at a projected daily dose of $3x10^6$ U/day subcutaneously. In addition, chemotherapy (see 6.1) may be administered to further control leukocyte counts. Patients with smouldering leukemia, CML in chronic phase, or multiple myeloma will receive Roferon for up to 4 weeks prior to the first infusion of donor cells. Patients with acute leukemia or CML in accelerated phase or blast crisis who relapse ≥3 months after SCT and who need debulking chemotherapy prior to DLI will receive Roferon when white blood cells counts are $\geq 0.3x10^9$/l and platelet counts are $\geq 30x10^9$/l following chemotherapy. Patients with primary refractory hematological malignancy or with a relapse < 3 months after SCT will receive Roferon as soon as blast cells have been detected.

8.2 *Duration of interferon therapy*

Treatment with Roferon will be continued throughout the infusions up to at least 6 months and preferably up to 1 year after the final infusion of cells in case of a partial or complete remission. In case of marrow aplasia Roferon will be stopped eight weeks after the last infusion. Roferon therapy may be reduced in dose if grade 3-4 GVHD toxicity occurs, or when white blood cells counts are $<2x10^9$/l in the absence of chemotherapy. Roferon will be stopped if granulocyte counts are $<1x10^9$/l following a complete response.

8.3 *Infusion of mononuclear cells*

Within 4 weeks after initiation of Roferon therapy mononuclear cell infusions will be started. For patients with smouldering leukemia, or CML in chronic phase, the first dose will be projected at 10^7 mononuclear cells/kg bodyweight. If GvHD maximally grade I and no partial or complete response is observed, 8-16 weeks later a dose of $3x10^7$ cells/kg mononuclear cells will be administered. Cryopreserved mononuclear cells from the donor will be thawed immediately prior to administration. The interval depends on clinical

effect, progression of the disease and concurrent complications. Again, 8-16 weeks later a projected dose of 10^8 cells/kg will be administered if GvHD maximally grade I in the absence of a clinical anti tumor response is observed. If GvHD ≥ grade II is observed, no further dose escalation will be performed. For patients with acute leukemia, CML in accelerated phase or blast crisis or patients with rapidly progressing multiple myeloma, who have received debulking chemotherapy, either or not followed by SCT, the initial dose will be aimed at $1x10^8$ cells/kg. In case of GvHD maximally grade I, while a persistent relapse of the malignancy is observed, treatment will be further continued. Four to eight weeks later a dose aimed at $3x10^8$ cells/kg will be administered. In general, if GvHD without clinical anti-leukemic effect is observed, the treatment will be postponed. If GvHD has been resolved, treatment will be reinstalled if no remission is observed. If during an interval between two leukocyte transfusions a partial or complete remission is observed, further dose escalating will be postponed. If during the intervals between the escalating doses of donor leukocytes clinical progression is observed that will enforce additional treatment, the interval between the various doses may be reduced to 4 weeks.

9.0 DONOR LEUKOCYTE COLLECTION AND CRYOPRESERVATION

9.1 *Collection of donor leukocytes*

Donor leukocytes will be collected by leukapheresis using citrate-phosphate-dextrose (CPD) as anti-coagulant. The leukapheresis procedure per mononuclear cell dose will be restricted to maximally 6 hours per session and maximally 12 hours in total with a maximal volume of blood to be processed of 20 liters per session. If the first dosis of lymphocytes administered to patient will be targeted at 10^7 mononucleair cells/kg bodyweight, the total amount that will be harvested during the first leukapheresis session will be aimed at $7x10^7$ mononuclear cells/kg bodyweight of the patient. The first dose (10^7 mononuclear cells/kg bodyweight) will be administered freshly. For the second dose, $6x10^7$ mononuclear cells/kg bodyweight of the patient will be cryopreserved in RPMI supplemented with 1% human albumin and 10% dimethylsulphoxide to allow 50% loss of the cells during thawing, aiming to infuse $3x10^7$ viable mononuclear cells/kg into the patient. In case of a first dose of 10^8 mononuclear cells/kg bodyweight the mononuclear cells will be administered freshly. 15 vials of 10^7 cells will be cryopreserved at the laboratory of Experimental Hematology as reference samples.

9.2 *Additional collection of donor leukocytes*

If no response is observed after administration of the second dose after SCT, additional leukapheresis will be performed. These cells will be administered freshly.

9.3 *Unrelated donors*

Unrelated donors will approached by Europdonor Foundations according to the usual rules and regulations for unrelated donors.

10.0 SECOND STEM CELL TRANSPLANTATION FOR APLASIA AFTER SUCCESSFUL DONOR LEUKOCYTE INFUSION

10.1 *Criteria for aplasia*

If due to a successful treatment with donor leukocytes as shown by a complete remission and the presence of donor lymphocytes in the peripheral blood, bone marrow aplasia may occur and persist, a second stem cell grafting may be performed. Prolonged aplasia of the recipient will be defined as renewed dependency on red blood cell transfusions, on platelet transfusions, in the absence of reticulocytes, and granulocytes $<0.1x10^9/l$ for a period of at least two weeks and hypocellular bone marrow. Stem cell grafting may take place as soon as 4 weeks following persistent aplasia.

10.2 *Donor stem cell harvest*

A second bone marrow graft or peripheral blood stem cell graft may be harvested not earlier than 3 months after the first harvest.

10.3 *T cell depletion of the stem cell graft*

In vitro T cell depletion of the stem cell graft may be performed by incubation of the unwashed stem cell buffycoat with 10 mg of Campath-1G antibody. The mixture will be incubated at room temperature for 30 minutes, and subsequently washed three time using HBSS to remove free Campath-1G antibody. Subsequently, the cell suspension is infused into the patient.

10.4 *Unrelated donors*

Unrelated donors will approached by Europdonor Foundations according to the usual rules and regulations for unrelated donors.

11.0 SPECIAL ORDERS

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

11.2 Patients will be hospitalized when they receive donor leukocyte infusion treatment until at least 2 hours after the infusion.

11.3 All patients will receive pneumocystes carinii prophylaxis preferentially with cotrimoxazol until 6 months after the last donor leukocyte infusion.

11.4 In case of GvHD following donor lymphocyte infusion, CMV antigenemia will be monitored. In case of increasing CMV-antigenemia, patients will be treated with ganciclovir.

12.0 TREATMENT OF GvHD AFTER DONOR LEUKOCYTE TREATMENT

12.1 *GvHD grade 1*

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

12.2 *GvHD grade 2*

Acute GvHD, grade 2, will be treated by the administration of methylprednisolone (MP) 5-10 mg/kg b.w./12 hr i.v. The dose MP will be reduced by 50% every 48 hr, depending on clinical symptoms of GvHD. No further dose escalation of donor leukocytes will be performed.

12.3 *GvHD grade 3 or 4*

Acute GvHD, grade 3 or 4, will be treated with cyclosporin A at a dose of 3 mg/kg i.v. and MP at a dose of 5-10 mg/kg bodyweight/12 hr i.v., reduced every 48 hrs by 50%. In case of persistent severe acute GvHD grade 3 or 4, treatment may be given by Campath-1G 5 mg/day during 5 days.

13.0 PRETREATMENT INVESTIGATIONS

13.1 *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.
4. Bone marrow aspirates from pelvis for cytology, cytogenetics and cryopreservation for immunological monitoring; a bone marrow biopsy for pathology will be collected if no adequate aspirate is obtained. In CML bone marrow analysis should be performed at most 6 weeks prior to the start of treatment with α-Interferon.
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, γGT.
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) (pretransplant).
16. Radiotherapy planning (pretransplant localization).
17. Screening for sensitization: allo antibodies against lymphocytes (pretransplant).
18. Sperm cryopreservation, if feasible (pretransplant).
19. Establishment of the presence of (residual) donor cells in the patient after transplant using restriction fragment length polymorphism (RFLP), or variable

numbers of tandem repeats (VNTR).

13.2 *Pretreatment observations of the donor*

1. History and physical examination; previous infections and transfusions.
2. Blood cell counts, differential, platelet count.
3. Bilirubin (direct and indirect), alkaline phosphatase, ASAT, ALAT, SLDH, γGT.
4. Serum creatinine, and glucose.
5. Hepatitis B, Hepatitis C, HIV, CMV, EBV, and toxoplasma serology .

14.0 STUDY PARAMETERS AFTER LEUKOCYTE TRANSFUSION

14.1 Interim history and physical examination prior to each leukocyte transfusion, and every other week between leukocyte infusions.

14.2 Blood cell count, differential, reticulocytes, platelet counts at least every other week during treatment. Thereafter as clinically indicated, but at least once a month for 4 months.

14.3 Creatinine, Urea, Na, K, Cl, Uric acid, Ca, glucose ASAT, ALAT, alkaline phosphatase, γGTP, bilirubine, SLDH, total protein, albumin, prior to mononuclear cell infusions and once every two weeks during treatment. Thereafter as clinically indicated, but at least once a month, for 4 months.

14.4 Surveillance cultures according to bacteriology guidelines.

14.5 At evaluation days (see schedule) 100 ml (or a leucapheresis if WBC are below $1.0x10^9/l$) of peripheral blood will be drawn for:

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

14.6 Prior to each leukocyte infusion, and 1, 2, 4 and 6 months after the last leukocyte infusion, and when clinically indicated, bone marrow aspirates will be taken for evaluation of morphology, phenotyping, cytogenetic analysis, and chimerism. From six months, follow up will be identical to the follow up after SCT.

14.7 Transfusates of donor mononuclear cells will be analyzed for the expression of CD14/45, CD2, CD3, CD4, CD8, and CD56.

15.0 CRITERIA OF EVALUATION

15.1 *Toxicity criteria*

15.1.1. 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.

15.1.2. GvHD will be graded using the criteria outlined in appendix I and II

15.2 *Response criteria in patients treated for relapse after SCT*

15.2.1. Hematologic complete response: normalization of blood counts in peripheral blood *and* bone marrow without further treatment, and disappearance of paraprotein in MM.

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

15.2.3 Complete response in NHL: disappearance of all localizations.

15.2.4 Partial response: reduction of more than 90% of malignant cells from bone marrow or peripheral blood, in AML, CML, or ALL.
Reduction of > 50% of tumor size in NHL. Reduction of > 50% of paraprotein in MM.

16.0 ETHICAL CONSIDERATIONS

Patients will be extensively informed about the status of their disease, the expected natural course, and possible other treatment modalities available, including low dose chemotherapy. All possible complications will be discussed. The patient is free to withdraw at any time from the study during donor cell treatment.

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APPENDIX I: KARNOFSKY PERFORMANCE - WHO PERFORMANCE STATUS

	Karnofsky	WHO
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%	1
Cares for self. Unable to carry on normal activity or to do active work.	70%	
Requires occasional assistance but is able to care for most of his needs.	60%	2
Requires considerable assistance and frequent medical care	50%	
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%	4
Moribund; fatal processes progressing rapidly.	10%	
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 forma- tion of bullea and desquamation	Bilirubin > 15 mg/dl (> 225 umol/L)	Severe abdominal pain with or without ileus

B. Grading of acute GVHD

Overall grade	Stage		
	Skin	Liver	Gut
I (mild)	1 or 2	0	0

II (moderate)	1-3	1	1
III (severe)	2 or 3	2 or 3	2 or 3
IV (life-threatening)	2-4	2-4	2-4

APPENDIX III: 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 NEUROTOXICITY	none	asymptomatic changes	symptomatic no tap required	tamponade tap required	tamponade surgery required	
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 pruritus vesiculation	moist desquamation ulceration	exfoliative dermatitis necrosis	

APPENDIX IV: MODIFIED WHO LIST AND GRADE OF TOXICITY

	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
					requiring surgical intervention	
Pain#	none	mild	moderate	severe	intractable	

TABLE 1 DONOR LEUKOCYTE TREATMENT

WEEKS																							
	Incl	0#	2	4	6	8#	10	12	14	16	18	20	22	24#	26	28	30	32	36	40	44	48	52
Hematology	X	X	X	X	X	X	X	X	X	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	X	X	X	X	X	X	X	X	X
BM aspirates	X	X		X		X		X		X		X		X		X		X		X			X
Paraprotein quantification (in MM)	X	X				X				X				X				X		X			X
Peripheral blood immunology, chimerism and cytogenetics	X	X		X		X		X		X		X		X		X		X		X		X	X
Infusion of mononuclear cells		10^7 /				$3x10^7$/ kg				10^8 /				$3x10^8$/ kg									

		kg								kg													
α-Interferon	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X