TREATMENT WITH DONOR DERIVED CMV SPECIFIC T CELL LINES IN PATIENTS AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (PHASE I/II STUDY)

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Study coordinator: Dr. R.M.Y. Barge

Writing committee: Dr. R.M.Y. Barge, Prof.dr. J.H.F. Falkenburg, Dr. I. Jedema, Dr. P. Meij, Dr. A. Wafelman, Prof.dr. R. Willemze, Dr. W.A.F. Marijt

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SUMMARY

The aim of the study is to establish the possible toxicity and therapeutic effect of treatment with donor derived CMV specific CD8+ and CD4+ T cell lines in patients following allogeneic stem cell transplantation. After receiving a stem cell transplant from a CMV+ donor, patients will be weekly monitored for CMV reactivation. Patients who are failing antiviral therapy will be treated with donor-derived CMV specific CD8+ T cell lines and if possible in combination with CMV specific CD4+ T cell lines. CMV specific T cell lines will be generated from peripheral mononuclear cells obtained by leucopheresis of the stem cell donor by in vitro stimulation of the mononuclear cells with synthetic CMV-peptides or recombinant pp65 protein. After isolation of the Interferon-γ secreting cells using the CliniMACS device the T cells will be expanded. The generation of T cell lines will be initiated as soon as patients fail antiviral therapy as defined by the persistence of positive CMV-DNA load after 2 weeks of antiviral therapy or recurrence of positive CMV-DNA load. In case patients are still CMV positive at the time the T cell lines are generated, the T cell lines will be infused intravenously. The procedure may be repeated two times.

1. INTRODUCTION

Cytomegalovirus (CMV) infection after allogeneic stem cell transplantation is frequently associated with life threatening invasive visceral disease. During the last few years the introduction of prophylaxic or preemptive administration of ganciclovir has resulted in a reduction in the mortality of CMV disease. The disadvantage of preemptive ganciclovir treatment is myelosuppression and nephrotoxicity which results in increased morbidity and mortality of allogeneic stem cell patients. Intensified immunosuppression and T cell depletion as increasingly performed for unrelated, mismatched or haplo-identical stem cell transplantations has further increased the incidence of CMV infection.

Cell mediated immunity represents an essential host factor in the control of persistent infection and a recovery from CMV disease. In healthy CMV+ individuals, a high frequency of CMV specific CD4+ and CD8+ mediate control of viral reactivation. Peripheral blood lymphocytes of the donor (DLI) usually contain CMV specific T cells and can therefore be used to control CMV infection. However this therapy is limited by a potential fatal complications caused by the alloreactive T cells that are also present in the donor lymphocyte infusion. In addition, there is a rather low frequency of CMV specific T cells in unselected donor lymphocytes preparations. Enrichment of virus specific T cells by in vitro culture before administration appears to reduce the risk of Graft versus Host disease and can effectively restore virus specific T cell responses. It has been demonstrated that transfer of CD8+ CMV specific cytotoxic T lymphocytes generated from an HLA-identical donor can result in a CD8+ T cell response in the recipient. In accordance with the use of EBV specific T cells in the treatment for EBV associated post-transplantation lymphoproliferative diseases it appears to be important to transfer both CD4+ and CD8+ virus specific T cells in order to establish long lasting viral immunity in patients with CMV disease after allogeneic stem cell transplantation.

Several transplant groups are currently investigating the use of CMV specific T cells for the treatment of CMV infection after allogeneic stem cell transplantation (Tübingen, Perugia, Seattle). Data from these groups show that it is possible to generate sufficient CD8+ CMV specific T cell lines. Infusion of these T cell lines at a dose of 3 x $10^7/m^2$ does not induce graft versus host disease even in patients undergoing transplantation with stem cells of a donor mismatched for 1-3 HLA-antigens.

In the GMP facility of the Leiden University Medical Center we have five years experience in the generation of leukemia reactive cytotoxic T lymphocytes as well as in HA-1 or HA-2 specific cytotoxic T cells. Recently, we modified and simplified our generation of specific T

cell line protocol by introducing the interferon-γ secretion assay as selection method. This method is now clinical grade available. Selection of T cells using interferon-γ secretion assay has also been used in our laboratory for production of donor-derived or CMV specific CD8+ T cells. In short, peripheral blood mononuclear cells obtained by leucopheresis of the donor are stimulated with HLA-restricted CMV synthetic peptides and incubated overnight. Magnetic enrichment of the interferon-γ secreting T cells is performed with a CliniMACS devise (Miltenyi Biotec, Germany). The isolated cells are expanded for 10 days in the presence of autologous serum and low dose IL-2. Using this protocol we have successfully generated several CD8+ CMV specific T cell lines. With this method sufficient numbers of CD8+ CMV specific T cells can be generated for adoptive transfer from CMV+ donors to their HLA-identical recipient. Only a limited amount of peripheral blood (500 ml) is necessary from the CMV positive donor. A drawback for the generation of CMV specific CD4+ cell lines is the inavailability of recombinant pp65 protein for the generation of a class-II respons (clinical grade recombinant pp65 is expected to be available in 2005).

The objectives of these studies are to determine the toxicity of administration of the donor derived CMV specific T cell lines in patients after allogeneic stem cell transplantation and to determine the possible therapeutic effect of treatment with these specific T cell lines.

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2. OBJECTIVES OF THE STUDY

1. To determine the toxicity of administration of donor-derived CMV-specific T-cell lines in patients after alloSCT.
2. To determine the possible therapeutic effect of treatment with donor-derived CMV-specific T-cell lines.

3. PATIENT SELECTION

Inclusion criteria

1. AlloSCT patients, receiving a transplant from a CMV positive donor and failing antiviral therapy, defined by the persistence of positive CMV-DNA load after 2 weeks of therapy or early relapse after therapy.
2. Availability of CMV-peptide in the context of HLA of the patient.
3. Adequate renal, liver, cardiac and pulmonary function.
4. Informed consent.

Exclusion criteria

1. At infusion of T-cells, treatment with corticosteroids in a dose of > 2 mg/kg
2. At infusion of T-cells, Graft versus Host > grade 2
3. Severe psychological disturbances
4. Severely limited life expectation

4. DONOR SELECTION

Inclusion criteria

1. CMV sero-positive
2. Informed consent

Exclusion criteria

1. Inability to tolerate leukopheresis due to psychological or medical reasons.

5. GENERATION OF DONOR DERIVED CMV SPECIFIC T-CELL LINES

After informed consent, a leukapheresis will be performed to isolate peripheral blood mononuclear cells from the donor of the patient. The total number of PBMC will be targeted at 2 x 10^9 CD3+ T cells. This target is sufficient to generate at least three T-cell lines. At the GMP facility CMV peptide will be added to the PBMC. CMV-specific T-cells recognizing their epitope (pp65 or IEA) in the context of the correct HLA molecule will secrete IFN-gamma,

allowing selection of these T cells using the cytokine capture assay (Milteny Biotec, Germany). Positive cells will be cultured for 10 days. Only if > 50% of the CD 8+ cells consist of CMV specific T-cells the culture will be considered eligible for clinical application. Target dose for administration is 3 x $10^7/m^2$ CD8+ T cells. Quality control will include sterility controls.

6. TREATMENT SCHEDULE

1. Monitor alloSCT patients for CMV viraemia: all patients after alloSCT are weekly monitored for CMV load by quantitive RT-PCR during at least 100 days. PCR-based preemptive anti-CMV therapy is started conform current treatment protocols (ganciclovir or valganciclovir) for 2 weeks of longer if necessary.
2. Criteria for treatment of positive CMV-DNA load: Ganciclovir treatment is started when CMV-DNA load in peripheral blood is > 10000 c/ml or 1000 c/ml and an increase of 1 log/week.
3. Generation of CMV specific CD8+ and CD4+ cell lines: when patients are failing anti-viral therapy as defined by the persistence of positive CMV-DNA load (> 1000 c/ml) after two weeks of anti-viral therapy or early recurrence of positive CMV-DNA load (> 10000 c/ml) the generation of donor derived T cell lines will start in the GMP facility.
4. Administration of T cell lines: When the CMV-DNA load is still positive (> 1000 c/ml) after generation of T cell lines, T cell lines will be administered intravenously in 30 minutes. Blood pressure, pulse will be monitored every 10 minutes administration and every 30 minutes after the infusion. The attending physician is present in the room of the patient. Patients must be off treatment with high dose corticosteroids (> 2 mg/kg) and no treatment with intensive cytostatic drugs is allowed. When the CMV-DNA load is negative after generation of T cell lines the T cell lines will be frozen and stored in nitrogen-vapour tank,.
5. In first five to ten patients (= cohort 1), 1-3 x $10^7/m^2$ CD8+ cells will be administered. If two weeks after the first administration the patient has shown no response in decline of CMV-DNA load, a second line will be generated. Infusion will be after two weeks when the patient is still CMV-DNA positive (> 1000 c/ml) at that time point. When the CMV-DNA load is negative after generation of T cell lines the T cell lines will be frozen.
6. If no response is observed in the first five to ten patients after two infusions of CD8+ cell lines and no restoration of CD8+ CMV specific immunity can be detected during monitoring we will generate pp65 protein specific $CD4^+$ and $CD8^+$ T-cell lines in the following patients (= cohort 2). If > 50% of the CD8+ cells are pp65 specific, we will administer the entire T cell lines at a dosis of 3 x $10^7/m^2$. If < 50% of the CD8+ cells

are pp65 specific, 1 x $10^7/m^2$ CD4+ T-cells will be purified from the pp65 protein specific T-cells and added to a CD8+ specific T-cell line.

7. (Val)ganciclovir treatment or other antiviral drugs will be continued conform standard anti-viral treatment protocols during T-cell therapy.

7. PRETREATMENT INVESTIGATIONS

1. History, physical examination and WHO performance
2. Blood cell counts, differential, quantitative platelet count.
3. Hepatitis B, Hepatitis C, HIV, CMV, EBV, toxoplasma serology.
4. Bilirubin (direct and indirect), alkaline phosphatase, ASAT, ALAT, SLDH, γGT, serum urea, creatinine, Na, K, uric acid, glucose.
5. EKG.
6. Chest X-ray.
7. CMV-DNA load
8. CMV-tetramer staining
9. CMV-IFN-γ secretion assay as marker for functional immunological response to CMV peptide.

8. STUDY PARAMETERS

1. Daily interim history and physical examination while hospitalized. Between T-cell line treatment cycles at least once every other week.
2. Blood cell count, differential, quantitative platelet counts, between t-cell treatment cycles at least once a week. Thereafter as clinically indicated, but at least once every two weeks for 4 months.
3. Creatinine, Urea, Na, K, Uric acid, glucose, ALAT, ASAT, alkaline phosphatase, bilirubin, SLDH, γGT, total protein, albumin, spectrum, once a week during T cell line treatment. Thereafter as clinically indicated but at least once every two weeks for 4 months. APTT, PT, FDP, fibrinogen during hospitalization on Tcell line treatment.
4. CMV-DNA load at day 1 and 2, followed by weekly for 8 weeks and monthly for 4 more months.
5. Quantification of CMV specific T cells by tetramer staining and assessment of their functionality (IFN-γ secretion assay) at day 1, 2, followed by weekly for 8 weeks and monthly for 4 more months.

9. CRITERIA OF EVALUATION

Toxicity criteria

1. General toxicity will be measured using the modified WHO criteria. In case of grade 3 or 4 toxicity during T cell line treatment, administration will be stopped.
2. GVHD will be graded using the criteria outlined in the appendix .

Response criteria

1. CMV-DNA load:
   - Partial response: reduction of CMV-DNA load of > 1 log/week of at least 2 weeks
   - Complete response: negativity of CMV-DNA load (< 100 c/ml)
   - Progressive disease: CMV disease or increase of CMV-DNA > 1 log
   - Stable disease: all other options
2. CMV-tetramer staining:
   - Response: increase of positive CMV-tetramer staining during time
3. IFN-γ secretion assay:
   - Retrospectively we will analyse in 30 cc of PB functional activity using cytokine capture assay.

10. ETHICAL CONSIDERATIONS

Declaration of Helsinki

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

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.

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.

11. 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.

12. 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						

MODIFIED WHO LIST AND GRADE OF TOXICITY						
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
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, no therapy required	symptomatic dys-function responsive to therapy	symptomatic dys-function non-responsive to therapy	
Pericarditis	none	asymptomatic changes	symptomatic no tap required	tamponade tap required	tamponade surgery required	
NEUROTOXICITY						
State of consious-ness	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 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	

MODIFIED WHO LIST AND GRADE OF TOXICITY						
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**Grade 5**
Vasculitis	none	restricted cutaneous	generalized cutaneous	hemorrhagic	systemic	
Allergic	no change	edema	bronchospasm	bronchospasm parenteral	anaphylaxis	
Cutaneous	no change	erythema	dry desquamation pruritus vesiculation	moist desquamation ulceration	exfoliative dermatitis necrosis requiring surgical intervention	
Pain#	none	mild	moderate	severe	intractable	

APPENDIX V: RECIPIENT STUDY PROCEDURE

	WEEKS											YEARS		
	Pre	0	2	4	6	8	10	12	18	26	38	1	2	5
Hematology	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Biochemistry	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Peripheral Blood (100cc)	X	X		X		X		X	X	X	X	X	X	X