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Vaccination with HA-1 peptide in patients showing minimal residual disease or mixed chimerism after allogeneic stem cell transplantation and donor lymphocyte infusion

A phase I/II study

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Pharmacy	Not applicable

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ALL	Acute lymphoblastic leukaemia
Allo-SCT	Allogeneic stem cell transplantation
AML	Acute Myeloblastic Leukemia
CCMO	Central Committee for Research in Humans (in Dutch: Centrale Commissie Mensgebonden Onderzoek)
CLL	Chronic lymphocytic leukaemia
CML	Chronic Myeloid leukaemia
DLI	Donor lymphocytes infusion
GVHD	Graft versus host disease
IMP	Investigational Medical Product
IMPD	Investigational Medical Product Dossier
HLA	Human leukocyte antigen
MDS	Myelodysplastic Syndrome
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MM	Multiple Myeloma
MUD	Matched unrelated donor
NMA	Non-myeloablative
PCR	Polymerase Chain Reaction
(S)AE	(Serious) Adverse Event
SUSAR	Suspected Unexpected Serious Adverse Reaction
WHO	World Health Organisation
WMO	Medical Research Involving Human Subjects Act (Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Objective	The main objectives of the phase I/II study are: 1. To determine the safety and toxicity of administration of HA-1 peptide vaccine in HLA-A2 and HA-1 positive patients who had undergone HLA-matched allogeneic stem cell transplantation followed by DLI from a HLA-A2 positive, HA-1 negative, donor showing persistent disease or mixed chimerism eight weeks after DLI (phase 1 study). 2. To evaluate whether an immunologic response can be induced by this vaccination program (primary endpoint of phase 2 study). 3. To evaluate whether an immunologic response influences chimerism and disease status (secondary endpoint).
Methodology	This is an single centre open label intervention study with a sequential phase I/II design.
Number of patients	Initially 12 patients will receive the HA-1 20mer peptide vaccine. In case of no toxicity, and immunologic response in less than 6 patients another 12 patients will receive the HA-1 20mer + the HA-1 9mer peptide vaccine. If an immunologic response occurs in 6 or more patients receiving the 20mer peptide vaccine, the vaccination program will be continued in order to include a maximum of 24 patients.
Main criteria for Inclusion (at time of DLI)	• Patients with AML, myelodysplasia (MDS), ALL, CML in accelerated phase or blastic transformation, CLL, MM or aggressive lymphoma, who underwent allo SCT (both myeloablative and non-myeloablative) followed by DLI for persistent mixed chimerism or smoldering disease • Patient and donor HLA-A2 positive, patient HA-1 positive, donor HA-1 negative. • WHO performance status of 0, 1 or 2 (see appendix) • No pregnancy, not breast feeding • Willing to use of effective contraception during the course of this trial and for at least three months after the last injection. • Life expectation of > 3 months • No severe psychological disturbances • No HIV positivity

Main criteria for vaccination (10 weeks after DLI)	• Mixed chimerism or persisting disease, 8 weeks after DLI. • No necessity of persistent treatment with high-dose corticosteroids (> 20 mg prednisone a day), chemotherapy or immunosuppressive drugs. • No rapidly progressive disease needing cytoreductive treatment • No overall GVHD grade 3 or 4 • At eight weeks after DLI no HA-1 specific immune response (defined by >0.2% of total CD8+ cells) in first six patients • At eight weeks after DLI no HA-1 specific immune response (defined by >1.0% of total CD8+ cells) in patients 7-24 if no toxicity >grade II in first three patients • Between six and eight weeks after DLI no doubling of percentage HA-1 specific CD8+ cells to a final percentage >0.2% • Informed consent according to the rules and regulations of the LUMC
Treatment	At week 0: DLI and inclusion in the study if informed consent is given At week 8: Bone marrow investigation for chimerism and/or disease evaluation. GVHD evaluation and measurement of HA-1 specific T cells **If no HA-1 specific response :** At week 10: First vaccination At week 13: Second vaccination At week 16: Third vaccination
Criteria of evaluation	Patients will be monitored for toxicity, graft versus host disease (GVHD), changing chimerism, disease status, and immunological response by appearance of HA-1 specific CD8+ T cells. At week 6: DLI; assessment of HA-1 specific CD8+ T cells At week 8: Bone marrow investigation for chimerism and/or disease/GvHD evaluation and measurement of HA-1 specific T cells From 10 - 26 weeks: immune response by measurement of HA-1 specific T cells (8 times, see Figure 3). At week 12,15,19 and 26: Bone marrow investigation for chimerism

	and/or disease/GvHD status
Statistical methods	Toxicity will be evaluated after the first 6 patients (with <0.2% HA-1 specific CD8+ cells before vaccination); if no patient experiences > grade II toxicity and/or worsening of GvHD to more than grade II, another 6 patients (with <1.0% HA-1 specific CD8+ cells before vaccination) will be treated using the 20mer vaccine . If 1 patient out of the first six experiences > grade II toxicity and/or worsening of GVHD to more than grade II, another 3 patients (with <0.2% HA-1 specific CD8+ cells before vaccination); will be treated with the 20mer vaccine. If < 2 (out of 9) patients experiences > grade II toxicity and/or worsening of GvHD to more than grade II, other patients (with <1.0% HA-1 specific CD8+ cells before vaccination) will be treated with the 20mer vaccine. Immunological and clinical effects will be evaluated after the first 12 patients treated using the 20mer vaccine. If six or more patients showed an immunological response, the vaccination program using the 20mer vaccine will be continued, aiming for a total number of 24 patients. If less than 6 patients showed an immunologic response, 12 additional patients will be entered and treated using the 20mer + 9mer vaccine If less than 6 patients showed an immunological response to the 20mer + 9mer vaccine, the vaccination strategy using current peptides will be considered inadequate.

Rationale: After allogeneic HLA-matched SCT, minor histocompatibility antigens (mHags) are the most likely targets for GvL associated immune reactivity. Among these mHags, HA-1 is expressed by both normal hematopoietic cells and their malignant counterparts. HA-1 specific T cells have been shown to be capable of eliminating HA-1 positive malignant (precursor) cells in HLA-A2 positive HA-1 positive patients after stem cell transplantation and DLI administration from a HA-1 negative donor. However, after DLI not all patients develop a HA-1 specific T-cell response and a durable GvL effect in this setting.

Objective: The main objectives of this study are:

- To determine the safety and toxicity of administration of HA-1 peptide vaccine in HLA-A2 and HA-1 positive patients who had undergone HLA-matched allogeneic stem cell transplantation followed by DLI from a HLA-A2 positive, HA-1 negative, donor showing persistent disease or mixed chimerism eight weeks after DLI (phase 1 study)
- To evaluate whether an immunologic response can be induced by this vaccination program (primary endpoint of phase 2 study)
- To evaluate whether an immunologic response influences chimerism and disease status (secondary endpoint).

Study design: This is a single centre open label intervention study with a sequential phase I/II design

Study population Patients with AML, myelodysplasia (MDS), ALL, CML in accelerated phase or blastic transformation, CLL, MM or aggressive lymphoma, who underwent allo SCT (both myeloablative and non-myeloablative) followed by DLI for persistent mixed chimerism or smoldering disease; patient and donor HLA-A2 positive, patient HA-1 positive, donor HA-1 negative.

Intervention Subcutaneous vaccination with a HA-1 peptide vaccine, ten weeks after DLI. In the absence of a strong HA-1 specific T cel response, the vaccination will be repeated twice with three weeks intervals. At the day of the first vaccination, 1 mg keyhole limpet hemocyanin (KLH, Biosyn) will be injected subcutaneously in order to evaluate the immune status of the patient. If after KLH injection no local skin reaction appears at the site of injection, the KLH injection will be repeated together with the following HA-1 vaccination, if given.

Main study parameters/endpoints: Patients will be monitored for toxicity, graft versus host disease (GVHD), changing chimerism, disease status, and immunological response by appearance of HA-1 specific CD8+ T cells.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Participating patients will visit the outpatient clinic once every two weeks for physical examination and blood sampling from the first vaccination until 16 weeks after vaccination. At this moment the standard care for patients after DLI is a two-weekly visit, so the patient has to perform no extra visits to the outpatient clinic. The total amount of blood which will be taken for study purposes will be maximally 140 cc in a three months period. Two extra bone marrow examinations will be performed (15 and 19 weeks after DLI). Theoretically, the risk of HA-1 peptide vaccination is the development of a strong immune response with severe acute GVHD or an anaphylactic reaction. However, as HA-1 is expressed only by hematopoietic cells we do not expect severe acute GVHD. Because HA-1 occurs in this group of patients by nature, we do not expect a systemic anaphylactic reaction. Local skin reactions on the site of injections are expected.

In our current treatment protocol, a patient can receive a second DLI three months after the first DLI in case of persisting disease or mixed chimerism in the absence of GVHD. If a patient participates in this vaccination study, the second DLI will be postponed maximally three months. However, in case of worsening chimerism status or progressive disease the patient can go off protocol en will be able to receive DLI as indicated by the treating physician.

If vaccination with HA-1 peptide indeed yields an immune response with changes in chimerism towards donor, there is no need for a next DLI, thus decreasing the risk of acute GVHD. In addition, an HA-1 specific immune response could be associated to a lower relapse rate.

1. INTRODUCTION AND RATIONALE

Allogeneic human-leukocyte-antigen (HLA)-matched hematopoietic stem cell transplantation (SCT) has been applied for many years as part of the treatment of hematologic malignancies. Transplantation of the patient with a stem cell graft results in hematologic and immunologic reconstitution after administration of chemotherapy, irradiation and immune suppression. The effect of allogeneic SCT on long term disease-free survival is not only the result of the conditioning regimen and hematologic reconstitution of the recipient. Clinical observations strongly suggest that donor T cells administered in the context of an allogeneic stem cell graft exhibit an antileukemic effect, the "graft versus leukemia" (GvL) reactivity. Depletion of T cells from the graft to prevent graft versus host disease (GVHD) resulted in an increased incidence of relapse after transplantation, illustrating the capacity of donor T cells to mediate GvL. Donor lymphocyte infusion (DLI) given to patients with recurrence of malignancy after allogeneic SCT, resulted in eradication of the disease in part of the patients, further demonstrating the efficacy of allogeneic T cells in exhibiting GvL reactivity. Mixed chimerism after allogeneic transplantation is associated with an increased relapse risk. Conversion of mixed chimerism towards 100% donor chimerism by DLI may be associated with a decrease in the risk of subsequent relapse of the disease.

After allogeneic HLA-matched SCT, minor histocompatibility antigens (mHags) are the most likely targets for GvL associated immune reactivity. mHags are immunogenic peptides derived from intracellular polymorphic proteins that can be presented by HLA class I or class II molecules, and can be recognized as non-self antigens by T cells from HLA-identical individuals. Based on the polymorphic nature of the genome, and the likelihood of disparity of mHags between donors and recipients in HLA-matched SCT, it seems likely that an allo-response against target antigens expressed on cells from the recipient may occur after transplantation or after DLI administration in most cases. Donor T cell responses against mHags broadly expressed on tissues from the recipient may result in severe GVHD as well as GvL after DLI. In contrast, T cells recognizing mHags preferentially expressed on hematopoietic cells from the recipient may result in elimination of recipient derived cells from hematopoietic origin, including the malignant cells, resulting in complete donor chimerism and cure of the disease without inducing severe GVHD. A number of mHags has been found to be selectively expressed in cells of hematopoietic origin

and may serve as tumor-specific antigens for vaccination when allogeneic transplantation has been performed for treatment of hematologic malignancies. These mHags include HA-1 and HA-2. HA-1 is expressed by both normal hematopoietic cells and their malignant counterparts and can be recognized in the context of HLA-A2.

The 9-mer VLHDDLLEA has been shown to be the peptide which is recognized in the context of the HLA-A2 by HA-1 specific cytotoxic T lymphocytes. This peptide is formed by proteosomal cleavage of the HMHA1 protein. Due to a single nucleotide polymorphism, approximately 69% of the HLA-A2 positive population present the histidin containing variant (VLHDDLLEA), whereas 31% only present the arginin-variant (VLRDDLLEA).

HA-1 specific T cells have been shown to be capable of eliminating HA-1 positive malignant (precursor) cells. Using HLA-2/HA-1 tetramers we have observed HA-1 specific T-cell responses in HLA-A2 and HA-1 positive patients treated with DLI from HLA-A2 positive, HA-1 negative donors. These responses occurred 5-7 weeks after DLI and were associated with a clinical antitumor response. However, such an immunological and clinical response was not observed in all HLA-A2 and HA-1 positive patients treated with DLI from HLA-A2 positive, HA-1 negative donors.

High frequencies of complete responses with DLI have been observed in patients with relapsed CML after transplantation. However, DLI in patients with relapsed AML, ALL, CLL, multiple myeloma and malignant lymphoma is less successful. It has been hypothesized that the malignant cells in these hematological malignancies may be poor Antigen Presenting Cells (APCs), not expressing the relevant co-stimulatory and/or adhesion molecules necessary for the induction of a mHag specific response in vivo. In donor-patient combinations with HA-1 disparity, vaccination with synthetic HA-1 epitope may augment the alloreactive GvL response in patients with minimal residual disease or mixed chimerism lacking a HA-1 specific T cell response after DLI.

The nonameric HA-1 peptide (VLHDDLLEA) presented by MHC class I is a possible candidate for use as a vaccine (Den Haan 1998, Hambach 2005). Immunological and clinical responses have been described in patients receiving Class I peptide vaccinations (Qazilbash 2007). Based on earlier studies, however, vaccination with a longer peptide containing the immunogenic epitope may yield better immune responses and less development of tolerance compared to vaccination with the

single epitope (Bijker 2007). In a preclinical model of human papillomavirus (HPV) 16-induced cervical cancer, it has been shown that vaccination with long peptides containing the CTL epitope leads to a good immunologic response.(Kenter, in press) Using a longer peptide, direct binding to MHC class I molecules does not take place and as a consequence, uptake, processing and presentation by professional APC is needed to induce HA-1 specific CTL, avoiding peptide-induced tolerance. In addition, vaccination with vaccines containing a long peptide may lead to antigen presentation in the context of MHC-II molecules, activating T-helper lymphocytes leading to a more robust immune response and memory T cell response. Therefore we have developed a 20mer peptide containing the HA-1 nonamer. We have shown that exposure of HLA-A2 positive, HA-1 negative APCs to the HA-1 20mer leads to processing and expression of the HA-1 nonamer in these APCs and recognition by HA1 specific CD8+ T cells. In the case that vaccination with the 20mer leads to tolerance, this will be restricted to the HA-1 peptide. Repeated DLI will still be able to induce anti tumor responses as those responses were shown to be directed towards several minor antigens (van Bergen 2007, Kloosterboer 2005)

In summary, a T-cell response specific for HA-1 appears to be important for a GvL effect in HLA-A2 positive HA-1 positive patients after transplantation and DLI administration from a HA-1 negative donor. However, after DLI not all patients develop a HA-1 specific T-cell response and a durable GvL effect. In order to improve the avidity and quantity of HA-1 specific T-cell responses we intend to implement a vaccination program for treatment of smoldering disease or mixed chimerism after DLI. In order to bypass possible peptide induced tolerance and to enhance specific CD4+ T cell reactivity, a 20mer long peptide will be used containing the 9mer sequence of the known HA-1 epitope.

2. OBJECTIVES

The main objectives of the phase I/II study are

- To determine the safety and toxicity of administration of HA-1 peptide vaccine in HLA-A2 and HA-1 positive patients who had undergone HLA-matched allogeneic stem cell transplantation followed by DLI from a HLA-A2 positive, HA-1 negative, donor showing persistent disease or mixed chimerism eight weeks after DLI (phase 1 study)
- To evaluate whether an immunologic response can be induced by this vaccination program (primary endpoint of phase 2 study).
- To evaluate whether an immunologic response influences chimerism and disease status (secondary endpoint).

3. STUDY DESIGN

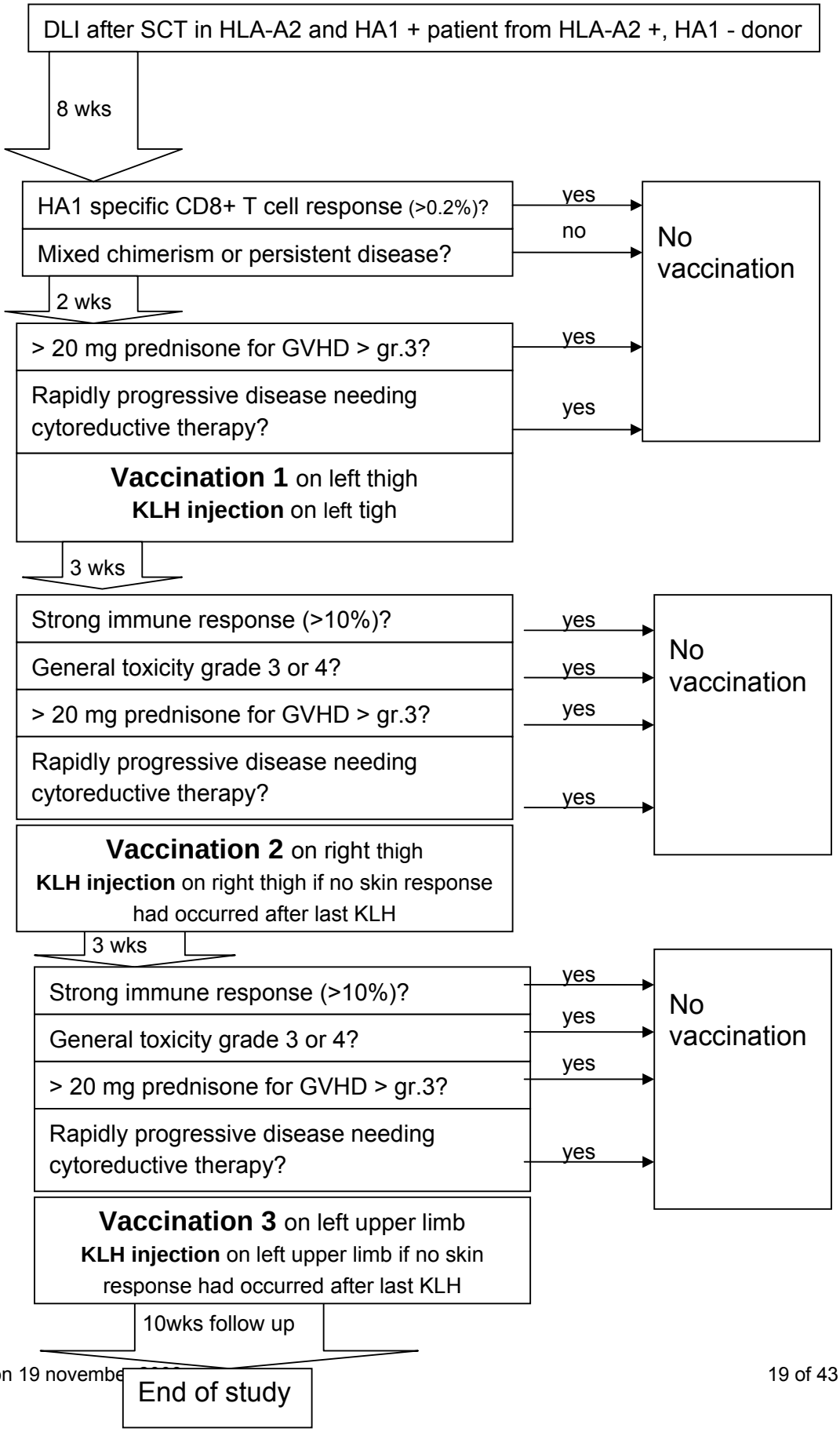
This is a phase I/II feasibility study to a vaccination program in patients with a hematological malignancy who show mixed chimerism or persistent disease after allogeneic SCT and DLI. Patients with AML, ALL, MM, CLL, malignant lymphoma, MDS or CML in accelerated or blastic phase showing mixed chimerism six months after allogeneic SCT, are treated in our institution with DLI ($3*10^6$ cells/kg if a family donor has been used and $1.5*10^6$ cells/kg in case of a matched unrelated donor). Patients who met inclusion criteria will be asked informed consent for this study at time of DLI. Following DLI, HA-1 specific CD8+ T cells will be measured with two week intervals. If eight weeks after DLI mixed chimerism or malignant disease persists, and no GvHD grade III or IV has developed, and no HA-1 specific immunologic response has occurred, patients will be eligible for receiving vaccination containing 300 µg 20mer peptide vaccine. At the day of the first vaccination, 1 mg keyhole limpet hemocyanin (KLH, Biosyn) will be injected subcutaneously on the controlateral thigh in order to evaluate immune status of the patient. Local skin reaction size and appearance of KLH reactive T cells will be used for determination of the immune status of the patient. The absence of a local skin reaction will be interpreted as a low or absent capacity to generate an immune response. In that case, the KLH injection will be repeated at the same time point as the following HA-1 vaccination in order to re-evaluate this capacity.
In the first six patients the presence of an initial immune response after DLI (HA-1 specific CD8+ cells >0.2% of total CD8+ cells), will be an exclusion criterion. However, if in the first three patients no grade III or IV toxicity has developed and no GVHD exceeding grade II was seen, the exclusion criterion for the presence of an immune response will be changed. Patients will be excluded from inclusion when HA-1 specific CD8+ cells exceed 1.0% of total CD8+ cells or after a doubling of the percentage of HA-1 specific CD8+ cells between 6 and 8 weeks with a total amount of HA-1 specific CD8+ cells exceeding 0.2% after 8 weeks.
The first twelve patients will receive vaccination with a HA-1 20mer peptide, ten weeks after DLI. Injection of this vaccine will be repeated after three and six weeks if GVHD does not exceed grade II. Before and after each vaccination, patients will be monitored for toxicity, immune response and clinical response. In case of grade 3 or 4 toxicity or rapidly progressive disease for which cytoreductive therapy is needed,

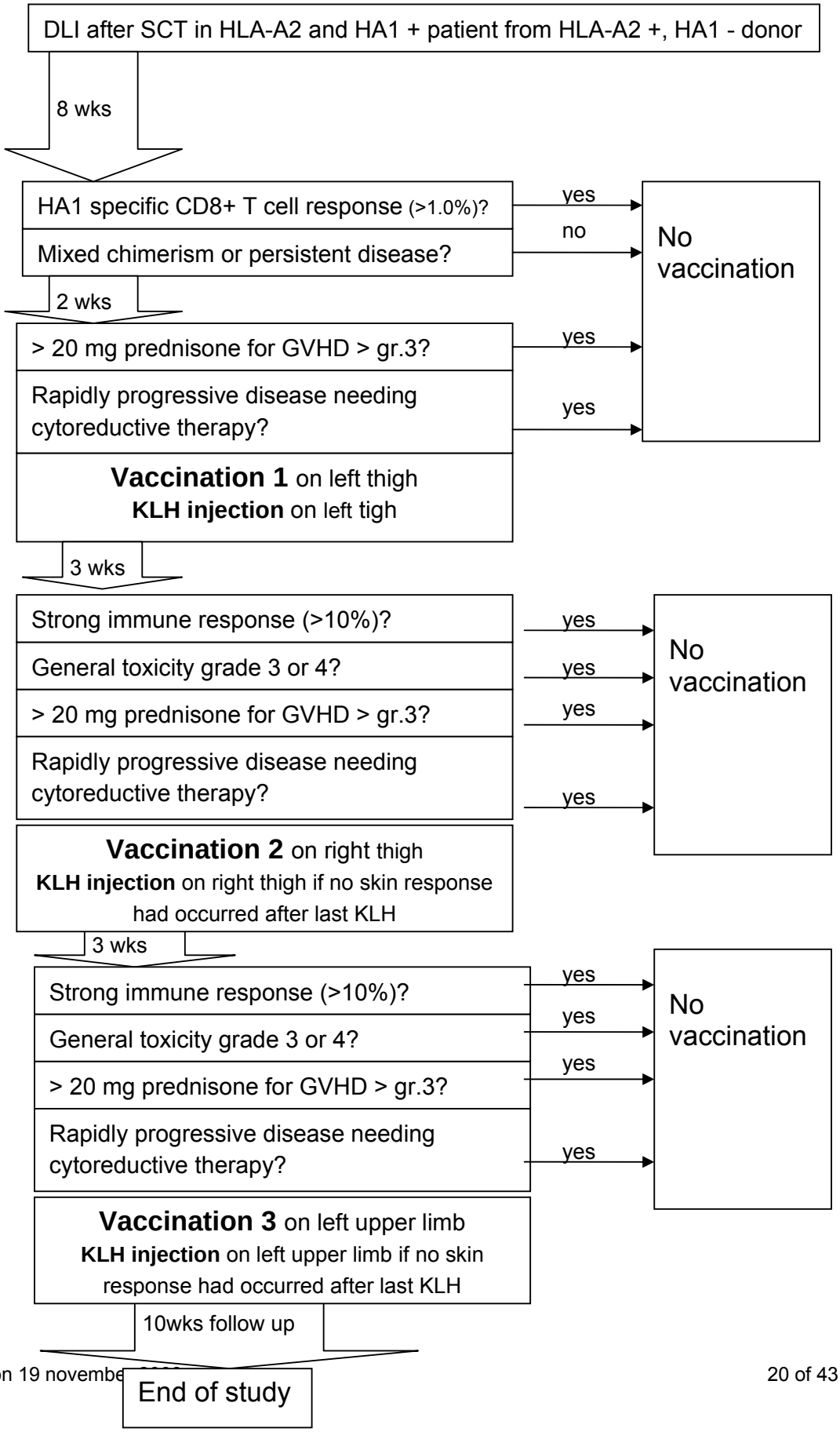
the vaccination program will be stopped. In case of a very strong immune response (appearance of >10% HA1 specific CD8+ T cells) the vaccination program will be stopped in order to avoid a possible severe systemic reactions after repeated vaccination.

If in the first twelve patients no general toxicity grade 3 or more has been observed and a complete immunological response after vaccination has been seen in less than 6 patients, the next twelve patients will be vaccinated with a peptide mixture of 300 µg 20mer in combination with 250 µg 9mer following the same time schedule (see Figure 2). If six or more patients showed an immunological response, the vaccination program using the 20mer vaccine will be continued, aiming for a total number of 24 patients.

Sixteen weeks after the first vaccination, patients go off the vaccination study and can be treated with a second DLI if needed. When a complete immune response accompanied with an ongoing clinical response was induced during the vaccination study, monitoring of the HA-1 specific immune response will be continued by determination of the amount of HA-1 specific T cells every two weeks for three months. If during monitoring a decrease in HA-1 specific T cells is observed, the vaccination program may be repeated in order to “reboost” the immune response.

Patients who were included in the study but did not met criteria for vaccination therapy, will be considered for repeated DLI three months after the first DLI according to current protocols.


DLI after SCT in HLA-A2 and HA1 + patient from HLA-A2 +, HA1 - donor
8 wks
HA1 specific CD8+ T cell response (>0.2%)?
yes
Mixed chimerism or persistent disease?
no
No vaccination
2 wks
> 20 mg prednisone for GVHD > gr.3?
yes
Rapidly progressive disease needing cytoreductive therapy?
yes
Vaccination 1 on left thigh
KLH injection on left tigh
3 wks
Strong immune response (>10%)?
yes
General toxicity grade 3 or 4?
yes
> 20 mg prednisone for GVHD > gr.3?
yes
Rapidly progressive disease needing cytoreductive therapy?
yes
No vaccination
Vaccination 2 on right thigh
KLH injection on right thigh if no skin response had occurred after last KLH
3 wks
Strong immune response (>10%)?
yes
General toxicity grade 3 or 4?
yes
> 20 mg prednisone for GVHD > gr.3?
yes
Rapidly progressive disease needing cytoreductive therapy?
yes
No vaccination
Vaccination 3 on left upper limb
KLH injection on left upper limb if no skin response had occurred after last KLH
10wks follow up
End of study


DLI after SCT in HLA-A2 and HA1 + patient from HLA-A2 +, HA1 - donor
8 wks
HA1 specific CD8+ T cell response (>1.0%)?
yes
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no
No vaccination
2 wks
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yes
Vaccination 1 on left thigh
KLH injection on left tigh
3 wks
Strong immune response (>10%)?
yes
General toxicity grade 3 or 4?
yes
> 20 mg prednisone for GVHD > gr.3?
yes
Rapidly progressive disease needing cytoreductive therapy?
yes
No vaccination
Vaccination 2 on right thigh
KLH injection on right thigh if no skin response had occurred after last KLH
3 wks
Strong immune response (>10%)?
yes
General toxicity grade 3 or 4?
yes
> 20 mg prednisone for GVHD > gr.3?
yes
Rapidly progressive disease needing cytoreductive therapy?
yes
No vaccination
Vaccination 3 on left upper limb
KLH injection on left upper limb if no skin response had occurred after last KLH
10wks follow up
End of study

4.0 STUDY POPULATION

4.1 Population

T-cell depleted stem cell transplantation (SCT) is performed according to standard procedures according to protocols LUMC 2003-1 (non-myeloablative conditioning) and LUMC 2003-4 (myelo-ablative conditioning). After SCT bone marrow aspiration is performed for morphological, immunophenotypical and chimerism analysis as clinically indicated but at least 1.5, 3, 6, 9, 12, 15, and 18 months after SCTT. If mixed chimerism or smoldering disease persists after six months, patients will be treated with donor lymphocyte infusion according to protocol ($3*10^6$ cells/kg if a family donor has been used and $1.5*10^6$ cells/kg in case of a family or matched unrelated donor, respectively). All patients receiving DLI who are HLA-A2 and HA1 positive with a HLA-A2 positive but HA1 negative donor, will be asked informed consent for this study at time of DLI.

4.2 Inclusion criteria at time of DLI

- Patients with AML, myelodysplasia (MDS), ALL, CML in accelerated phase or blastic transformation before transplantation, CLL, MM or aggressive lymphoma, who underwent allo SCT (both myeloablative and non-myeloablative) followed by DLI for persistent mixed chimerism or smoldering disease.
- Patient and donor HLA-A2 positive, patient HA-1 positive, donor HA-1 negative.
- WHO performance status of 0, 1 or 2 (see appendix)
- Female patients of childbearing potential must be neither pregnant nor breastfeeding and must agree to use effective contraception (birth control pills, condoms, approved implant, or IUD) during the course of this trial and for at least three months after the last injection.

4.3 Exclusion criteria at time of DLI

- Life expectation of < 3 months
- psychological disturbances
- Severely limited life expectation due to diseases other than the malignancy

- HIV positivity

4.4 Criteria for vaccination 10 weeks after DLI

- Mixed chimerism or persisting disease 8 weeks after DLI
- No necessity of persistent treatment with high-dose corticosteroids (> 20 mg prednisone a day), chemotherapy or other immunosuppressive drugs.
- No rapidly progressive disease
- No GVHD grade 3 or 4
- No HA-1 specific immune response (defined by >0.2% of total CD8+ cells in first six patients and defined by >1.0% of total CD8+ cells in patients 4-24 if no toxicity >grade II in first three patients). No important increase in percentage HA-1 specific CD8+ cells between 6 and 8 weeks after DLI (defined as a doubling of this percentage resulting in a percentage of > 0.2%)
- Informed consent given by patient

4.5 Sample size calculation

This is an exploratory study, so no power calculations can be made. Based on a yearly inclusion of at least three patients, we will be able to include 12 patients in a four years period. If the 20mer peptide-vaccination yields an immunological response in 6 or more patients, we consider this vaccination strategy as successful and will continue the vaccination program in twelve other patients, aiming for a total number of 24 patients. If an immunological response is seen in less than 6 patients, we want to include another 12 patients for vaccination with the combination of the 20mer and 9mer peptide.

5.0 TREATMENT OF SUBJECTS

5.1 Treatment administered

- 20mer vaccine

The 20mer peptide (see chapter 6.0: investigational product) is dissolved in 20 mM Phosphate Buffered Saline / Montanide ISA 51 50/50 v/v, being the adjuvant which is used in most of the vaccination trials in cancer immunotherapy (Qazilbash, 2007 Kenter, in press). The vaccine will be administered by subcutaneous injection at a vaccination dose of 300 μg. Injections will be administered three times separated by 3 weeks intervals at different anatomical sites. All vaccinations will be administered by trained personal and supervised by a medical doctor. After injection, patients will be monitored in the hospital for three hours,.

- 20mer and 9mer combination vaccine

The combination vaccine with 9mer and 20mer peptides (see chapter 6.0: investigational product is dissolved in 20 mM Phosphate Buffered Saline / Montanide ISA 51 50/50 v/v. The vaccine will be administered by subcutaneous injection at a vaccination dose of 300 μg of the 20mer and 250 μg of the 9mer. Injections will be administered three times separated by 3 weeks intervals at different anatomical sites. All vaccinations will be administered by trained personal and supervised by a medical doctor. After injection, patients will be monitored in the hospital for three hours,.

5.2 *Use of co-intervention*

At the day of the first vaccination, 1 mg of the neo-antigen keyhole limpet hemocyanin (Immucothel®, Biosyn) will be injected subcutaneously on the controlateral thigh in order to evaluate immune status of the patient. Local skin reaction size and appearance of KLH reactive T cells will be used for determination of the immune status of the patient. Immucothel is a registered drug for the topical treatment of bladder carcinoma. It is immunogenic in virtually 100% of vertebrate animals. After subcutaneous injection local erythema is seen in control subjects with a normal immune response, but erythema is not seen in a subset of patients who had underwent SCT, suggesting a diminished immune response. See appendix III for

Summary Product Characteristics.
Patients should use effective contraception during the course of this trial and for at least three months after the last injection.

5.3 Escape medication

Patients are allowed to use all medication which is prescribed to them by their treating hematologist. However patients who are described high dose systemic corticosteroids (> 20 mg prednisone a day) will not be able to receive further HA-1 vaccinations. In case of severe acute GVHD developing after the vaccination, patients will be treated with immunosuppressive drugs as indicated in current protocols (see appendix IV) .

6.0 INVESTIGATIONAL MEDICAL PRODUCT

6.1 Name and description of investigational medical product

- 20mer vaccine

The subcutaneous 20mer HA-1 vaccine consists of a single peptide representing the amino-acid sequence 133-152 (LKECVLHDDLLEARRPRAHE) of the HA-1 protein encoded by the gene KIAA0223. The peptide is produced in the Interdivisional GMP-Facility of the LUMC (IGFL), Department of Clinical Pharmacy and Toxicology. Technical details regarding the IMP, the production process, as well as a summary of preclinical toxicity studies is presented in the Investigational medical Product Dossier (IMPD) that accompanies this protocol. Montanide ISA 51 is used as an adjuvant.

- 20mer and 9 mer combination vaccine

This investigational medical product (IMP) consists of a peptide that comprises LKECVLHDDLLEARRPRAHE**,** representing the amino-acid sequence 133-152 of the HA-1 protein encoded by the gene KIAA0223, in combination with a peptide that comprises VLHDDLLEA, representing the amino-acid sequence 137-145 of the HA-1 protein encoded by the gene KIAA0223. The peptides are produced in the Interdivisional GMP-Facility of the LUMC (IGFL), Department of Clinical Pharmacy and Toxicology. Technical details regarding the IMP, the production process, as well as a summary of preclinical toxicity studies is presented in the Investigational medical Product Dossier (IMPD) that accompanies this protocol. Montanide ISA 51 is used as an adjuvant.

6.2 Summary of known and potential risks and benefits

See the Investigational medical Product Dossier (IMPD) that accompanies this protocol. Local toxicity at the vaccination site includes erythema, pruritis and edema, could be seen, due to the use of Montanide. Potential benefit of the vaccination is a specific immune response toward HA1 antigene presenting cells leading to improved chimerism status, which is associated to a lower recurrence rate of the hematological disease. As HA1 is restricted to the hematopoietic system, we do not anticipate severe systemic acute GVHD or systemic anaphylactic reaction. However, Mild GVHD (only requiring local therapy) is associated with Graft versus leukemia effects and is therefore an anticipated and accepted side effect in this study.

If vaccination with HA-1 peptide indeed yields an immune response with changes in chimerism towards donor, there is no need for a next DLI, thus decreasing the risk of acute GVHD. Besides, an HA-1 specific immune response could be associated to a lower relapse rate.

6.3 Description and justification of route of administration and dosage

The HA-1 vaccine will be administered in the thigh or the limb, via subcutaneous injection. Dose and route were chosen on the basis of a phase I trial with a HPV16 E6/E7 vaccine, consisting of 13 overlapping long-peptides produced at the IGFL in end-stage cervical carcinoma patients recently performed in our institution. Subcutaneous vaccination of this HPV16 E6/E7 vaccine at a dose of 300 μg in the presence of Montanide has been shown to induce strong HPV16 specific T cell immunity and was without toxicity higher then grade 2.

6.4 Preparation and labeling of the Investigational Medicinal product

Preparation, labelling and dispensing of the IMP will be performed at the Department of Clinical Pharmacy and Toxicology as specified in Standard Operating Procedures (SOPs) according the Good Manufacturing Practice for hospitals (GMP-z).

6.5 Drug accountability

Procedures for the shipment, receipt, disposition, return and destruction of the investigational medicinal products are specified in SOPs and will be performed by the Department of Clinical Pharmacy and Toxicology according to GMP-z.

7.0 METHODS

7.1 Study endpoints

- The primary study endpoint of the phase I study is to determine the safety of administration of HA-1 peptide vaccine in HLA-A2 and HA-1 positive patients who had undergone HLA-matched allogeneic SCT followed by DLI from a HA-1 negative, HLA-A2 positive donor showing persistent disease or mixed chimerism eight weeks after DLI.
- The primary study endpoint of the phase II part of the study is to evaluate whether an immunologic response can be induced by this vaccination program
- A secondary endpoint is to evaluate whether an immunologic response is accompanied with a clinical response.

7.2 Study procedures

Pretreatment investigations prior to vaccination study

- History and physical examination
- Blood cell counts, differential, quantitative platelet count.
- Measurement of HA-1 specific CD8+ T cells using tetramers and measurement of HA-1 specific CD4+ T cells by the interferon-gamma production assays.
- Bone marrow aspirates for for chimerism and/or disease evaluation
- Chimerism analysis using short tandem repeat (STR)-PCR to determine the percentage of residual recipient cells).
- Other staging procedures as appropriate, related to sites of malignancy (radiological evaluation in case of lymphoma as underlying disease, paraprotein levels in case of multiple myeloma)
- Bilirubin (direct and indirect), alkaline phosphatase, ASAT, ALAT, LDH, γGT.
- Albumin and protein electrophoresis.
- Serum urea, creatinine, Na, K, uric acid, glucose.
- At the day of the first vaccination, 1 mg keyhole limpet hemocyanin (KLH, Biosyn) will be injected subcutaneously on the same site in order to evaluate immune status of the patient. Local skin reaction size and appearance of KLH

reactive T cells will be used for determination of the immune status of the patient. If no local skin reaction has been seen at the site of KLH injection, KLH injection will be repeated with following HA-1 vaccinations.

Investigations during follow-up (see Figure 3)

- Intensive interim history and physical examination will be done. Local skin reaction size at the site of KLH injection will be measured one week after the vaccination.
- Blood cell count, differential, quantitative platelet counts, creatinine, Urea, Na, K, Uric acid, glucose, ALAT, ASAT, alkaline phosphatase, bilirubin, LDH, gammaGT, total protein, albumin, at regular timepoints (see Figure 3)
- Blood will be drawn for measurement of HA-1 specific CD8+ T cells using tetramers and measurement of HA-1 specific CD4+ T cells using the interferon-gamma production assays. When a complete immune response accompanied with an ongoing clinical response was induced during the vaccination study, monitoring of the HA-1 specific immune response will be continued by determination of the amount of HA-1 specific T cells every two weeks for three months.
- Bone marrow aspiration for chimerism and/or disease evaluation will be done 2, 5, 9 and 16 weeks after the first vaccination.
- In case of lymphoma as underlying disease, radiological evaluation will be done 4, 8 and 16 weeks after the first vaccination.
- In case of persistent skin lesion on vaccination site skin biopsy, one week after vaccination, skin biopsy will be done if feasible for isolating local HA-1 specific T cell response. A maximum of two biopsies will be performed in each patient.

Figure 3: evaluation during vaccination protocol (first eight weeks after SCT every two weeks HA-1 specific T cells.

Weeks after DLI:

8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
X B R		X v K	S E	X B	X V K	S R	X B	X v K	s	R	B X			X		X		X B R

X: Venous blood sampling for evaluation of immune response and clinical response
V: vaccination
K: KLH injection (in absence of a local skin reaction after previous KLH injection)
E: Evaluation of local skin reaction at the site of KLH injection
S: Possibility for skin biopsy if persistent ongoing local reaction (maximal two biopsies per patient)
B: bone marrow aspiration
R: Radiological evaluation in case of malignant lymphoma

7.3 Evaluation criteria

- *Safety evaluation during treatment*

General toxicity will be measured using the modified WHO criteria. In case of grade 3 or 4 toxicity after vaccination, vaccination schedule will be stopped in this patient. Local toxicity of vaccination site includes erythema, pruritis and edema at the vaccination site and will not be considered as major toxicity for which vaccinations should be stopped.
GVHD will be graded using the criteria outlined in the appendix. GVHD grade 1 or 2 developing after treatment will not be considered as a major toxicity for which vaccination should be stopped because limited GVHD often is associated to a GvL effect and therefore can be a desirable response. Treatment for GVHD will be initiated following the current treatment protocol (Appendix IV)

- *Evaluation of immunological response during treatment*

If >1% of all CD8+ lymphocytes at any time point are HA-1 specific CD8+ T cells, an complete immune response will be considered to be present. If the percentage is < 1.0% and higher than 0.2% but has been doubled during two weeks, a partial immune response will be considered to be present.

- *Evaluation of clinical response during treatment*

If bone marrow chimerism changes towards donor after vaccination, this will be considered as a clinical response.

7.4 Special orders

Hematological supportive care will involve prophylactic platelet transfusions when counts are < 10 x 10^9/l and leukocyte-free red blood cell transfusions as clinically indicated. All blood products will be irradiated with 25 Gy. Patients will be observed for four hours after injection of the vaccine for occurrence of hypersensitive or anaphylactoid reaction.
In case of rapid progressive disease for which cytoreductive therapy (chemotherapy or clonal antibody therapy) is necessary according to the treating physician, patient will go off protocol.

7.5 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. Such patients will be considered as lost for follow-up. The reason(s) for withdrawal must be obtained and documented, e.g. patient refusal, with medical reasons to be specified. The investigator can decide to withdraw a subject from the study for urgent medical reasons. After enrolment of a patient, the investigator may start treatment concomitant to that specified in the protocol if this is considered to be in the patient's best interest, but the reasons for doing so should be recorded. If such concomitant treatment does not conflict with the in-and exclusion criteria, the patient remains on study and will keep on receiving treatment according to the protocol.

7.6 Follow-up of withdrawn subjects

Any withdrawn patient will be followed up to obtain at least safety information (i.e. collection of adverse events). Every effort will be made to perform as much as possible the efficacy assessments as well.

7.7 Premature termination of the study

The investigator reserves the right to terminate the study for reasons of safety,

important ethical issues or severe non-compliance. Reason for premature termination of the study can be the occurrence of serious adverse events. In (prematurely) terminating the study, the sponsor and the investigator will ensure that adequate consideration is given to the protection of the best interests of the patients. This study may also be ended or suspended by competent authorities.

8.0 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 jeopardise the subjects' health. The investigator 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 this clinical trial, whether or not considered related to the intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded and will be reported to the principal investigator.

A serious adverse event is any untoward medical occurrence or effect that at any dose results in death;
is life threatening (at the time of the event);

- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- is a new event of the trial likely to affect the safety of the subjects, such as an unexpected outcome of an adverse reaction, major safety finding from a newly completed animal study, etc.

All SAEs will be reported within 24 hours to the principal investigator.

8.2.1 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 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, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

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 as indicated.

9.0 STATISTICAL EVALUATION

9.1 Planned sample size

At the department of hematology of the LUMC, about forty allogeneic stem cell transplantations are performed for malignant hematological diseases each year. Based on historic results, in about 10% of those transplantations the patient is HLA-A2 and HA-1 positive and the donor HLA-A2 positive and HA-1 negative. In a minority of patients an HA-1 specific CD8+ response is seen after DLI, so we expect to be able to start the vaccination program in three patients on a yearly base. If after twelve patients vaccinated with the 20mer, an immune response has been seen in less than 6 patients, the vaccination strategy will be changed and a combination of the 20mer and the 9mer will be used in another twelve patients. If the 20mer peptide-vaccination yields an immunological response in 6 or more patients, we consider this vaccination strategy as successful and will continue the vaccination program in twelve other patients, aiming for a total number of 24 patients.

9.2 Power calculation

As this is an exploratory study, no power calculations can be made.

10.0 ETHICAL CONSIDERATIONS

10.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki (version 2004) and in accordance with the Medical Research Involving Human Subjects Act (WMO)

10.2 Recruitment and consent

The treating haematologist will inform potential participants about this study and explain him or her about the informed consent procedure. All patients will be informed of the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she 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 is allowed to refuse further participation in the protocol whenever he/she wants. This will not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study. The patient is given the information letter. During a second visit, information will be repeated if necessary and participation to the study will be considered.

10.3 Benefits and risks assessment, group relatedness

Potential benefit of participation to this study is a changing chimerism status towards donor. This is associated to decreased risk of subsequent relapse risk and the need for a second DLI.

10.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor (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.

- € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
- € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
- € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation 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. As the development of GVHD is an anticipated effect of this vaccination study, the insurance does not apply for morbidity and mortality due to GVHD.

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11.0 ADMINISTRATIVE ASPECTS AND PUBLICATION

11.1 Handling and storage of data and documents

Patients will be included after verification of eligibility by the IKW Data Center. A list of questions to be answered during the inclusion procedure is included in the registration check-list. This check-list should be completed by the responsible investigator before the patient is included.
Standard questions:

- Eligibility criteria: all inclusion criteria will be checked
- Date of written informed consent

The patient's code will be entered in the CRF. Nine weeks after the inclusion, the treating hematologist will be contacted by the principal investigator in order to check eligability for vaccination. If the patient meets the criteria for vaccination, he or she will be vaccinated. A list of questions to be answered before vaccination is included in the registration check-list. This check-list should be completed by the responsible investigator before the patient receives vaccination

Questions to be asked before vaccination:

- Is the patient using systemic immunosuppressive treatment for GVHD (>20 mg corticosteroids a day)?
- Is the patient suffering progressive malignant disease needing cytoreductive treatment?
- Was a HA1 specific CD8+ T cell response found at eight weeks after DLI? (see Chapter Study design for definitions)
- Did bone marrow investigation at eight weeks after DLI show mixed chimerism or persisting disease?

The completed check-list must be signed by the responsible investigator and returned to the data center. The IKW Data Center will be responsible for handling and storage of all CRFs.

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 patient's last control visit.

In case the study is ended prematurely, the investigator will notify the CCMO, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor 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

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

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