LUMC 2010-03

TREATMENT WITH CMV PP65-SPECIFIC T CELLS GENERATED BY USE OF A CMV PP65 PROTEIN-SPANNING PEPTIDE POOL IN PATIENTS WITH CMV REACTIVATION OR CMV DISEASE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION

(October 2011)

PROTOCOL TITLE

Treatment with CMV pp65-specific T cells generated by use of a CMV pp65 protein-spanning peptide pool in patients with CMV reactivation or CMV disease after allogeneic stem cell transplantation

Protocol ID	**LUMC 2010-03**
Short title	**Treatment with CMV-specific T cells after stem cell transplantation**
Version	**1.2**
Date	**October 2011**
EudraCT number	**2010-024307-27**
TOL number	**NL35080.000.11**
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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AlloSCT	allogeneic stem cell transplantation
BUN	blood urea nitrogen
CCMO	Central Committee on Research Involving Human Subjects
CMV	cytomegalovirus
CRF	case report form
CTCAE	common terminology criteria for adverse events
DLI	donor lymphocyte infusion
EBV	Epstein-barr virus
ECG	electrocardiogram
GCP	good clinical practice
GMP	good manufacturing practice
GvHD	graft versus host disease
HLA	human leukocyte antigen
IGFL	Interdivisional GMP Facility LUMC
IFNg	interferon-gamma
IMPD	investigational medical product dossier
LDH	lactate dehydrogenase
LUMC	Leiden University Medical Center
MHC	major histocompatibility complex
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
SAE	serious adverse event
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reaction
UPN	unique patient number
WHO	world health organization
WMO	medical research involving human subjects act (wet medisch-wetenschappelijk onderzoek met mensen)

DEFINITIONS

CMV reactivation: CMV DNA load >1000 cp/ml in serum as measured by polymerase chain reaction (PCR).

CMV disease: organ dysfunction (pneumonitis, enteritis, retinitis, encephalitis, hepatitis, and bone marrow suppression) due to CMV infection.

CMV reactivation treatment failure: persistent CMV DNA load of more than 1000 cp/ml or CMV disease after 2 weeks of adequate treatment with antiviral therapy or relapse of CMV DNA load of more than 1000 cp/ml within 4 weeks after adequate

treatment with antiviral therapy or contraindication for treatment with antiviral therapy at the discretion of the physician.

Complete response: Complete disappearance of CMV DNA load for at least 4 weeks.

Partial response: reduction of CMV DNA load of > 1 log but no complete disappearance of CMV DNA load for at least 2 weeks.

Stable disease: no significant change (<1 log) in viral DNA load and no complete disappearance of CMV DNA load for at least 4 weeks.

Progressive disease: persistent increase in CMV DNA load of > 1 log during 4 weeks or new CMV disease.

SUMMARY:

Treatment with CMV pp65-specific T cells generated by use of a CMV pp65 protein-spanning peptide pool in patients with CMV reactivation or CMV disease after allogeneic stem cell transplantation (alloSCT)

Objectives:

- To assess the feasibility, tolerability and safety of administration of donor derived CMV pp65-specific T cells in patients with CMV reactivation or CMV disease after alloSCT.
- To determine the presence of CMV-specific T cells at different time points after infusion of CMV pp65-specific T cells.
- To evaluate whether administration of CMV pp65-specific T cells in patients with persistent CMV reactivation or CMV disease after alloSCT leads to complete or partial responses.

Population:

- Recipients of alloSCT (age 0-75 years) with CMV reactivation who fail antiviral therapy (defined as **CMV reactivation treatment failure**: persistent CMV DNA load of more than 1000 cp/ml or CMV disease after 2 weeks of adequate treatment with antiviral therapy or relapse of CMV DNA load of more than 1000 cp/ml within 4 weeks after adequate treatment with antiviral therapy or contraindication for treatment with antiviral therapy at the discretion of the physician) ***or***
- Patients who develop **CMV disease** (organ dysfunction (pneumonitis, enteritis, retinitis, encephalitis, hepatitis, and bone marrow suppression) due to CMV infection).
- Number of patients to be treated with CMV pp 65 specific T cells will be 15.

Inclusion criteria:

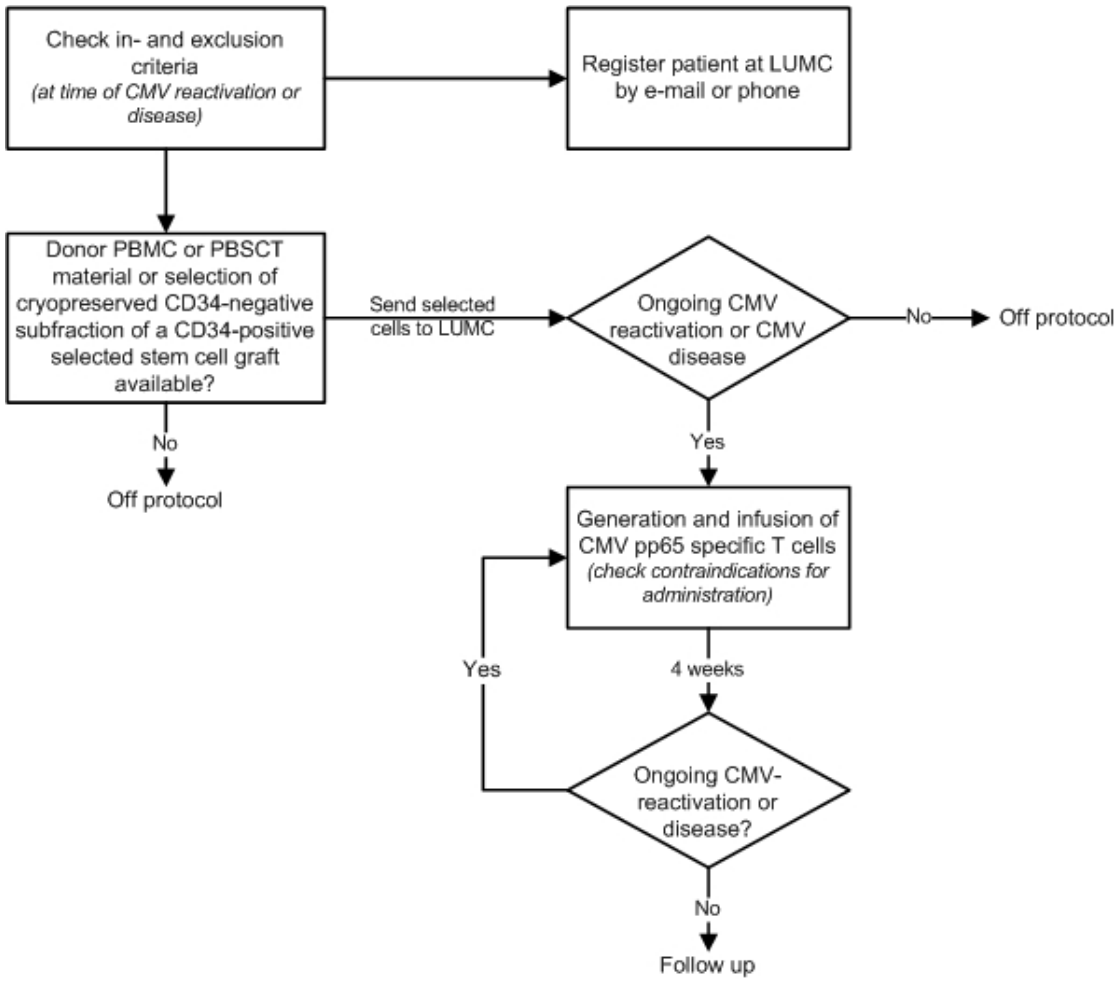
- age 0-75 years
- recipient of alloSCT for standard indication according to national- and European Group for blood and Marrow Transplantation-guidelines (see appendix D)
- Possibility to obtain PBMC by leukapheresis from the CMV seropositive donor or availability of peripheral blood stem cell graft (PBSCT) or of a CD34-negative subfraction of a CD34-positively selected PBSCT product of the donor prepared and cryopreserved at a GMP-facility or stem cell center.
- **CMV reactivation treatment failure** (persistent CMV DNA load of more than 1000 cp/ml or CMV disease after 2 weeks of adequate treatment with antiviral therapy or relapse of CMV DNA load of more than 1000 cp/ml within 4 weeks after adequate treatment with antiviral therapy or contraindication for treatment with antiviral therapy at the discretion of the physician) or **CMV disease** (organ dysfunction (pneumonitis, enteritis, retinitis, encephalitis, hepatitis, and bone marrow suppression) due to CMV infection).

- Written informed consent by the patient and/or parent(s) or legal guardian(s).

Exclusion criteria:

- Life expectation < 3 months.
- End stage irreversible multi-system organ failure.
- Pregnant or lactating women.
- Severe psychological disturbances.
- Patient HIV positive.
- Donor HIV positive.

Study design for treatment of CMV reactivation and/or CMV disease with allogeneic CMV pp65-specific T cells



Investigational product:
The CMV pp65-specific T cell product will be generated from PBMC from a donor leukapheresis product, from a peripheral blood stem cell graft (PBSCT material) or from a CD34-negative subfraction of a CD34 positively selected stem cell graft prepared and cryopreserved at a GMP-facility or stem cell center, whichever is available.
PBMC will be exposed to the CMV pp65-derived 15 mer peptide pool, after which CMV-pp65-specific T cells will start to produce Interferon gamma (IFNg). After overnight incubation, IFNg secreting T cells will be isolated using the CliniMACS® Cytokine Capture System. The positively isolated fraction will be washed, after which the percentage of IFNg- and CD137-positive T cells will be determined. When ≥ 20% of the T cells are IFNg and/or CD137–positive the CMV pp65 T cell product will be released. The number of cells that will be infused in the patient will be determined by the number of non-specific T cells in the product. The maximal amount of non-specific T cells in the product is < 0.3 x 10^6/kg body weight in patients transplanted with related donors and < 0.15 x 10^6/kg body weight in case of unrelated donors. After release, the CMV-pp65-specific T cell product will be resuspended in a solution of NaCl 0,9%, supplemented with human albumin (2%), after which the product can be administered.

Investigational treatment:
CMV pp65-specific T cells are a cell therapy product that will be administered after alloSCT in case of CMV reactivation treatment failure or CMV disease. The CMV pp65-specific T cells will be derived from a CMV seropositive donor. The product will contain an aimed dose of 10-20 x 10^6 total T cells. This dose is based on the amount of cells we expect to isolate on the basis of the currently performed phase I/II clinical study (LUMC 2004-01) on the treatment of refractory CMV reactivation using CMV pp65-specific CD8+ T cells and preclinical data of the current strategy. All cells isolated will be infused if ≥ 20% of the T cells are IFNg and/or CD137 positive and the maximal amount of non-specific T cells in the product is < 0.3 x 10^6/kg body weight in patients transplanted with related donors and < 0.15 x 10^6/kg body weight in case of unrelated donors. If the number of non-specific T cells in the product exceeds these criteria, the infused dose will be adjusted accordingly. The cells will be administered intravenously at the department of Hematology of the LUMC. In case of ongoing CMV reactivation or CMV disease and no severe toxicity, the procedure may be repeated 2 times with at least 4 weeks interval.

Risk/benefit:
Potential benefit of participation to this study is a reduction of the CMV DNA load which may lead to a reduction of development of severe CMV disease or cure of CMV disease. A potential risk of participation to this study is development of acute GvHD.

Contraindications for administration of CMV pp65-specific T cells:

- Life expectation < 6 weeks.
- End stage irreversible multi-system organ failure.
- Acute GvHD overall grade ≥ III.
- Treatment with corticosteroids in an equivalent dose of >0.5 mg/kg prednisone.
- No CMV reactivation or CMV disease.

Study endpoints:

- The number of events of acute GvHD, death and all other adverse events.
- The number of CMV-specific T cells at different time points after infusion of CMV pp65-specific T cells.
- The number of complete responses or partial responses of CMV reactivation or CMV disease after infusion of CMV pp65-specific T cells.

Follow-up:

Follow-up of patients will be performed until 6 months after last infusion of CMV pp65-specific T cells or until subsequent DLI, whichever comes first.

In adults:

Weeks after infusion	0	1	2	3	4	5	6	7	8	12	16	20	24
Infusion of cells	x				(x)				(x)				
History and PE	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood	x	x	x	x	x	x	x	x	x	x	x	x	x
CMV DNA	xxx	x	x	x	x	x	x	x	x	x	x	x	x
CMV tetramer	xx	x	x	x	x	x	x	x	x	x	x	x	x
Chimerism							x			x			x
xxx day 0, 1, 2 xx day 0, 1													

The volume of blood for quantification of CMV-specific T cells by tetramer staining and functional assays (intracellular cytokine staining) is 50 ml.
The volume of bone marrow for chimerism analysis is 10ml.
In children:
Sampling of blood will be according to study protocol “adoptive immunotherapy for adenovirus infections after allogeneic stem cell transplantation in children” as approved by the CCMO. PBMC samples (20ml) will be taken and stored at 2 week intervals for the investigation of the presence and function of CMV-specific T cells until 3 months after last infusion. Blood sampling for this specific protocol will be combined with blood sampling for regular monitoring of the transplantation as much as possible.

Interim analysis:

After the first 3, 6 and 10 patients an interim analysis will be performed by the principal investigator.

Premature termination of the study:
In case of increase of >1 point of overall grade acute GvHD in 2 out of the first 3 patients, or 3 out of the first 6 patients, or 4 out of the first 10 patients, the study will be closed temporarily for inclusions and the outcomes in all patients will be discussed in order to decide whether potential beneficial effects of the treatment could overcome non-beneficial effects of GvHD.

End of study:
End of the study is defined as 6 months after the infusion of CMV pp65-specific T cells of patient number 15 or at time of subsequent DLI of patient number 15.

1. INTRODUCTION AND RATIONALE

Patients with hematological diseases can be successfully treated with allogeneic stem cell transplantation (alloSCT). After transplantation, patients suffer from a prolonged period of immune deficiency resulting in an increased risk of opportunistic infections, including cytomegalovirus (CMV) reactivation. CMV reactivation after alloSCT can cause serious disease with high mortality in case off absence of CMV-specific T cells. Treatment with antiviral drugs is associated with myelosuppression and nephrotoxicity and CMV reactivation often relapses. Reconstitution of the CMV-specific T-cell repertoire directed against immunodominant proteins, like CMV pp65, in the first year after alloSCT has been demonstrated to confer sustained protection from CMV disease. Furthermore, for the long-term protection against CMV the development of CMV-specific T-cell immunity has been found to be essential.
It has been demonstrated that adoptive transfer of donor derived CD8+ CMV-specific cytotoxic T lymphocytes can result in a CMV-specific CD8+ T cell response and CMV clearance in the recipient. Currently, a phase I/II clinical study (LUMC 2004-01) on the treatment of refractory CMV reactivation using CMV pp65-specific CD8+ T cells is performed at the department of Hematology at the Leiden University Medical Center (LUMC). In the good manufacturing practice (GMP) facility of the LUMC (IGFL) CMV pp65-specific CD8+ T cells are generated by stimulating donor- or patient-derived peripheral blood mononuclear cells (PBMC) with a CMV pp65-derived human leukocyte antigen (HLA)-A02- and/or HLA-B07-restricted peptide. After stimulation CD8+ T cells specific for these peptides will start to produce Interferon-gamma (IFNg) and the specific CD8+ T cells can be isolated based on this IFNg production. After isolation, the T cells are cultured for 7 -14 days, after which they are adoptively transferred to patients. Since the introduction of protocol LUMC 2004-01, sixteen CMV pp65-specific CD8+ T cell lines have been generated. Of those T cell lines, eight have been infused in six patients without any side effects. All treated patients cleared the CMV within weeks after infusion. Isolation of CMV pp65-specific T cells from a donor using this protocol is only possible when the donor is CMV seropositive. Due to the HLA restriction (HLA-A02 or HLA-B07) and the time frame needed for the generation of the CMV-specific CD8+ T cell line, this treatment regimen could not be applied to all patients. Furthermore, only CMV-specific CD8+ T cells were generated, while clinical observations have indicated that CMV-specific CD4+ T cells may promote development and persistence of a CMV-specific CD8+ T cell response in-vivo. Therefore, to increase the potential clinical use of CMV-specific T cells and to increase the in-vivo potential of the CMV-specific T cells we will start a new clinical protocol, in which we will use a CMV pp65 protein-spanning peptide pool of 15-mer peptides for the simultaneous generation of donor- derived CMV pp65-specific CD8+ and CD4+ T cell product. Using this peptide pool both CMV pp65-specific CD4+ and CD8+ T cells can be isolated irrespective of HLA typing of the donor. Secondly, the isolated CMV pp65-specific T cells will be directly administrated after isolation to decrease the level of manipulation of these T cells and to shorten the period between

the onset of CMV disease and the infusion of the CMV-specific cells allowing more accurate therapeutic intervention.

2. OBJECTIVES

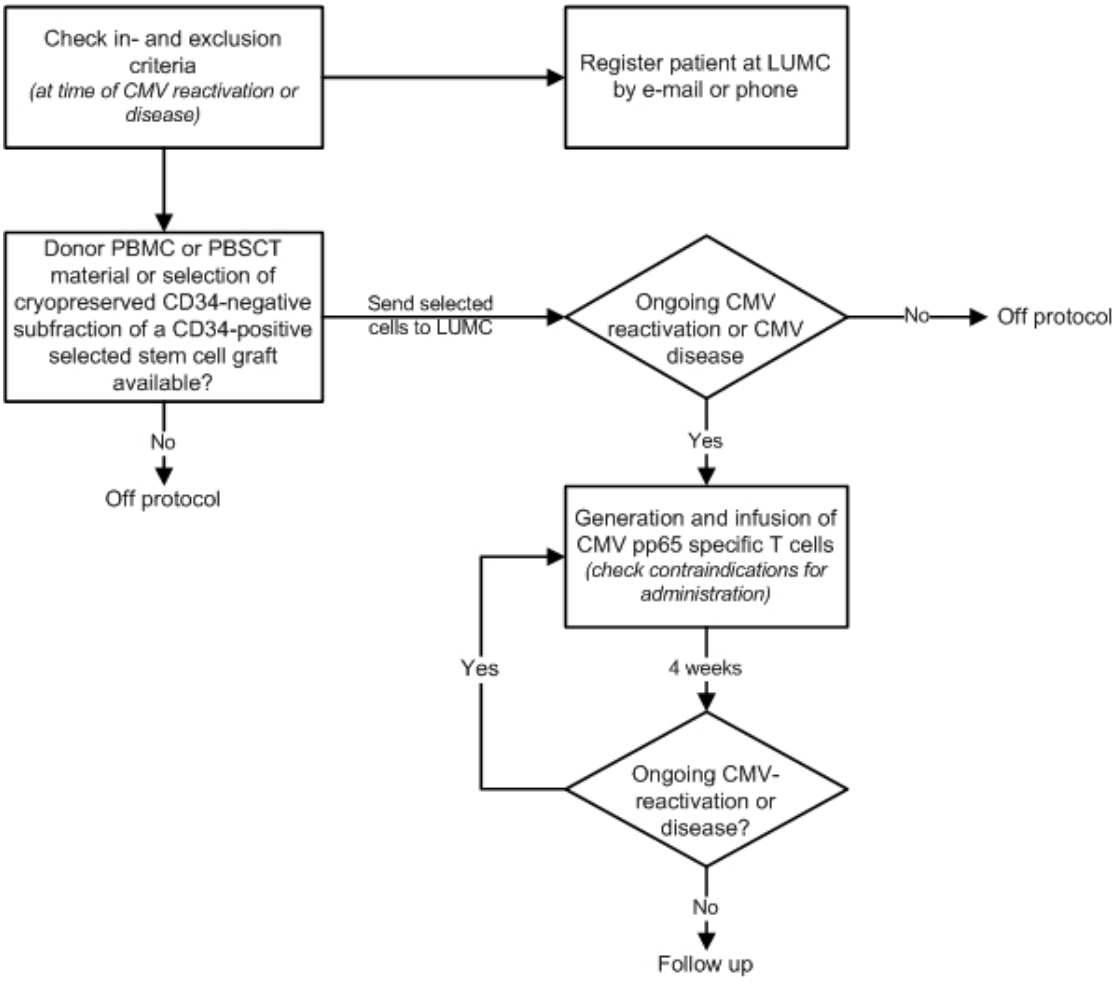
- To assess the feasibility, tolerability and safety of administration of donor derived CMV pp65-specific T cells in patients with CMV reactivation or CMV disease after alloSCT.
- To determine the presence of CMV-specific T cells at different time points after infusion of CMV pp65-specific T cells.
- To evaluate whether administration of CMV pp65-specific T cells in patients with persistent CMV reactivation or CMV disease after alloSCT leads to complete or partial responses.

3. STUDY DESIGN

This is an open-label non-randomized phase I/II feasibility study to treat patients with persistent CMV reactivation or CMV disease after alloSCT with administration of CMV pp65-specific T cells generated by use of a CMV pp65 protein-spanning peptide pool.

Patients who receive an alloSCT from a CMV-positive donor are monitored weekly for CMV reactivation using PCR for the detection of CMV DNA. In case of CMV reactivation (defined as CMV DNA load >1000 cp/ml) patients will be treated with antiviral therapy consisting of valganciclovir, ganciclovir or foscarnet.
For patients with CMV reactivation who fail antiviral therapy (defined as **CMV reactivation treatment failure**: persistent CMV DNA load of more than 1000 cp/ml or CMV disease after 2 weeks of adequate treatment with antiviral therapy or relapse of CMV DNA load of more than 1000 cp/ml within 4 weeks after adequate treatment with antiviral therapy or contraindication for treatment with antiviral therapy at the discretion of the physician) or develop CMV disease (organ dysfunction (pneumonitis, enteritis, retinitis, encephalitis, hepatitis, and bone marrow suppression) due to CMV infection), CMV pp65-specific CD4+ and CD8+ T cells will be generated from donor PBMC by overnight in vitro stimulation with pp65 CMV peptide pools. CMV-specific CD4+ and CD8+ T cells will be isolated based on their IFNg production and administered to the patient directly after quality control. If alloSCT was performed using CD34 positive cell selection and the CD34 negative subfraction has been cryopreserved at a GMP facility, this fraction can also be used for selection of CMV-specific T cells. Antiviral therapy will be continued after infusion of CMV pp65-specific T cells according to standard antiviral treatment protocols at the discretion of the physician.
In case of ongoing CMV reactivation or CMV disease the infusion of CMV pp65-specific T cells may be repeated 2 times with at least 4 weeks interval. The patient will be monitored for adverse events and for effect on CMV DNA load. Follow-up of patients will be performed until 6 months after infusion of CMV pp65-specific T cells or until subsequent DLI, whichever comes first.

Study design for treatment of CMV reactivation and/or CMV disease with allogeneic CMV pp65-specific T cells.



4. STUDY POPULATION

4.1 Population

- Recipients of alloSCT (age 0-75 years) with CMV reactivation who fail antiviral therapy (defined as **CMV reactivation treatment failure**: persistent CMV DNA load of more than 1000 cp/ml or CMV disease after 2 weeks of adequate treatment with antiviral therapy or relapse of CMV DNA load of more than 1000 cp/ml within 4 weeks after adequate treatment with antiviral therapy or contraindication for treatment with antiviral therapy at the discretion of the physician) ***or***
- Patients who develop **CMV disease** (organ dysfunction (pneumonitis, enteritis, retinitis, encephalitis, hepatitis, and bone marrow suppression) due to CMV infection).
- Number of patients to be treated with CMV pp 65 specific T cells will be 15.

4.2 Inclusion criteria

- age 0-75 years
- recipient of alloSCT for standard indication according to national- and European Group for blood and Marrow Transplantation-guidelines (see appendix D)
- Possibility to obtain PBMC by leukapheresis from the CMV seropositive donor or availability of peripheral blood stem cell graft (PBSCT) or of a CD34-negative subfraction of a CD34-positively selected PBSCT product of the donor prepared and cryopreserved at a GMP-facility or stem cell center.
- **CMV reactivation treatment failure** (persistent CMV DNA load of more than 1000 cp/ml or CMV disease after 2 weeks of adequate treatment with antiviral therapy or relapse of CMV DNA load of more than 1000 cp/ml within 4 weeks after adequate treatment with antiviral therapy or contraindication for treatment with antiviral therapy at the discretion of the physician) or **CMV disease** (organ dysfunction (pneumonitis, enteritis, retinitis, encephalitis, hepatitis, and bone marrow suppression) due to CMV infection).
- Written informed consent by the patient and/or parent(s) or legal guardian(s).

4.3 Exclusion criteria

- Life expectation < 3 months.
- End stage irreversible multi-system organ failure.
- Pregnant or lactating women.
- Severe psychological disturbances.
- Patient HIV positive.
- Donor HIV positive.

4.4 Sample size calculation

We consider the infusion of CMV pp65-specific T cells a success when the response rate is at least 66 % (p1; 2/3 of patients). Based on our historical experience, we

estimate the rate of responses with pharmacotherapy alone but without immune intervention to be 30% (=p0) for the targeted patient population. Given the high laboratory effort and associated costs of treatment with CMV pp65-specific T cells, the trial should have a power of 90% (beta-error 0.1). With the usual alfa-error of 0.05, the sample size as calculated according to the standard Fleming design is 15 treated patients under these assumptions. On the basis of the currently performed phase I/II clinical study (LUMC 2004-01) on the treatment of refractory CMV reactivation we expect to include 5-7 patients per year for a period of 3 years. We expect no excess in number of events of acute GvHD, death and all other adverse events.

5. TREATMENT OF SUBJECTS

5.1 Investigational product

The CMV pp65-specific T cell product will be generated from PBMC from a leukapheresis product, from a peripheral blood stem cell graft (PBSCT material) or from a CD34-negative subfraction of a CD34 positively selected stem cell graft from a CMV seropositive donor prepared and cryopreserved at a GMP-facility or stem cell center, whichever is available.
PBMC will be exposed to the CMV pp65-derived 15 mer peptide pool, after which CMV-pp65-specific T cells will start to produce Interferon gamma (IFNg). After overnight incubation, IFNg secreting T cells will be isolated using the CliniMACS® Cytokine Capture System. The positively isolated fraction will be washed, after which the percentage of IFNg- and CD137-positive T cells will be determined. When ≥ 20% of the T cells are IFNg and/or CD137 positive the CMV pp65 T cell product will be released. The numver of cells that will be infused in the patient will be determined by the number of non-spedific T cells in the product. The maximal amount of non-specific T cells in the product is < 0.3 x 10^6/kg body weight in patients transplanted with related donors and < 0.15 x 10^6/kg body weight in case of unrelated donors, the CMV pp65 T cell product will be released for administration.
After release, the CMV-pp65-specific T cell product will be resuspended in a solution of NaCl 0,9%, supplemented with human albumin (2%), after which the product can be administered.

5.2 Investigational treatment

CMV pp65-specific T cells are a cell therapy product that will be administered after alloSCT in case of CMV reactivation treatment failure or CMV disease. The CMV pp65-specific T cells will be generated from a CMV seropositive donor. The product will contain an aimed dose of 10-20 x 10^6 total T cells. This dose is based on the amount of cells we expect to isolate on the basis of the currently performed phase I/II clinical study (LUMC 2004-01) on the treatment of refractory CMV reactivation using CMV pp65-specific CD8+ T cells and preclinical data of the current strategy. All cells isolated will be infused if ≥ 20% of the T cells are IFNg and/or CD137 positive and the maximal amount of non-specific T cells in the product is < 0.3 x 10^6/kg body weight in patients transplanted with related donors and < 0.15 x 10^6/kg body weight in case of unrelated donors. If the number of non-specific T cells in the product exceeds these criteria, the infused dose will be adjusted accordingly. The cells will be administered intravenously at the department of Hematology of the LUMC. In case of ongoing CMV reactivation or CMV disease and no severe toxicity, the procedure may be repeated 2 times with at least 4 weeks interval.

5.3 Collection of donor PBMC

Donor PBMC will be collected by leukapheresis using acid citrate dextrose-anticoagulant (ACD-A) as anticoagulant. 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 with an aimed number of 1 x 10^8 PBMC/kg body weight. Donor PBMC that are not used for generation of CMV pp65-specific T cells will be cryopreserved for future usage as unselected DLI.

5.4 Contraindications for administration of CMV pp65-specific T cells

- Life expectation < 6 weeks.
- End stage irreversible multi-system organ failure.
- Acute GvHD overall grade ≥ III.
- Treatment with corticosteroids in an equivalent dose of >0.5 mg/kg prednisone.
- No CMV reactivation or CMV disease.

5.5 Use of co-intervention

Antiviral therapy will be continued after infusion of CMV pp65-specific T cells according to standard antiviral treatment protocols at the discretion of the physician.

5.6 Escape medication

Patients will receive routine clinical care for alloSCT patients. All medication and other treatments administered will be noted and continued if considered necessary by the treating physician.

5.7 Escape administration of cultured CMV pp65-specific T cell line

If after generation of the CMV pp65-specific T cells by peptide stimulation and IFNg isolation the release criteria for direct administration of the cells are not met, the patient will go off protocol. When between 5 – 20% of the isolated T cells are IFNg positive the T cell product will be cultured in vitro to enrich for the specific T cells. If After 7 – 10 days of culture the CMV pp65-specific T cell line meets the release criteria, the cells will be administered to the patient if clinically indicated.

6. INVESTIGATIONAL MEDICINAL PRODUCT

6.1 Name and description of Investigational Medicinal Product

CMV pp65-specific T cells are a cell therapy product. This particular product consists of a T cell suspension which is generated from PBMC, PBSCT material or CD34 negative cell fraction from a donor (allogeneic T cell product). CMV pp65-specific T cells are isolated from the starting material under Good Manufacturing Practice (GMP) conditions following Standard Operating Procedures (SOPs) of the Interdivisional GMP Facility LUMC (IGFL) and the Department of Hematology. Technical details regarding CMV pp65-specific T cells and the production process are presented in the Investigational Medicinal Product Dossier (IMPD).

6.2 Summary of findings from non-clinical studies

CMV pp65-specific CD8+ and CD4+ T cells are circulating within the peripheral blood of CMV infected individuals for the control of CMV. CMV-specific T cells can be isolated from PBMC. We have demonstrated that stimulation of PBMC from CMV infected individuals with the CMV pp65 protein-spanning 15-mer (11 mer overlapping) peptide pool induced simultaneous activation of both CMV pp65-specific CD8+ and CD4+ T cells, irrespective of the HLA type. Activated CMV-specific CD8+ and CD4+ T cells both produce IFNg and CMV pp65-specific T cells can be isolated from PBMC based on their IFNg production using the cytokine capture system from Miltenyi Biotec. Isolation of IFNg-secreting cells after stimulation with the CMV pp65 15 mer peptide pool resulted in efficient enrichment of CMV-specific CD8+ and CD4+ T cells recognizing multiple CMV pp65-derived epitopes. Isolated CMV pp65-specific CD4+ and CD8+ T cells retain their functionality and can be activated upon antigenic stimulation.
For a more extensive overview of preclinical data on CMV-specific T cells, please refer to the IMPD.

6.3 Summary of findings from clinical studies

Since the introduction of protocol LUMC 2004-01 for the treatment of therapy-resistant CMV reactivation or disease after alloSCT, twelve donor-derived CMV-specific CD8+ T cell lines have been generated. Of those cell lines, eight have been infused in six patients with no side effects. All treated patients cleared the CMV within weeks after infusion.
For a more extensive overview of the clinical studies on CMV-specific T cells, please refer to the IMPD.

6.4 Summary of known and potential risks and benefits

Risks:
The CMV pp65-specific T cell product is released when 20% of the T cells is specific for CMV pp65, indicating that 80% of the cells might be not specific for CMV pp65.

The consequence of this approach is that no exact information is obtained concerning the reactivity of IFNg- and CD137-negative T cells in the CMV pp65-specific T cell product. To minimize the risk of GvHD to a minimum, the amount of IFNg/CD137 negative T cells administered in the T cell product will be < 0.3 x 10^6/kg body weight in case of a related donor and < 0.15 x 10^6/kg body weight in case of an unrelated donor.
CMV pp65-specific T cells will be generated for the treatment of CMV reactivation or CMV disease in patients after alloSCT. To increase the potential clinical use of CMV-specific T cells and to increase the in vivo potential of the CMV-specific T cells CMV pp65-specific T cell lines will be generated using CMV pp65 peptide pool, which is not restricted to certain HLA-types and will induce CD4+ and CD8+ T cell responses. Furthermore, the CMV pp65-specific T cells will be isolated after one day of culture and will be directly administrated after isolation. In this way we can shorten the period between the onset of refractory CMV reactivation/CMV disease and the infusion of the CMV-specific cells and furthermore we expect that the in vivo potential of the T cells will be increased. For more information on the overall risk and benefit assessment, please refer to the IMPD.

6.5 Description and justification of route of administration and dosage

The CMV pp65-specific T cells will be administered intravenously. The aimed dose of CMV pp65-specific T cells of 10-20 x 10^6 total T cells is based on the amount of cells we expect to isolate on the basis of the currently performed phase I/II clinical study (LUMC 2004-01) on the treatment of refractory CMV reactivation using CMV pp65-specific CD8+ T cells and preclinical data of the current strategy. All cells isolated will be infused to maximize the effect if ≥ 20% of the T cells are IFNg and/or CD137 positive and the maximal amount of non-specific T cells in the product is < 0.3 x 10^6/kg body weight in patients transplanted with related donors and < 0.15 x 10^6/kg body weight in case of unrelated donors. The maximal amount of non-specific T cells administered in the T cell product is based on the current protocol for unselected DLI at 3 months in which comparable numbers of unselected CD3 positive cells are administered with acceptable toxicity. If the number of number of non-specific T cells in the product exceeds these criteria, the infused dose will be adjusted accordingly.

6.6 Dosages, dosage modifications and method of administration

The CMV pp65-specific T cells will be infused intravenously over 30 minutes via a peripheral intravenous access, or, if already in place, an in-situ venous access device.
The aimed dose of CMV pp65-specific T cells is 10-20 x 10^6 total T cells, with >20% IFNg secreting T cells and containing < 0.3 x 10^6/kg body weight T-cells with unknown specificity in case a patient was transplanted with an HLA-matched sibling and < 0.15 x 10^6/kg body weight T-cells with unknown specificity in case a patient was transplanted with an HLA-matched unrelated donor.

6.7 Preparation and labeling of Investigational Medicinal Product

Preparation, labeling and dispensing of the CMV pp65-specific T cells will be performed at the IGFL as specified in SOPs according to GMP. Please refer to the IMPD.

6.8 Drug accountability

Procedures for shipment, receipt, disposition, return and destruction of the CMV pp65-specific T cells are specified in SOPs and will be performed by the IGFL according to GMP.

7. METHODS

7.1 Study endpoints

- The number of events of acute GvHD, death and all other adverse events.
- The number of CMV-specific T cells at different time points after infusion of CMV pp65-specific T cells.
- The number of complete responses or partial responses of CMV reactivation or CMV disease after infusion of CMV pp65-specific T cells.

7.2 Study procedures

7.2.1 **Pretreatment investigations**

- History, physical examination, WHO performance score (see Appendix A) and weight.
- CMV DNA load.
- Blood cell counts, differential, platelet count.
- Sodium, potassium, BUN, creatinine, liver enzymes, total bilirubin, albumin, LDH, glucose.
- Quantification of CMV-specific T cells by tetramer staining and functional assays (intracellular cytokine staining)
- Chest X-ray.
- Assessment of chimerism.

7.2.2 **Investigations during treatment**

Contraindications for administration of CMV pp65-specific T cells will be checked before the administration procedure of CMV pp65-specific T cells.
The product will be infused intravenously over 30 minutes via peripheral intravenous access, or, if already in place, an in-situ central venous access device. Actual doses of infused CMV pp65-specific cells will be documented.
Close monitoring of vital signs (temperature, pulse, respiratory rate, blood pressure, and oxygen saturation) will be measured and documented before CMV pp65-specific T cell infusion and every 15 minutes during a 1 hour period after infusion.

7.2.3 **Investigation during follow-up in adults**

- Interim history and physical examination including WHO performance score, weight, signs of toxicity, infection and GvHD daily during hospitalization or weekly for 8 weeks, followed by monthly for 4 more months.
- Blood cell counts, differential, platelet count, sodium, potassium, BUN, creatinine, liver enzymes, total bilirubin, albumin, LDH and glucose weekly for 8 weeks, followed by monthly for 4 more months.
- CMV DNA load at day 1 and 2, followed by weekly for 8 weeks and monthly for 4 more months.

- Quantification of CMV-specific T cells by tetramer staining and functional assays (intracellular cytokine staining) will be performed at the LUMC from blood samples from day 1 and thereafter weekly samples from week 1-8 and monthly samples from month 3-6.
- Assessment of chimerism at 6 weeks, 3 months and 6 months.

Weeks after infusion	0	1	2	3	4	5	6	7	8	12	16	20	24
Infusion of cells	x				(x)				(x)				
History and PE	x	x	x	x	x	x	x	x	x	x	x	x	x
Blood	x	x	x	x	x	x	x	x	x	x	x	x	x
CMV DNA	xxx	x	x	x	x	x	x	x	x	x	x	x	x
CMV tetramer	xx	x	x	x	x	x	x	x	x	x	x	x	x
Chimerism							x			x			x
xxx day 0, 1, 2 xx day 0, 1													

The volume of blood for quantification of CMV-specific T cells by tetramer staining and functional assays (intracellular cytokine staining) is 50 ml.
The volume of bone marrow for chimerism analysis is 10ml.

7.2.4 **Investigation during follow-up in children**

- Interim history and physical examination including WHO performance score, weight, signs of toxicity, infection and GvHD daily during hospitalization or weekly for 8 weeks, followed by monthly for 4 more months.
- Sampling of blood will be according to study protocol “adoptive immunotherapy for adenovirus infections after allogeneic stem cell transplantation in children” as approved by the CCMO.

PBMC samples (20ml) will be taken and stored at 2 week intervals for the investigation of the presence and function of CMV-specific T cells until 3 months after last infusion. Blood sampling for this specific protocol will be combined with blood sampling for regular monitoring of the transplantation as much as possible.

7.3 Evaluation criteria

7.3.1 **Safety evaluation after treatment**

General non GvHD toxicity will be scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 (see appendix B) In case of grade 3 or 4 toxicity after infusion of CMV pp65-specific T cells, further administrations of CMV pp65-specific T cells will be cancelled in that patient.
Acute GvHD will be graded using the criteria outlined in appendix C. In case of grade 3 or 4 acute overall GvHD after infusion of CMV pp65-specific T cells, further administrations of CMV pp65-specific T cells will be cancelled in that patient.

7.3.2 **Evaluation of clinical response**

Complete response: Complete disappearance of CMV DNA load for at least 4 weeks.
Partial response: reduction of CMV DNA load of > 1 log but no complete disappearance of CMV DNA load for at least 2 weeks.
Stable disease: no significant change (<1 log) in viral DNA load and no complete disappearance of CMV DNA load for at least 4 weeks.
Progressive disease: persistent increase in CMV DNA load of > 1 log during 4 weeks or new CMV disease.

7.3.3 **Evaluation of presence of CMV pp65-specific T cells**

Quantification of CMV-specific T cells will be performed by tetramer staining and functional analysis of CMV-specific T cells by intracellular cytokine staining.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The treating clinician can decide to withdraw a subject from the study for urgent medical reasons.

7.5 Premature termination of the study

In case of increase of >1 point of overall grade acute GvHD in 2 out of the first 3 patients, or 3 out of the first 6 patients, or 4 out of the first 10 patients, the study will be closed temporarily for inclusions and the outcomes in all patients will be discussed in order to decide whether potential beneficial effects of the treatment could overcome non-beneficial effects of GvHD.

7.6 End of study

End of the study is defined as 6 months after the infusion of CMV pp65-specific T cells of patient number 15 or at time of subsequent DLI of patient number 15.

8. SAFETY REPORTING

8.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the CCMO if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the CCMO, except insofar as suspension would jeopardize the subjects' health. The investigation will take care that all subjects are kept informed.

8.2 Adverse and serious adverse events

Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational drug. All adverse events reported spontaneously by the subject or observed by the investigator will be recorded.

A serious adverse event (SAE) is any untoward medical occurrence or effect that at any dose results:
- in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing inpatients' hospitalization;
- in persistent or significant disability or incapacity.

All SAEs will be reported through the web portal *ToetsingOnline* to the CCMO, within 15 days after the sponsor has first knowledge of the serious adverse reactions. SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

8.2.1 **Suspected unexpected serious adverse reactions (SUSAR)**

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.
Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information.
All SUSARs during this study will be reported to the CCMO and the competent authority through the web portal *ToetsingOnline* within 15 days. For fatal or life-threatening cases, a preliminary report will be send within 7 days, followed by a definite repost within 8 days.

8.2.2 **Annual safety report**

The investigator will submit, once a year throughout the clinical trial, a safety report to the CCMO.

This safety report consists of:
- a report of progress of the study
- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system.
- a report concerning the safety of the subject, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the CMV pp65-specific T cells.

8.3 Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures, and/or referral to a medical specialist.

9. STATISTICAL EVALUATION

9.1 Statistical design

This is an open-label non-randomized phase I/II feasibility study to treat patients with persistent CMV reactivation or CMV disease after alloSCT with administration of CMV pp65-specific T cells.

9.2 Planned sample size

We aim to include 15 patients. On the basis of the currently performed phase I/II clinical study (LUMC 2004-01) on the treatment of refractory CMV reactivation we expect to include 5-7 patients per year for a period of 3 years.

9.3 Power calculation

We consider the infusion of CMV pp65-specific T cells a success when the response rate is at least 66 % (p1; 2/3 of patients). Based on our historical experience, we estimate the rate of responses with pharmacotherapy alone but without immune intervention to be 30% (=p0) for the targeted patient population. Given the high laboratory effort and associated costs of treatment with CMV pp65-specific T cells, the trial should have a power of 90% (beta-error 0.1). With the usual alfa-error of 0.05, the sample size as calculated according to the standard Fleming design is 15 treated patients under these assumptions. We expect no excess in number of events of acute GvHD, death and all other adverse events.

9.4 Statistical analysis

Descriptive statistics and univariate and multivariate analysis will be used to characterize the study population. The endpoint of analysis of treatment toxicity will be done primarily by tabulation of the incidence of side effects with CTCAE grade 2 or more (see Appendix B) or acute GvHD overall grade III or more (see Appendix C). Actuarial competing risk estimates of probability of death will be split by cause of death where a difference will be made between death due to CMV disease and death due to side effects of the treatment. The endpoints of presence of CMV-specific T cells and number of complete remissions and partial remissions of CMV reactivation or CMV disease after administration of CMV pp65-specific T cells will be documented.

9.5 Interim analysis

After the first 3, 6 and 10 patients an interim analysis will be performed by the principal investigator.

10. ETHICAL CONSIDERATION

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

10.2 Recruitment and consent

The treating physician will inform the patient (and in case of a minor their parents or guardians) about this study and explain the patient (and in case of a minor their parents or guardians) about the informed consent procedure. All patients (and in the case of a minor their parents or guardians) will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which the patient will be exposed. They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. It will be emphasized that the participation is voluntary and that the patient (and in case of a minor their parents or guardians) is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. The patient (and in case of a minor their parents or guardians) is given the information letter. During a second visit, information will be repeated if necessary and participation to the study will be considered.

For children below the age of 12 years written informed consent will be obtained from both parents or guardians. Children aged between 12-17 years of age must give their written informed consent, unless these children are not capable to give their consent, together with both parents or guardians. Only after written informed consent is obtained will the participants be entered into the study.

10.3 Benefits and risks assessment

Potential benefit of participation to this study is a reduction of the CMV DNA load which may lead to a reduction of development of severe CMV disease or cure of CMV disease. A potential risk of participation to this study is development of acute GvHD.

10.4 Compensation for injury

The chance of injury as a result of this study is extremely small. However, the LUMC has insured this risk by taking a liability insurance, which is in accordance with article 7, subsection 6 of the WMO.

The LUMC (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003).

This insurance provides cover for damage to research subjects through injury or death caused by the study.
1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11. ADMINISTRATIVE ASPECTS AND PUBLICATION

11.1 Handling and storage of data and documents

Patients will be included after verification of eligibility by the office of the department of hematology. A list of questions to be answered during the inclusion procedure is included in the registration check-list (see appendix D), including inclusion criteria and date of written informed consent. This check-list should be completed by the physician before the patient is included. Patients will be assigned for the purpose of the study and publication, a unique patient number (UPN).
Data will be recorded on Case Report Forms (CRF) and will be entered after validation into a computer system for subsequent tabulation and analyses. The data will be handled confidentially and, as far as possible, anonymously.
The investigator will retain the originals of the source documents generated for a minimum of ten years after the study is completed. After this all documents will be archived according to Good Clinical Practice (GCP) regulations. The results of the study will be published in recognized medical journals if applicable. If so, the patient identity will not be revealed.

11.2 Annual progress report

The principal investigator will submit a summary of the progress of the trial to the CCMO once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

11.3 End of study report

The investigator will notify the CCMO of the end of the study within a period of 8 weeks. The end of the study is defined as the last patients' last control visit. This will be 6 months after the infusion of CMV pp65-specific T cells of patient number 15 or at time of subsequent DLI of patient number 15.
In case the study is ended prematurely, the investigator will notify the CCMO including the reason for the premature termination.
Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the CCMO.

11.4 Public disclosure and publication policy

A writing committee will be responsible for the publication of the results of this study.

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APPENDIX A

WHO performance score

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

APPENDIX B

Common toxicity Criteria

The grading of toxicity and adverse events will be done using the NCI Common Terminology Criteria for adverse events, CTC version 4.0, published May 28, 2009. A complete document (78 pages) may be downloaded from the following site: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

APPENDIX C

Grading of GvHD

Acute GvHD

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 erythro-derma with formation of bullea and desquamation	Bilirubin > 15 mg/dl (> 225 umol/L)	Severe abdominal pain with or without ileus

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

Chronic GvHD

Limited Localized skin involvement and/or liver function abnormalities

Extensive Generalized skin involvement or localized skin involvement and/or liver function abnormalities + other organ involvements

APPENDIX D

Standard indications for alloSCT including:

In adults:
Acute lymphoblastic leukemia/lymphoma
Acute myeloid leukemia
Chronic lymphocytic leukemia
Chronic myelogenous leukemia
Hodgkin lymphoma
Non Hodgkin lymphoma
Myelodysplastic syndromes
Myeloproliferative neoplasms
Plasma cell myeloma
Severe aplastic anemia
Sickelcell disease
Thalassemia

In children:
Acute lymphoblastic leukemia/lymphoma
Acute myeloid leukemia
Chediak-Higashi syndrome
Chronic myelogenous leukemia
Congenital immunedeficiencies
Diamond-blackfan anemia
Fanconi anemia
Griscelli-Siccardi syndrome
Hemophagocytic lymphohistiocytosis
Juvenile myelomonoccytic leukemia
Myelodysplastic syndromes
Non Hodgkin lymphoma
Paroxysmal nocturnal hemoglobinuria
Resistent Langerhans histiocytosis
Severe aplastic anemia
Thalassemia