MYELOABLATIVE STEM CELL TRANSPLANTATION USING HLA-IDENTICAL FAMILY DONORS

2003-04

(follow up of 95-05)

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1. INTRODUCTION AND BACKGROUND

1.1. Allogeneic stem cell transplantation (SCT) after intensive chemotherapy and total body irradiation (TBI) leads to longterm 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 leukaemia transplanted in first chronic phase or in patients with the myelodysplastic syndrome.

1.2 To prevent acute graft-versus-host disease (GVHD) patients routinely receive intermittently methotrexate(MTX) or cyclosporin-A during the first 100 days following SCT. Even with this prophylactic therapy, however, approximately 50% of all patients treated for aplastic anaemia or leukaemia develop moderate to severe (grade II-IV) acute GVHD following stem cell transplantation from HLA-identical sibling donors. At least 15% of patients die from complications directly related to GVHD.
Patients with GVHD may also be more susceptible to develop interstitial pneumonitis, which is fatal in almost 80% of patients affected. Approximately 30% of patients surviving the immediate post-transplant period develop chronic GVHD, a complication associated in some cases with considerable morbidity, increased susceptibility to serious infections and occasional mortality.

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 marrow grafts. Even in HLA-identical sibling combinations the incidence of this complication has been reported to rise as high as 10-30%. The use of peripheral blood stem cell as a source of stem cells has significantly decreased the risk of graft failure. Preliminary evidence indicates that matching not only for HLA but also for the non-HLA or so-called minor antigens between donor and recipients might diminish the risk of graft failure and/or graft versus host disease. A drawback of in vitro T cell depletion is the increased risk of recurrence of the leukemia following transplantation in particular in patients with CML.

1.4 Campath-1 (CDw52) antibodies recognize a small glycoprotein which is expressed on the surface of human lymphocytes and monocytes. Campath-1M (rat IgM) has been used extensively to control graft versus host disease and graft rejection in bone marrow transplant recipients by depletion of T cells in the graft (in-vitro) and in recipients (in-

vivo). Since the in-vivo administration of Campath-1M has considerable side-effects, additional antibodies retaining the same specificity have been developed. Campath-1G (rat IgG2b) and Campath-1H (human IgG1) bind to the human Fc-receptors and are also effective for lysis in-vivo.

We recently reported our results with Campath incubation of the graft. In a retrospective study we compared the results of allo peripheral SCT versus alloBMT. A significant shorter duration of hospital stay was observed in the peripheral stem cell patients. Graft failure was a minor problem in this group of patients. In the present follow-up study, grafts will be treated with in-vitro incubation of Campath-1H.

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2. OBJECTIVES OF THE STUDY

2.1 To determine whether in-vitro incubation of the graft with Campath-1H prevents graft rejection and delayed engrafment.

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

2.3 To assess hematologic and immunologic recovery.

2.4 To determine survival and leukaemia-free survival.

3. PATIENT SELECTION

3.1 Entry criteria

3.1.1
- Patients with acute myelogenous leukemia (AML) or acute lymphoblastic leukemia (ALL) in remission.
- Patients with the following myelodysplastic syndromes: refractory anaemia with excess of blasts (RAEB) with life threatening pancytopenia, and RAEB in transformation and other myelodysplasias with life threatening features.
- Patients with chronic myelogenous leukaemia (CML) in chronic phase, in accelerated phase or in second chronic phase.
- Patients with intermediate/high grade non-Hodgkin's lymphoma with initial positive bone marrow in remission.
- Myeloma, progressive stage II or stage III.
- Patients with aplastic anemia, failing ATG or other benign hematological diseases.

Patients are eligible regardless of previous chemotherapy or radiotherapy.

3.1.2 Age under 60

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

3.1.4 Informed consent according to rules and regulations of the Leiden University Hospital.

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

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

3.2 Exclusion criteria

3.2.1 Life expectancy severely limited by diseases other than leukaemia.

3.2.2 Severe psychological disturbances.

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

4. DONOR SELECTION

4.1 HLA-identical family members will be selected as donors. In case more than one donor is available, the age, CMV status and sex of the donor will be taken in 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 HLA-matched family 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 (localization).
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

The pretransplant observations of the donor are performed of protocol LUMC 2003-01.

5.3 Conditioning regimen

Recipients receiving donor peripheral stem cells will be treated with cyclophosphamide

(60 mg/kg b.w.) on days -6 and -5 and single dose total body irradiaton (TBI) on day -1. Recipients receiving a bone marrow graft will be treated with TBI on day -8 and cyclophosphamide on days -6 and -5. Stem cells will be infused on day 0.

The conditioning without TBI consists of Busulphan i.v. (0.8 mg/kg x 4) on day -9, -8, -7, -6 and cyclophosphamide 60 mg/kg on day -6, -5.

- 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 on one day, 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.

5.4 Additional treatment:

5.4.1 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 gastrointestinal tract. Skin biopsies or rectal mucosal biopsies will be performed to confirm GVHD. Acute GVHD, grade I-II, will be treated by 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. Progression of GVHD during this treatment or severe acute GVHD (grade III-IV) will be treated with horse ATG (20 mg/kg b.w.) during 5 days.

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

5.4.3 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.

5.4.4 Non engraftment

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

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

5.4.6. 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.

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

5.4.7 Hematological supportive care will involve prophylactic platelet transfusions when counts are < $10x10^9$/l and leucocyte-free red blood cell transfusions as clinically indicated. AII 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 g/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** 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 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 will include reconstitution of peripheral blood counts following lethal chemoradiation preparation. T-cell reconstitution will be monitored with several monoclonal antibodies (CD1, CD2,

CD3, CD4, CD8, HNK-1) and indirect immunofluorescence. 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 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 erythro-derma with formation of bullea and desquamation	Bilirubin > 15 mg/dl (> 225 umol/L)	Severe abdominal pain with or without ileus

B. Grading of acute GVHD

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

APPENDIX 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 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 III

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 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						
Arythmia	none	sinus tachyardia > 110 at rest	unifocal PVC atrial arrhythmia	multifocal PVC	ventricular tachycardia	
Function	none	asymptomatic but abnormal cardiac sign	transient sympto-matic dysfunction,	symptomatic dys-function responsive to therapy	symptomatic dys-function non-responsive to	

MODIFIED WHO LIST AND GRADE OF TOXICITY						
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
			no therapy required		therapy	
Pericarditis	none	asymptomatic chang-es	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 re-flexes	severe paresthesia and/or mild weakness	intolerable pares-thesia 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 in-tervention	

MODIFIED WHO LIST AND GRADE OF TOXICITY						
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
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.