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Phase I Study: Hespecta vaccination in HPV+ lesions

Study number: NL48274.000.14

Speetjens FM, *et al. J Immunother Cancer* 2022; 10:e005016. doi: 10.1136/jitc-2022-005016

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Protocol ID	HPV16HH01
Short title	Phase I Study: Hespecta vaccination in HPV+ lesions
ABR Dossiernummer	NL48274.000.14
EudraCT number	2014-000658-12
Version	3
Date	May 24, 2016
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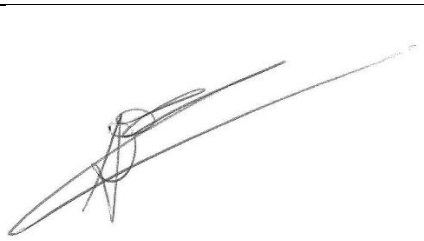
Principal Investigators: Prof. dr. H. Gelderblom		28 juni 2016

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SYNOPSIS

Rationale: Human papillomavirus (HPV) has been found to be associated with several types of premalignant lesions and cancer. HPV16 is the far most common HPV type detected in these tumors and premalignant lesions. HPV16 encodes the two tumor-specific oncoproteins E6 and E7. In most humans the virus is cleared. However, in some individuals, infection results in an uncontrolled persistent HPV16 infection, that due to expression of the viral oncoproteins E6 and E7 may lead to the formation of malignancies. Moreover, these oncoproteins maintain the malignant state of the transformed cells. The virus-specific interferon-γ (IFNγ)-producing CD4+ helper T cells (Th1 cells) and CD8+ cytotoxic T-lymphocytes (CTL) are able to recognize peptides processed from the highly immunogenic E6 and play a critical role in the elimination and/or control of the virus. Studies in patients with HPV associated tumors have shown that the spontaneous HPV-specific T-cell responses, are weak and fail to sufficiently control tumor outgrowth. Preexisting T-cell responses specifically directed against E6 and E7 in patients with HPV related tumors are associated with better outcome after treatment. Since the HPV16-transformed tumor cells constitutively express the two HPV16 encoded E6 and E7 oncoproteins, these viral antigens are considered to be excellent targets for immunotherapeutic vaccine strategies aiming at reinforcing the tumor-specific T-cell response. Previous vaccination studies showed that the use of our first generation HPV16 synthetic long peptides vaccine (HPV16-SLP) was safe and highly immunogenic in patients with HPV-induced anogenital lesions. Vaccination of patients with cervical cancer (CxCa) also resulted in the induction of HPV16-specific T-cell responses but the nature and strength of the induced T-cell responses was not sufficient for the regression of these tumors. Specifically, it was concluded that the polarization of the T-cell response to Th1 (IFNγ-response) was not optimal and a much stronger CD8+ T-cell response was required for clinical efficacy. These results initiated the development of new HPV16 vaccination strategies that are able to polarize the induced Th1 response and obtain strong CD8+ T-cell cytotoxicity. One of these developments consists of conjugating two of the HPV16 E6 SLP to Amplivant®, a synthetic Toll-like receptor (TLR) 2 ligand. These two peptides cover the most immunodominant regions of the overlapping HPV16-SLP set and contain both Th and CTL epitopes. Peptide-conjugated Amplivant® has been selected because it is acknowledged for its capacity to strongly enhance antigen presentation by dendritic cells (DCs), enhance T-cell priming and cause superior induction of effective anti-tumor CTL responses in mouse tumor models, compared to a mixture of free TLR ligand and peptide. In preclinical murine studies, Amplivant®-conjugated SLP showed 10 to 100 times higher bioactivity compared to unconjugated SLP, in terms of induced immune responses. In addition, the quantity and quality of human T-cell responses, and especially the HPV16-specific CD8+ T-cell response, in cancer patients could be markedly enhanced by ex vivo stimulation with Amplivant®-conjugated SLPs. Here we propose a phase I study to establish the biological activity using this highly promising novel therapeutic vaccine concept named: Hespecta (HPV E Six Peptide Conjugated To Amplivant®), to induce HPV16 E6-specific T-cell responses.

Objectives:

Primary objective: to determine the biological activity of Hespecta namely its ability to induce HPV16 E6-specific T-cell immunity within a proposed dose escalation of 1, 5, 20 or 50 µg/peptide - conjugate in patients with HPV16+ tumors or premalignant lesions.

Secondary objectives: to study safety of Hespecta by collecting all adverse events according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE).

Study design: This is a single center, translational dose escalation phase I trial.

Study population: 24 patients (≥ 18 years of age) screened after diagnosis and standard treatment for HPV16+ tumors or premalignant lesions..

Intervention: Patients first treated with curative intent for HPV16+ premalignant lesions or tumors will be vaccinated intradermally (i.d.) in four dose escalation groups (1, 5, 20 or 50 µg/peptide). Each dose group exists of 6 patients. The dose range is based on a 10-100 fold higher bioactivity of Amplivant® conjugated peptides in preclinical studies. The starting dose of this dose escalation trial is based on preclinical murine experiments showing that a dose of 1 µg per Amplivant® conjugated peptide resulted in no severe toxicity and a suboptimal immune response in that the magnitude of the immune response increased when mice were given a higher dose of vaccine. We reason that this provides the best scientific basis for the first dose to start with. As Amplivant® conjugated peptides are expected to induce T-cell responses more effectively compared to unconjugated peptides, the lowest dose known (50 µg per peptide) of unconjugated peptides able to induce T-cell responses is used as highest dose in this trial. From the starting dose an ascending dose range is proposed, evaluating four doses (1, 5, 20, 50 micrograms per peptide). Notably, the higher the dose the smaller the dose-fold increase between the doses (5, 4, 2.5 times), this should allow for maximal safety. Patients will be vaccinated three times with an interval of three weeks with a fixed dose of Hespecta (i.e. no escalating dose within the patient). Vaccination will start with the i.d. injection of the lowest dose. The decision to start enrolment at the next dose level will be made by assessing the safety after 4 out of 6 patients at the previous dose level have completed the first follow up visit after the third vaccination. The Principal Investigator (PI) will employ listings of individual patient data related to baseline characteristics, safety, and study medication administration for the patients necessary to assess per above before decision to start enrolment at the next dose level. The PI will assess information per above for relevant patients, summarize findings, and give a recommendation whether or not he endorses the start of enrolment at the next dose level. The summary and recommendation from the PI, including all necessary individual patient background material, should be reviewed and commented on by the Independent Data Monitoring Committee (IDMC) members. After having received the opinion of the IDMC, the PI can issue a written approval (or disapproval) regarding the start of enrolment at the next dose level. If in two or more patients, grade 3 or 4 vaccine related toxicity occurs the dose escalation phase will be discontinued. If in the lowest dose group two patients experience grade 3 or 4 vaccine related toxicity, there is no safe dose and the study will be discontinued.

Main study parameters/endpoints: Primary objective is to determine the biological activity of Hespecta that is able to induce HPV16 E6-specific T-cell mediated immunity within a proposed dose escalation of 1, 5, 20 or 50 µg/peptide-conjugate in patients with HPV16+ tumors or premalignant lesions. Blood samples will be drawn and used in an array of complementary immunological assays (HPV16-specific proliferation assay, IFNγ-ELISPOT, cytokine bead array and multiparameter flow

cytometry analysis) to assess the biological activity of Hespecta. These established assays are performed at 4 different time points (baseline and 3 time points after start of vaccination). Vaccine-induced immunity in the different assays is defined as a post-vaccination response that is at least 3 times higher than the pre-vaccination response. Biological activity is identified if it is seen in at least 2 assays at 2 consecutive follow-up time points after the baseline assessment, in line with CVCTWG criteria. All adverse events (AE) will be collected according to CTCAE v4.0.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Patients will visit the clinic during the trial six or even additional times (screening visit, three vaccination visits, and three follow up visits (if no abnormalities are found during the first follow up visit, the second follow visit may take place by telephone,).These follow up visits are combined with regular treatment follow up visits. Potential risks with the current trial are mainly linked to the toxicity related to the treatment compounds utilized in Hespecta. Although the rabbit toxicity testing of Hespecta (see IMPD brochure) did not show any severe side effects, it is expected from previous results with HPV16-SLP, that the most important AEs of Hespecta may consist of fever, chills, nausea, malaise, fatigue and local reactions at the vaccination site including pain, redness, swelling and itching. Generally the AEs after vaccination with HPV16-SLP did not exceed grade 2 according to the CTCAE criteria. Almost all patients, vaccinated with HPV16-SLP formulated in Montanide ISA51 experienced local injection site reactions (grade 2). After vaccination, ulceration/abscess formation occasionally occurred, estimated to be in between 1 and 5% of vaccinations, due to the use of this Montanide as adjuvant. The current vaccine is administered without Montanide, and therefore, it is expected that these local reaction will be less severe. Allergic reactions have occurred in a few patients after HPV16-SLP vaccinations, which could be controlled with antihistamines. Vaccinations should therefore only be administered in a clinic where immediate treatment of severe allergic reactions is possible. Thus, after vaccinations, patients will be closely monitored during one hour after injection to provide means for immediate treatment of allergic reactions. In addition to the standard of care, the patients participating in the trial will receive three vaccinations with Hespecta for the induction of a strong and broad CTL response against HPV16 E6. Study patients will not be paid for their participation however any study-related travel expenses will be reimbursed.

1. INTRODUCTION AND RATIONALE

1.1. HPV16-induced tumors and premalignant lesions

Human papillomavirus (HPV) is one of the most common sexually transmitted viruses worldwide. It is known that 80% of the population in his/her life is infected with HPV [1]. More than 150 different HPV types are known [2]. These give rise to an array of cutaneous or mucosal epithelial lesions, mostly benign hyperplasia such as warts or papillomas. Some types have a causal role in precancerous lesions or tumors of the cervix, vulva, vagina, penis, anus and areas of the head and neck. HPV16 is the most common HPV type found in the six cancers associated with HPV.HPV16 encodes the two tumor-specific oncoproteins E6 and E7. The E6 oncoprotein binds and induces the degradation of the p53 tumor suppressor protein via an ubiquitin-mediated process disrupting the p53 pathway, while E7 protein binds and degrades the retinoblastoma protein. Their combined action leads to uncontrolled cell cycle progression [3,4]. The HPV16 E6 and E7 proteins are causally involved in cancer formation and its maintenance, and accordingly are constitutively expressed in all HPV-transformed tumor cells. Hence they are attractive tumor-specific targets for T-cell based immunotherapy [5].

1.2. Immunity to HPV

Only a small percentage of HPV16 infected individuals develops high-grade squamous intraepithelial lesions whereas, in the remainder, the virus disappears spontaneously. There is much evidence to support the view that host-dependent immunologic status and HPV induced immune evasion are responsible for persistent HPV infection and subsequent development of neoplasia. Therefore, the role of the immune system, not only in viral clearance but also in tumor antigen recognition, is particularly relevant in the case of HPV induced carcinogenesis [6]. Comprehensive studies of HPV16-specific immunity in protected individuals have revealed the presence of circulating HPV16-specific CD4+ T helper (Th) cells and CD8+ cytotoxic T lymphocytes (CTLs). These cells are directed against the viral E6 and E7 oncoproteins and migrate to areas where viral antigen is presented. In general, HPV-specific CD4+ T-cell immunity comprises both Th type 1 (Th1; i.e.IFNγ-producing) and Th2 (IL-5-producing) T cells that are reactive to a broad array of epitopes within these antigens [7,8,9,10]. In individuals in whom spontaneous regression of HPV-induced genital lesions occurs, this is coincident with the infiltration of CD8+ CTLs and CD4+ Th cells into the lesions [11,12,13]. Spontaneous regression is also associated with the presence of circulating CD4+ and CD8+ T cells specific for these HPV early antigens [14]. Remarkably, when patients with HPV-induced high-grade dysplasia spontaneously mount an HPV-specific Th1 response, these individuals display a better clinical outcome, and when treated with immune stimulators, such as the toll like-receptor 7-agonist Imiquimod, may display complete regression of disease [15,16]. In addition, when such patients develop a strong and broad HPV-specific IFNγ-producing CD4+ and CD8+ T-cell response after vaccination, this is associated with complete regression of the lesions [17,18,19].

Tumors can be infiltrated by different immune cells, including CD4+ and CD8+ T cells. A dense infiltration with CD8+ T cells and not the suppressive regulatory T cells (Tregs) is correlated to better clinical outcome for patients with head and neck cancer as well as cervical cancer [20,21,22,23,24,25].

Notably, for head and neck cancer, the HPV+ tumors displayed a more intense immune infiltration than their HPV-negative counterparts, and this is related to better clinical responses to standard therapy [26,27,28]. Our own studies showed that the majority of tumor infiltrating lymphocytes (TIL) obtained from HPV16+ tumors comprised a broad repertoire of CD4+ and CD8+ T cells reactive to HPV16[29,30]. More recently, we were able to show that TIL from HPV16+ head and neck tumors often responded directly ex-vivo to HPV16 E6 and/or E7 antigens, a sign that they were already active in vivo and may mediate an antitumor effect. There are strong indications that presence of HPV-specific T cells is associated with better clinical outcome for patients with HPV+ tumors.

In conclusion, it is thought that the development of HPV-induced tumors is associated with lack of effective HPV-specific immunity prohibiting clearance of HPV-infected cells. Presence of HPV-specific immunity, although often weak in patients with HPV+tumors, is associated with better survival after treatment compared to patients that lack HPV-specific immunity. Therefore, this indicates that reinforcement of HPV-specific T-cell immunity may improve clinical outcome of patients with HPV16+ tumors and premalignant lesions.

1.3. Vaccine development

The CxCa registered preventive vaccines (Cervarix®, Gardasil®) against HPV16/18 act by the induction of antibodies against the viral L1 protein, which is the outer (envelop) protein of HPV16/18. The L1 protein is not expressed in de basal squamous epithelial cells, in which the virus persists after infection. Therefore, anti-L1 antibodies are totally ineffective to combat the infection, once a person has become infected [31]. HPV16-infected basal squamous epithelial cells, however, do express intracellularly the early viral proteins of HPV16, including the two oncogenic proteins E6 and E7. HPV E6/E7 peptides are presented in the context of human leukocyte antigen (HLA) class I and class II molecules at the cell surface. Because the HPV E6/E7 proteins only occur intracellularly and not at the cell surface of infected cells, only T cell-mediated immunity can efficiently attack these cells. As shown above, patients with HPV-induced disease usually show weak HPV-specific immune responses. Appropriate vaccination may overcome this deficit. In the Leiden University Medical Center (LUMC) a HPV16 synthetic long peptide vaccine (HPV16-SLP) was developed. The HPV16-SLP vaccine formulated in Montanide ISA51 (Seppic, France) was shown to be safe and highly immunogenic in patients with HPV-induced anogenital lesions. In patients with high grade vulvar intraepithelial neoplasia (VIN3), a precursor of vulvar cancer, vaccination was followed by complete and partial regressions [17,32], indicating that the vaccine was clinically active. Vaccination of patients with CxCa also resulted in the induction of HPV16-specific T-cell responses but the nature and strength of the induced T-cell responses was not of the same quality as in patients with VIN3, and vaccination alone was not sufficient for the regression of these tumors [33,34]. Specifically, it was concluded that the polarization of the T-cell response to Th1 (IFNγ-response) was suboptimal and a much stronger CD8+ T cell response was required. Additionally, alternatives for Montanide were preferred as the use of Montanide was occasionally associated with late (beyond 1 year) severe vaccine site reactions. Together, this supports the further development and clinical testing of new generation therapeutic HPV16 vaccines. One of these developments consists of conjugating HPV16 E6 SLP to a Toll-like

receptor (TLR) ligand, to improve more effective presentation of HLA-class I-restricted CD8+ T-cell epitopes by professional antigen presenting cells (APC).

1.4. Toll-like receptors

To achieve a state of effective antitumor immunity, a triad of immune-related hallmarks can be defined. First, a therapy should be targeted towards the professional APC, the dendritic cell (DC). This goal can be met by making use of the broad array of receptors that DCs express. At the same time, a DC should be matured in such a way that a process is set into action that will ultimately lead to presentation of tumor antigens in their HLA class I or II molecules, combined with the up-regulation of co-stimulatory molecules and the production of pro-inflammatory cytokines (e.g. IL-12). Third, the antigen that is delivered to the DC should be highly tumor specific in order to achieve antitumor immunity and evade tolerance induction. These three hallmarks can also potentially be combined in one molecule. Conjugating antigen to a targeting molecule, such as a TLR ligand, enables the efficient delivery of tumor-derived antigen to DCs while maturing these DCs to induce (cross-) presentation to tumor-specific Th cells and CTLs.

TLR are sensing receptors expressed mainly on cells of the innate immune system. These receptors recognize pathogen-associated molecules, have been proven to be important in detection of infectious agents and act as a communicator between innate and adaptive immune responses. To date, 13 mammalian TLRs have been identified, most of which have been studied in detail and have had specific ligands identified. The ligands of the different receptors are small, usually repetitive, specific molecules derived from microorganisms. These can be cell wall components (like LPS or Pam3CysSK4, binding to TLR4 or TLR2, respectively) or intracellular molecules (like bacterial DNA that activates TLR9 or RNA that binds to TLR7 and TLR3). The receptors are expressed either on the cell surface or in the endosomal organelles. This compartmentalization of the individual TLR correlates with the type of ligands with which they interact. The TLR ligands are generally small well-defined molecules which can be synthetically produced under GMP conditions in quantities and purity suited for clinical studies. Based on their potent biological activity TLR ligands can be regarded as modern, molecularly well-defined adjuvants for enhancing immunity against cancer.

1.5. TLR ligand-peptide conjugates

A number of groups have shown that the conjugation of antigens to different types of TLR targeting ligands resulted in therapeutically effective immune responses against viruses, bacteria and tumors (reviewed in [35]). The TLR9 ligand CpG is frequently used in these models but is less suitable for human application since TLR9 expression is limited to the plasmacytoid subset of DCs in humans. The use of lipopeptides for effective peptide vaccination of humans has already been shown in the late eighties [36]. More recently it became clear that these lipopeptides were actually ligands for TLR2, a receptor that is expressed on all DC subtypes.

By comparing several different TLR2 ligands for optimal activation of human DCs, we selected Pam3CysSK4 for the proposed clinical study. Pam3CysSK4 is generally a relatively weak TLR ligand but it has excellent targeting properties to DCs. Based on the published structure of the TLR2/TLR1

heterodimer co-crystalized with its Pam3CysSK4 ligand [37] we have slightly modified this synthetic TLR ligand. In one of the fatty acid chains a carbon atom was changed for nitrogen leading to a potential hydrogen bond between this residue and the TLR pocket increasing its affinity. This novel ligand is recently patented and is now registered as “Amplivant®” by the LUMC spin-off company ISA pharmaceuticals. Indeed, we could functionally show improved activity of Amplivant® in DCs and human TLR2 reporter cells compared to native Pam3CysSK4. This significant activity improvement by a minimal molecular change of the relative weak Pam3CysSK4, with retained DC targeting capacity, favored the Amplivant® ligand for clinical application.

We showed that a SLP conjugated to Amplivant® induces DC maturation to the same extent as the free TLR ligand, proving that the maturating potential of Amplivant® is not affected by conjugation to a peptide. Subsequently, peptide was taken up more rapidly in DCs when conjugated to Amplivant®, both in vitro and in vivo. Besides, conjugation of peptide to Amplivant® greatly enhanced in vitro antigen presentation compared to a mixture of free Amplivant® and peptide. Moreover, the peptide conjugate enhanced T-cell priming in vivo. Investigation of antigen-processing routes in mice showed that major histocompatibility (MHC) class I presentation was dependent on endosomal acidification, proteasomal cleavage, and Transporter associated with antigen processing translocation. The uptake of peptide conjugated to Amplivant® was independent of expression of the TLR. However, TLR expression and downstream TLR signalling were shown to be essential for DC maturation and CD8+ T-cell priming. The uptake of the Amplivant®-peptide conjugate was dependent on clathrin-coated pits and caveolae formation [38]. Targeting DCs with a Amplivant®–peptide conjugate was shown to lead to the formation of an intracellular antigen depot. This depot enables prolonged antigen presentation and subsequent T-cell priming, which was not observed when DCs were pulsed with the peptide only (short OVA-derived SIINFEKL peptide) [39]. One of the reasons for DCs to require sustained peptide presentation for several days is the time needed to migrate from the infection site to draining lymphoid organs. Indeed, 2 weeks after subcutaneous vaccination of TLR2-ligand-peptide conjugates, a very effective CTL priming was observed, induced with 50- to 100-fold lower doses of conjugate than with mixtures of peptide with free TLR ligand [40]. Therefore, next to DC maturation signals, proper antigen targeting and handling by DC are essential for optimal vaccine formulations. This improved TLR ligand, Amplivant® has been conjugated to two HPV16 E6 SLP, thereby creating the therapeutic vaccine used in the current study protocol.

1.6. Selection of two antigenic E6 synthetic long peptides.

Previous clinical vaccination trials in patients with HPV16-induced lesions were performed with a mix of 13 overlapping SLP derived from HPV16 E6 and E7 oncoprotein sequences. We have selected the most immunodominant regions of the overlapping SLP set, based on extensive immunomonitoring studies using circulating lymphocytes obtained from healthy donors [10] to determine the naturally occurring HPV16 responses and from patients in the various clinical vaccination trials i.e. patients with end stage CxCa [34,41], with VIN lesions [17,18], with CIN lesions [42] as well as of tumor infiltrating lymphocytes (TILs) in patients with HPV16+ CxCa and OSCC [30,43,44]. Two SLP sequences were selected from the E6 protein namely E6 71-95 and E6 127-158 for which we found detectable T-cell

responses in 40-60% of the vaccinated patients and healthy donors. Both Th and CTL responses were found against these two peptide sequences. In addition, we used internet-based epitope prediction databases to screen these two sequences for potential T-cell epitopes presented in the various HLA molecules. The IEDB and SYFPEITHI databases were used to predict epitopes in peptides E6 71-95 and 127-158 for HLA class I and II alleles. In the table below the numbers of predicted HLA class I-binding and class II-binding epitopes are listed for the most frequently occurring HLA-alleles in the human population. Based on these findings we can expect that at least 50 - 75% of the patients that will be injected with a combined mixture of these two antigens will respond to vaccination with an HPV16-directed T-cell response.

	E6 71-95	E6 127-158
HLA-A*01	4	1
HLA-A*02	3	2
HLA-A*03	3	4
HLA-A*11	3	4
HLA-A*2402	8	2
HLA-A*26	4	0
HLA-B*0702	3	2
HLA-B*0801	2	4
HLA-B*15	2	0
HLA-B*51	2	1
7 HLA-class II alleles	12	13

1.7. Toxicity study

Both selected E6 SLP were conjugated with the above mentioned Amplivant® TLR2 ligand under GMP conditions. These conjugates have been tested in rabbits for general toxicity in same dosing groups as planned in this clinical study. The results of these experiments have been described in the investigator brochure (IB). No toxicity was observed.

1.8. Dendritic cell maturation by the two clinical conjugates

The Amplivant® as TLR-ligand in the two conjugated E6 SLP was able to induce maturation (activation) of both murine and human monocyte-derived DCs (moDCs). In both cases the DCs specifically produced the essential T-cell activating cytokine interleukin 12 (IL-12) upon stimulation with the conjugates. Also, essential co-stimulatory markers such as CD86, CD80 and CD83, as well as MHC class II molecules, were up-regulated on the cell surface of stimulated DCs. On the other hand, production of the anti-inflammatory cytokine IL-10 was almost undetectable upon stimulation.

Peripheral blood mononuclear cells (PBMC) were also stimulated with the conjugates and the pro-inflammatory cytokine IL-1β could be measured in response to stimulation.

1.9. Human T-cell activation

Human HPV16-specific CD4+ or CD8+ T-cell clones, isolated from the TIL populations of CxCa patients, who were capable of spontaneously mounting a HPV16-specific T-cell response and of which it was known that they recognized an epitope within the two Amplivant®-conjugated SLPs, were used to analyze the quality of the conjugates. After exposure to conjugate-loaded DCs, both HPV16-specific CD4+ and CD8+ T-cell clones from these patients strongly proliferate and produce IFNγ, showing that the conjugated SLPs are taken up by the DCs and efficiently processed and presented in both HLA class I and II. The CD8+ T-cell clone proliferated to a much greater extent in response to conjugate-loaded DCs compared to SLP-loaded DCs or DCs that were incubated with a mixture of TLR-ligand and SLP. Approximately 100-fold lower concentrations of conjugate were sufficient for the same level of CD8+ T-cell activation than free TLR-ligand mixed with SLP. The tested CD4+ T-cell clones proliferated to a similar extent to conjugate-loaded DCs as to SLP- or mixture-loaded DCs. These data show that the use of these conjugates has great advantages for stimulating CD8+ T-cell responses to the HPV16 E6 epitopes in the conjugates, whereas the conjugates have no disadvantages with respect to induction of CD4+ T-cell responses to epitopes in the conjugates. In addition, we determined whether the two clinical conjugates are effective in the stimulation of cancer patient-derived T-cells. Upon stimulation of freshly isolated TILs with the conjugated SLP, CD4+ TILs produced significantly more IL-2 than TILs stimulated with unconjugated SLP. In addition, CD4+ T-cells from a lymph node of a cervical cancer patient strongly produced both IFNγ and IL-2 and displayed a more activated phenotype in response to stimulation with conjugated SLP. Taken together, these data indicate that the quantity and quality of T-cell responses, and especially the HPV16-specific CD8+ T-cell response, in cancer patients can be markedly enhanced by ex vivo stimulation with Amplivant®-conjugated SLPs.

1.10. Intradermal vaccination

The vaccine will be injected i.d., thereby targeting the vaccine to a dense network of cutaneous DCs, among others Langerhans cells and dermal DC. Intradermal vaccination strategies for vaccination have been tested in many cancers [45,46,47] and have met with some clinical benefit [48,49]. It was shown before that an i.d. challenge with the HPV16 SLP injected at a dose of 10 μg per peptide in healthy subjects is safe and results in the induction of an HPV16-specific T-cell response in at least 13 out of 19 injected subjects as reflected by the appearance of skin reactions, the migration of HPV16-specific T-cells into the skin and/or an increase in the number of circulating HPV16-specific CD4+ and CD8+ T cells [9]. The 10 μg dose of non-conjugated SLP was also i.d. injected in CxCa patients but failed to induce a response [24], suggesting that the dose of the conjugated peptide should at least be tested at a higher dose. Together, this shows that already at low concentrations of antigen an i.d. vaccination can induce HPV16-specific T-cell responses in comparison to higher doses used for subcutaneous (s.c.) injection. The dose needed to establish a strong T-cell response in HPV16+

cancer patients by reactions is unknown and therefore a dose finding study will be needed. The use of Amplivant®-conjugated SLPs will assumingly induce proper Th1/CTL polarization. I.d. vaccination instead of s.c. vaccination is warranted to allow omission of the Montanide oil-depot used so far for s.c. vaccine injection, because of the occasionally late (1 year) vaccine site reactions [42] and discomfort.

1.11. Summary

Based on the literature data and our own experience we propose a phase I clinical study to determine the biological activity of a vaccine within the predefined dose range that is able to induce HPV16 E6-specific T-cell mediated immunity. In addition this study will report on the safety of this second generation HPV16 vaccine consisting of selected two long E6-derived peptides covering multiple potential CTL and Th epitopes both coupled to a TLR ligand in a group of patients screened after diagnosis and standard treatment for HPV16+ induced tumors and premalignant lesions..

2. OBJECTIVES

Primary objective:

- To determine the biological activity of Hespecta that is able to induce HPV16 E6-specific T-cell immunity within a proposed dose escalation of 1, 5, 20 or 50 µg/peptide - conjugate in patients with HPV16+ tumors or premalignant lesions.

Secondary objective:

- To study safety of Hespecta by collecting all AE's according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE)

3. STUDY DESIGN

This single center phase I trial will determine the biological activity of Hespecta within the proposed dose range that induces HPV16 E6-specific immunity. In addition, this study will evaluate toxicity of vaccination with Hespecta. This translational dose escalation phase I trial will be performed in 4 dose groups (1, 5, 20 or 50 µg/peptide), each group consisting of 6 patients (see paragraph 4.4). Patients will receive i.d. injections three times with a fixed dose of Hespecta at an interval of every three weeks. Patients diagnosed with HPV16+ (pre-)malignant lesions will be informed of this study. They will be included at least one month after the last standard treatment (for details see paragraph 4). Patients will be recruited at the LUMC, including referrals from other hospitals. The HPV type will be determined in tumor tissue by the department of pathology using standard operating procedures (SOPs). After obtaining informed consent, patients will be screened for eligibility in the study. Safety will be assessed during the study by collecting all AE according to the CTCAE version 4 (see paragraph 7.1). Venous blood samples at different time points will be obtained for immunomonitoring and clinical parameters for safety establishment. Immunological responses will be determined in isolated PBMCs of all vaccinated patients at the different time points (for details see paragraph 7). The decision to start enrolment at the next dose level will be made by assessing the safety after 4 out of 6 patients at the previous dose level have completed the first follow up visit after the third vaccination. The Principal Investigator (PI) will employ listings of individual patient data related to baseline characteristics, safety, and study medication administration for the patients necessary to assess per above before decision to start enrolment at the next dose level. The PI will assess information per above for relevant patients, summarize findings, and give a recommendation whether or not he endorses the start of enrolment at the next dose level. The summary and recommendation from the PI, including all necessary individual patient background material, should be reviewed and commented on by the Independent Data Monitoring Committee (IDMC) members. After having received the opinion of the IDMC, the PI can issue a written approval (or disapproval) regarding the start of enrolment at the next dose level. If in two or more patients CTCAE grade 3 or 4 treatment related toxicity occurs the dose escalation phase will be discontinued. If in the lowest dose group two patients experience CTCAE grade 3 or 4 treatment related toxicity, there is no safe dose and the study will be discontinued.

4. STUDY POPULATION

4.1. Population (base)

Only in a minority of persons, HPV16 infection persists and subsequently (pre)malignant lesions develop. It is unknown in which persons the virus will be eradicated and in whom it will persist [6]. It is generally thought that immune related factors determine which persons clear and do not clear HPV16 infections [6,50]. As the targeted population of this therapeutic vaccine consists of patients with HPV16+ tumors or premalignant lesions, this vaccine should be tested in patients whose primary HPV infection has not been cleared. Previous experience showed that the vaccine-induced immune response differs between patients with pre-malignant lesions who have cleared their lesions [17,18,32] and patients with HPV16+ recurrent or metastatic cervical cancer. The latter patients showed much weaker vaccine-induced immune responses and in contrast to VIN patients with robust vaccine-induced immune responses did not show clinical benefit [41]. They showed not only a weaker immune response than successfully vaccinated VIN patients, but also to patients with high-grade premalignant lesions who have been successfully vaccinated. Notably, similar spontaneous responses against the HPV16 oncoproteins were observed in healthy individuals who have successfully cleared the virus [10]. Patients with premalignant lesions (CIN1-3, VIN3) had more side effects after vaccination with the first generation of Montanide emulsion SLP vaccine which emphasizes the need for new vaccine formulation [51]. As it is not possible to distinguish healthy individuals who are able to eliminate HPV16 from those who are not, it has been decided to perform this study in patients who are candidate for the improved second generation Hespecta vaccine, i.e. either suffering from a HPV16+ premalignant lesion or a non-metastatic HPV16+ malignancy for which they have received the standard (curative intent) treatment. Premalignant lesions associated with HPV16 consist of vulval (VIN), cervical (CIN), anal (AIN) and Vaginal intraepithelial neoplasia (VAIN). HPV16+ malignant tumors can arise in the cervix, vulva, vagina, penis, anus and areas of the head and neck. A total of 24 eligible adult males or females in good clinical condition will be included in this trial.

4.2. Inclusion criteria

Patients must meet all the following criteria in order to be included in the study:

1. Histological or cytological documented evidence of HPV16 positive (pre)malignant lesion following standard treatment
2. Patients with a tumor should have no evidence of residual disease based on physical examination at the completion of curative intent therapy
3. At least four weeks and less than sixteen weeks after last anti-tumor treatment
4. Willing and able to comply with the protocol and to provide informed consent in accordance with institutional and regulatory guidelines
5. Patients must be 18 years or older

6. Patients of child-bearing potential should test negative using a serum pregnancy test and agree to utilize effective contraception during the entire treatment and follow-up period of the study (up to 2 months after the last vaccination)
7. Patients must be in good general health and ambulatory, with an ECOG performance status of 0 or 1 (appendix 1)

4.3. Exclusion criteria

Patients who meet the following exclusion criteria will not be eligible for admission to the study:

1. Radiotherapy, chemotherapy or other potentially immunosuppressive therapy administered within 4 weeks prior to the enrolment visit
2. History of an autoimmune disease or other systemic intercurrent disease that might affect the immunocompetence of the patient, or patients receiving immunosuppressive therapy, except for topical application
3. History of a second malignancy except curatively treated low-stage tumors with a histology that can be differentiated from the current tumor or premalignant lesion
4. Receipt of another investigational product within the previous 4 weeks or at any time during the study period
5. Receipt of prior HPV directed immunotherapy
6. Hematology and biochemistry:
   - Absolute Neutrophil Count (ANC) < 1.5 x 10^9/L, or platelet count < 100 x 10^9/L or hemoglobin < 6 mmol/L.
   - Serum (total) bilirubin > 2 x upper normal limit (ULN);
   - Aspartate Aminotransferase (ASAT) or Alanine Aminotransferase (ALAT) > 2.5 x ULN;
   - Alkaline phosphatase levels > 2.5 x ULN;
   - Serum creatinine > ULN or calculated clearance </= 40 mL/min/1.73 m^2 for patients with serum creatinine levels above the institutional normal value
7. Human immunodeficiency virus (HIV), chronic hepatitis B or C infection.
8. Any condition that in the opinion of the investigator could interfere with the conduct of the study

4.4. Sample size calculation

According to the clinical development paradigm for cancer vaccines as set by the Cancer Vaccine Clinical Trial Working Group (CVCTWG) [52] the current trial satisfies the criteria for being a proof-of-principle trial. The dose and schedule are investigated in proof of- principle trials, however, a definitive answer on the optimal setting for further trials should not be expected from a first-in-human study. For cancer vaccines, there may not be any linear association between dose, immunogenicity and clinical end points, therefore, the vaccine acute safety profile is not dose dependent. It is expected that the vaccine produces the desired biological effect across a broad-dose range. Primary end point used in this study is to determine biological activity i.e. T-cell immune response to the vaccine. Four different assays are used to evaluate the immunogenicity of Hespecta. These established complementary assays are conducted with blood samples taken at 4 time points (baseline and 3 follow-up time points after vaccine administration) , and are fully described in standard operating procedures (SOPs), including predefined criteria for positive and vaccine-induced response, and have been used and validated in a number of vaccine trials as published by our laboratory [17,32,33]. An immune response is identified if it was seen in at least 2 assays at 2 consecutive follow-up time points after the baseline assessment, in line with CVCTWG criteria. For proof-of-principle trials CVCTWG suggests a cohort size of at least 6 patients per dose [52]. As the CVCTWG already stated, the body of literature for optimizing the size of conventional phase 1 trials has little applicability to designing a proof-of-principle cohort trial because of the differences in the types of outcome measures and objectives. Here a cohort size of 6 patients per dose is used to determine safety and immunogenicity of Hespecta.

5. TREATMENT OF SUBJECTS

5.1. Investigational product/treatment

The vaccine consists of the Amplivant® TLR-ligand conjugated to 1) the HPV16 E6 71-95 peptide and 2) the HPV16 E6 127-158 peptide, each harboring multiple potential Th and CTL epitopes (see paragraph 1.6). The design of these long peptides was based on high immunogenicity in patients vaccinated with the HPV16-SLP and healthy donor who spontaneously responded to these peptides. Amplivant® was chosen for bioactivity (see paragraph 1.5) on human DCs and feasibility concerning GMP production (see IMPD)

5.2. Use of co-intervention (if applicable)

The effect of the vaccine could be modified by immunosuppressive therapy, which is therefore an exclusion criterion (see section 4.3). Other therapeutic options like surgery or chemotherapeutic treatment should be avoided. Patients must agree to utilize effective contraception (female of child-bearing potential only) during the entire treatment period of the study and during follow-up.

5.3. Escape medication (if applicable)

No specific antidotes for the Hespecta vaccine are available. Appropriate drugs and medical equipment to treat acute anaphylactic reactions should be available, and study personnel will be trained to recognise and treat anaphylaxis. Supportive measures will be used as indicated in case of AEs. When side-effects from the vaccination occur it is allowed to use paracetamol for pain relief of the local injection site, or anti-histamines when an allergic reaction occurs at the site of vaccination to alleviate the complaints.

6. INVESTIGATIONAL PRODUCT

6.1. Name and description of investigational product(s)

Hespecta, consisting of two HPV16 E6 synthetic peptides (E6 71-95 and E6 127-158) conjugated to the Amplivant® TLR-ligand, is produced in the Interdivisional GMP-Facility LUMC (IGFL) of the LUMC Department of Clinical Pharmacy and Toxicology. Technical details regarding the Investigational Medicinal Product (IMP) or the production process are listed in the IMP Dossier (IMPD) that accompanies this protocol. Immunological and preclinical data concerning the IMP are described in the Investigator's Brochure (IB) accompanying this protocol.

6.2. Description and justification of route of administration and dosage

Two HPV16 E6 peptides conjugated to Amplivant® will be reconstitued in DMSO / Water For Injections (WFI) 20/80 v/v in a total volume of 0.10 mL. The peptides are dissolved in this volume at a dose of 1 µg per peptide (first group), of 5 µg per peptide (second group), 20 µg per peptide (third group) or 50 µg per peptide (fourth group). The dose needed to establish a strong T-cell response in HPV16+ cancer patients by i.d. injection is unknown. A dose escalation of 1, 5, 20 or 50 µg per Amplivant® conjugated peptide (respectively 100 times to 10 times lower doses than unconjugated SLP) was chosen.

The dose range is based on a 10-100 fold higher bioactivity of Amplivant® conjugated peptides in preclinical studies [40]. The starting dose of this dose escalation trial is based on preclinical murine experiments showing that a dose of 1 µg per Amplivant conjugated peptides resulted in no severe toxicity and a suboptimal immune response in that the magnitude of the immune response increased when mice were given a higher dose of vaccine. We reason that this provides the best scientific basis for the first dose to start with. Fifty µg of unconjugated peptides dissolved in Montanide ISA51 and injected s.c. induced a strong T-cell response in CxCa patients as well as in CIN patients [33,51]. As Amplivant® conjugated peptides are expected to induce T-cell responses more effectively compared to unconjugated peptides, the lowest dose known (50 µg per peptide) of unconjugated peptides able to induce T-cell responses is used as highest dose in this trial. From the starting dose of 1 µg per peptide an ascending dose range is proposed, evaluating four doses (1, 5, 20, 50 micrograms per peptide). Notably, the higher the dose the smaller the dose-fold increase between the doses (5, 4, 2.5 times), this should allow for maximal safety. Vaccination will be carried out three times with three weeks intervals, at least one month after the last conventional treatment. The vaccine will be injected i.d. at alternating sites of the thigh or the upper arm. The number of vaccinations and spacing of vaccine doses with intervals of three weeks is based on our previous immunotherapy trials with HPV16-SLP. We have chosen to inject i.d. because the vaccine targets to a dense network of cutaneous DCs. This i.d. injection instead of s.c. vaccination is warranted to get rid of the oil-depot with Montanide adjuvant used for s.c. injection, as this can occasionally be associated with late (1 year) severe vaccine site reactions [42]. GMP produced clinical grade Amplivant®-conjugated HPV16E6-SLP were used to pre-

clinically test the potential toxicity of this new generation vaccine in animals. No mortality occurred and no adverse systemic effects were observed at 5 or 60 µg and was very well tolerated in New Zealand White rabbits, as described in detail in de IB.

6.3. Preparation and labeling of Investigational Medicinal Product

Just before administration, the vaccine is reconstituted, prepared with excipients, and labelled for each individual subject, according to standard procedures at the Department of Clinical Pharmacy and Toxicology of the LUMC. The reconstituted and ready-to-administer vaccine contains both HPV-Amplivant® peptides dissolved in dimethylsulfoxide/water for injection 20/80 v/v. One dose for i.d. administration contains 1, 5 20 or 50 µg of each peptide in a total volume of 0.10 mL.

6.4. Drug accountability

Drug accountability will be recorded by the responsible staff members of the Department of Clinical Pharmacy and Toxicology of the LUMC according to standard procedures.

7. METHODS

7.1. Main study parameter/endpoint

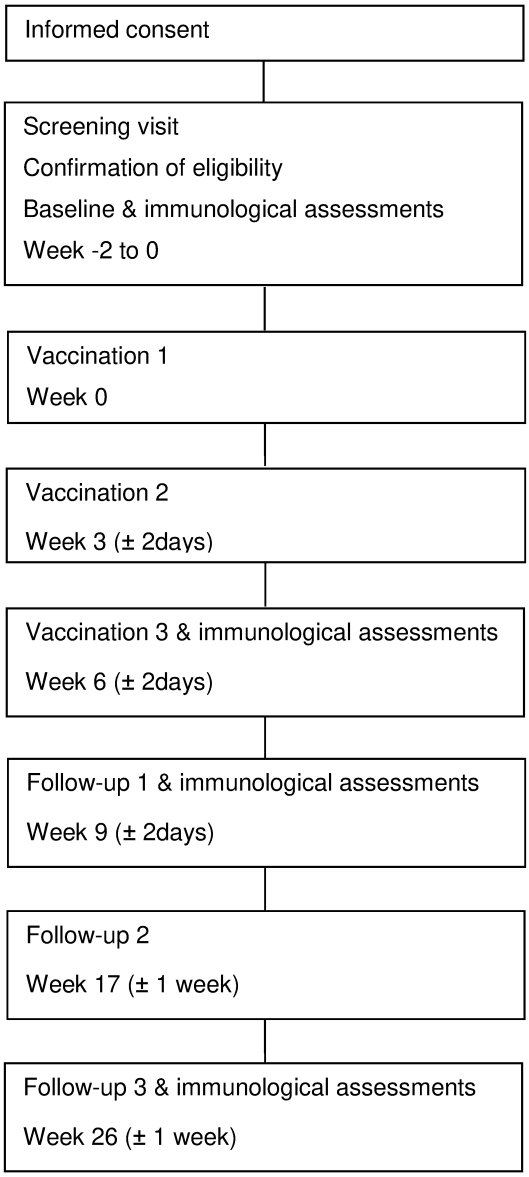
Primary objective is to determine the biological activity of Hespecta that is able to induce HPV16 E6-specific T-cell immunity within a proposed dose escalation of 1, 5, 20 or 50 µg/peptide - conjugate in patients with HPV16+ tumors or premalignant lesions. Blood samples will be drawn and used in an array of complementary immunological assays (HPV16-specific proliferation assay, IFNγ-ELISPOT, cytokine bead array and multiparameter flow cytometry analysis) to assess the biological activity of Hespecta. These established assays are performed at 4 different time points (baseline and 3 time points after start of vaccination). Vaccine induced immunity in the different assays is defined if the response after vaccination is at least 3-fold higher than the pre-vaccination response. Biological activity is identified if it was seen in at least 2 assays at 2 consecutive follow-up time points after the baseline assessment, in line with CVCTWG criteria.

7.2. Secondary study parameters/endpoints

Safety will be determined by the incidence rate at each dose level based on the following safety parameters: adverse events (AE) and serious (SAE), changes in hematology and chemistry values, including those associated with hepatic and renal function, and assessment of physical examinations, vital signs and performance status using NCI-CTCAE version 4.0 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7.3. Study procedures

7.3.1. Flowchart of treatment and examinations



7.3.2.Schedule of events

Study Event	Enrollment	Vaccination			Follow up		
	Screening and confirmation of eligibility[1] Week -2 to 0	First Week 0[1]	Second Week 3 (± 2days)	Third Week 6 (± 2days)	First Week 9 (± 2days)	Second Week 17 (± 2 weeks)	Third Week 26 (± 2 weeks)
Medical history / demographics	X						
In- / exclusion criteria	X						
Physical examination	X	X	X	X	X	X[7]	X
Concomitant medication	X	X	X	X	X	X	X
Blood collection (Hematology, Chemistry)[2]	X		X	X	X		X
Serum pregnancy test[3]	X		X[4]	X[4]			
Blood collection (immunology)[5]	X			X	X		X
Adverse events according to CTCAE criteria		X	X	X	X	X	X
Hespecta injection		X	X	X			
Vital signs	X	X[6]	X[6]	X[6]	X	X[7]	X

[1]: Baseline and first vaccination visit may be done on the same day; [2] In detail described in paragraph 7.8;[3]: Female of child-bearing potential only; [4]: Urinary pregnancy test also allowed; [5]: HPV16 specific proliferation assay, IFNγ ELISPOT and cytokine array; at baseline and early follow up visit also ex-vivo cytokine staining; [6]: Before and after vaccination; [7]. Shall not be performed if this visit is done by phone

7.4. Schedule

7.4.1. Screening and confirmation of eligibility (Week -2 to week 0)

No screening assessments will be made or any procedure performed before the patient has given written informed consent. If possible screening and first vaccination visit will be done on the same day. The following assessments will be made and procedures will be performed during the screening period (up to 2 weeks before the first vaccination):

- Medical history
- Collection of patient demographic information
- Evaluation of inclusion/exclusion criteria
- Standard physical examination
- Documentation of concomitant medications
- Documentation of baseline status of HPV16+ tumor or premalignant lesion (date of diagnosis, prior therapy, extent of lesions)
- Collection of blood samples for safety parameters (Chemistry and hematology, see paragraph 7.8)
- Collection of blood samples for immunology
- Serum pregnancy test (female of child-bearing potential only)
- Vital signs (heart rate, respiratory rate, body temperature and blood pressure)

7.4.2. Vaccination 1 (Week 0)

Prior to vaccination:

- Inspect the intended vaccination site for any skin abnormalities. Only continue vaccination when the skin is intact
- Vital signs (heart rate, respiratory rate, body temperature and blood pressure)

Vaccination:

- Inject the vaccine i.d. in either one of the thighs or of the upper arms. Injection may only be done by qualified study staff under medical supervision.

1 hour after each vaccination:

- Vital signs (heart rate, respiratory rate, body temperature and blood pressure)
- Examination of the inoculation site
- Registration of AE (with special emphasis on the vaccination sites)

7.4.3. Vaccination 2 (Week 3 ± 2days) and vaccination 3 (Week 6 ± 2days)

Before second and third vaccination:

- Inspect the intended vaccination site for any skin abnormalities
- Standard physical examination
- Vital signs (heart rate, respiratory rate, body temperature and blood pressure)
- Collection of blood samples for safety parameters (Chemistry and hematology, see paragraph 7.8)
- Collection of blood samples for immunology (only before third vaccination)
- Documentation of concomitant medications
- Urine pregnancy testing (female of child-bearing potential only)
- Documentation of AE (with special emphasis on the vaccination site)
- Inspection and measure of previous inoculation sites

Vaccination:

- Inject the vaccine i.d., in either one of the thighs or of the upper arms. Injection may only be done by qualified study staff under medical supervision.

1 hour after each vaccination:

- Vital signs (heart rate, respiratory rate, body temperature and blood pressure)
- Examination of the inoculation site, and also of previous inoculation sites
- Registration of AE (with special emphasis on the vaccination sites)

7.4.4.Follow up for immunological and safety assessments (Week 9 ± 2 days)

Patients will return to the clinic 3 weeks ± 2 days after the last vaccination for assessment of the immunological parameters.

- Standard physical examination
- Documentation of concomitant medications
- Collection of blood samples for safety parameters (Chemistry and hematology, see paragraph 7.8)
- Collection of blood samples for immunology
- Documentation of AE (with special emphasis on the vaccination sites)
- Inspection and measure of previous inoculation sites

7.4.5.Follow up for immunological and safety assessments (Week 17± 1 week), (Week 26 ± 1 week)

- Patients will return to the clinic 11 weeks ± 1 week) and 20 weeks ± 1 week after the last vaccination for assessment of the immunological and safety parameters. These visits are combined with regular follow up visits. If during the first follow-up visit (week 9) no abnormalities are found, it is to be expected that also at the second monitoring visit no abnormalities are found. Therefore if no abnormalities are found during the first monitoring

visit, the second monitoring visit may take place by telephone (week 17).Standard physical examination

- Documentation of concomitant medications
- Collection of blood samples for safety parameters (Chemistry and hematology, see paragraph 7.8), only week 26 ± 1 week
- Collection of blood samples for immunology, only week 26 ± 1 week
- Documentation of AE (with special emphasis on the vaccination sites)
- Inspection and measure of previous inoculation sites

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 and without any explanation. Such patients will be considered as withdrawals. 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. Replacement of individual subjects after withdrawal

Patients who withdraw from the study due to unacceptable toxicity of the study drugs will not be replaced. In order for patients to be eligible for immunological evaluations they have to fulfil all of the below listed criteria:

- Patients should have received at least two doses of Hespecta
- For immunological assessment the patients should have given blood samples for immunological assays (i.e. at baseline (week 0), and at least two of the following time points: before vaccination 3 (week 6) at first follow up, 3 weeks after the third vaccination (week 9),or at late follow up (week 26). Blood samples should have sufficient cell numbers of PBMCs (≥ 40 x 10^6). Patients of whom PBMC counts are not sufficient will be replaced to be able to determine the objectives of this study.

7.7. Follow-up of subjects withdrawn from treatment

Any withdrawn patient will be followed up to obtain at least safety information (i.e. collection of adverse events) only when this patient received at least one vaccination. In addition, every effort should be made to perform as much as possible the efficacy assessments.

7.8. Premature termination of the study

Both the investigator and the sponsor reserve 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 SAE or suspected unsuspected serious adverse reactions (SUSAR). 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 or by the Central Committee on Research involving Human Subjects (CCMO).

7.9. Description of assessments

7.9.1. Medical history

Specific information will be recorded on the case report form (CRF) relating to any prior or existing medical conditions/surgical procedures involving the following: infectious diseases, oncologic diseases, allergic, metabolic/endocrine/nutritional, hematopoietic, musculoskeletal, dermatologic, head/ears/eyes/nose/throat (HEENT), breasts, respiratory, cardiovascular, gastrointestinal/hepatic, genitourinary/renal, neurologic, and psychiatric/psychosocial.

7.9.2. Standard physical examinations, and vital signs

The standard physical examination will include the following observations/measurements: general appearance, skin, HEENT, lymph nodes, heart, lungs, breasts, abdomen, extremities/joints, neurological, mental status, and vital signs, including systolic and diastolic blood pressures (mmHg), heart rate (beats/minute), respiratory rate (breaths/minute), and temperature (degrees of Celsius).
If a clinically relevant worsening in a baseline standard physical examination parameter is observed upon vaccination, the change will be documented as an AE on the AE page of the CRF. Clinical relevance is defined as any variation in physical findings, which has medical consequences that result in an alteration in medical care. The investigator will continue to monitor the patient until the parameter returns to baseline level or until the investigator determines that follow-up is no longer medically necessary.

7.9.3. Blood collection

For hematology and biochemistry: 12.5 ml of venous blood (1 x 4 ml + 1 x 8.5 ml) will be obtained during the screening visit, before second and third vaccination (week 3 and 6, respectively) and at follow up (week 9 and week 26). For immunological assessment: 81 ml (9 x 9 ml) of venous blood will be obtained before vaccination (week 0), before third vaccination (week 6), at first follow up (week 9) and at third follow up (week 26). A total volume of 386.5 ml blood will be drawn during this trial.

7.9.4.Serum chemistry and hematology tests

Serum chemistry profile: albumin, alkaline phosphatase (AP), alanine transaminase (ALT), aspartate transaminase (AST), urea, calcium, chloride, creatinine, gamma glutamyl transferase (γGT), gamma globulin, phosphate, potassium, sodium, total bilirubin, total protein.

Hematology: INR, aPTT, white blood cell (WBC) count with differential, hemoglobin, hematocrit, platelet count, red blood cell (RBC) count. For these two analyses methods a total of 15 mL blood will be collected by venous puncture.

A serum pregnancy test will be performed in females of child-bearing potential only at the screening visit. These patients will also have a urine pregnancy test performed prior to each vaccination.

The Investigator must score all abnormal laboratory values as either clinically relevant, or not clinically relevant. Clinical relevance is defined as any variation in laboratory parameters that has medical consequences that result in an alteration in the patient's medical care. If clinically relevant worsening from baseline levels is noted, the changes will be documented on the AE page of the CRF. The Investigator will also assess the relationship of all clinically relevant abnormal values to study medication as being none, remote, possible, probable, or definite. The Investigator will continue to monitor the patient with additional laboratory assessments until (1) values have reached normal range and/or baseline levels, or, (2) in the judgment of the investigator, the abnormal values are not related to the administration of study medication or other protocol-specific procedures.

7.9.5.Immunological assessments

7.9.5.1. HPV16 E6 and E7 peptide-specific proliferation assay

HPV16 E6 and E7 peptide-specific proliferation is determined using a short-term (6-day) assay. This assay has been published and found to be related to clinical outcome [10,18,32,53,54]. The average and standard deviation of the 8 medium only control wells are calculated and the cut-off is defined as this average plus 3xSD. The stimulation index (SI) is calculated as the average of 8 experimental wells divided by the average of the 8 medium control wells. A positive proliferative response is predefined as a SI of at least 3 and the counts of at least 6 out of the 8-wells must be equal or above the cut-off value [7].

7.9.5.2. HPV16 E6 and E7 peptide-specific cytokine bead array

Supernatants from the peptide-specific proliferation assay are used to perform a cytokine bead array and measure cytokine production (IFNγ, TNFα, IL-4, IL-5, IL-10, and IL-2). Positive antigen-specific cytokine production (in pg/ml) is defined as a cytokine concentration above the detection limit and >2x the concentration of the medium control. A vaccine-induced response is defined as a value (SI or cytokine level) which is at least 3-fold higher than the pre-vaccination response [7].

7.9.5.3. HPV16 E6 and E7 peptide-specific IFNγ-ELISPOT for CD4+ T-cells

The 4-day IFNγ-ELISPOT for the detection of HPV16 E6 and E7 peptide-specific CD4+ T cells is our standard and validated assay. Spots are counted with a fully automated computer-assisted-video-imaging analysis system (BioSys 5000). Specific spots are calculated by subtracting the mean number of spots in quadruplicate wells + 2xSD of the medium only control from the mean number of spots in experimental wells. Antigen-specific T-cell frequencies are considered to be increased compared to non-responders when specific T-cell frequencies are ≥ 1/10,000. T-cell frequencies are considered to be boosted by the vaccine when they were at least 3-fold higher than those prior to vaccination [10,55,56,57].

7.9.5.4. Ex-vivo HPV16 E6 and E7 peptide-specific responses

We have developed and validated a multiparameter flow cytometry assay. In this assay the HPV16-specific cytokines IFNγ TNFα, and/or IL-2 producing CD4+ and/or CD8+ T cells can be simultaneously enumerated in combination with the activation markers CD154 and/or CD137. For this long overlapping HPV16 E6 and E7 peptides will be used to be able to detect both CD4 and CD8 T-cell responses in a HLA-independent setting as these long peptides harbor all potential CD4 and CD8 epitopes for every individual patient. Moreover, due to increased sensitivity in comparison to commonly applied intracellular cytokine staining (ICS) also low frequency antigen-specific T-cell responses can be detected directly ex vivo by using freshly isolated or cryopreserved PBMCs [58]. A positive response is defined as a frequency of activation marker expressing or cytokine producing T cells in the peptide stimulated condition which is twice above the medium only control. A vaccine-induced response is indicated by a 3-fold increase in frequency of antigen-specific T-cells (activation markers and/or cytokine production) compared to baseline values.

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 reviewing 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 investigator will take care that all subjects are kept informed.

8.2. AEs, SAEs and SUSARs

8.2.1. Adverse events (AEs)

AEs are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2. Serious adverse events (SAEs)

A 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;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- any other important medical event that may not result in death, be life threatening, or require hospitalization, may be considered a serious adverse experience when, based upon appropriate medical judgment, the event may jeopardize the subject or may require an intervention to prevent one of the outcomes listed above.

The sponsor will report a SAE through the web portal *ToetsingOnline* to the CCMO that approved the protocol, within 15 calendar days after the sponsor has first knowledge of the SAE.

A SAE that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 calendar days after the responsible investigator has first knowledge of the SAE. This is for a preliminary report with another 8 days for completion of the report.

8.2.3. Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Investigator's Brochure (IB) for an unauthorized medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the CCMO:

- SUSARs that have arisen in the clinical trial that was assessed by the CCMO;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the CCMO.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the CCMO. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern.
The expedited reporting of SUSARs through the web portal ToetsingOnline is sufficient as notification to the competent authority.

The expedited reporting will occur not later than 15 calendar days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 calendar days for a preliminary report with another 8 calendar days for completion of the report.

8.3. Annual safety report

In addition to the expedited reporting of SAEs and SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the CCMO and the competent authority.
This safety report consists of:

- a list of all suspected (unexpected or expected) SAEs, along with an aggregated summary table of all reported SAEs, 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.

8.4. Follow-up of AE

All AEs 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, and/or referral to the general physician or a medical specialist.

SAEs and SUSARs need to be reported till end of study within the Netherlands, as defined in the protocol

8.5. Independent Data Monitoring Committee (IDMC)

An IDMC will review and evaluate safety and efficacy data collected during the study, and assesses reports on cumulated SAEs and SUSARs at pre-specified time-points. Based on this review the IDMC should provide recommendations to the Principal Investigator (PI) and the sponsor regarding the ongoing scientific and ethical integrity of the study based on the data it has reviewed and the progress report of the study in reference to the study protocol. The IDMC also has to approve advancement to the next dose level, based on information provided by the PI (see appendix 2).

A charter describing the roles and responsibilities of the independent IDMC for the trial, including the timing of meetings, methods of providing information to and from the IDMC, frequency and format of meetings, and relationships with other committees, can be found as Appendix 2: Independent Data Monitoring Committee (IDMC) Charter.

9. STATISTICAL ANALYSIS

All patients receiving at least one vaccination will be included in the evaluation of safety. In order for patients to be included in the evaluation of the HPV16 E6/E7-specific T-cell responses they have to fulfil all of the below listed criteria:

- Patients should have received at least two doses of Hespecta;
- For immunological assessment the patients should have given blood samples for immunological assays (i.e. at baseline (week 0), and at least two of the following time points: before vaccination 3 (week 6) at first follow up, 3 weeks after the third vaccination (week 9),or at late follow up (week 26).Blood samples should have sufficient cell numbers of PBMCs (≥ 40 x 10^6). Patients of whom PBMC counts are not sufficient will be replaced to be able to determine the objectives of this study.

9.1. Patient characteristics and immunogenicity

The demographics and baseline characteristics of enrolled patients will be summarized by dose level of Hespecta. Concomitant medications and significant non-drug therapies prior to and after the start of the study drug will be listed by subject and summarized by name and dose cohorts by means of contingency tables.

The HPV-specific immune responses to the Hespecta vaccine will be described using descriptive statistics and 95% confidence intervals.

9.2. Safety

The safety analysis will be performed on all subjects receiving at least one dose of study medication. Adverse events (AEs) will be tabulated by system organ class using the MedDRA dictionary, and by severity according to CTCAE criteria, version 4.0.

10. ETHICAL CONSIDERATIONS

10.1. Regulation statement

This study will be conducted in accordance with the ethical principles that have their origins in the Declaration of Helsinki, and in compliance with the approved protocol, with the guidelines for good clinical practice (GCP), and with applicable regulatory requirements.

10.2. Recruitment and consent

Patients will be recruited from the group of patients visiting or referred to the departments of medical oncology of the LUMC. Written informed must be obtained in accordance with ICH-GCP guidelines using the approved ICF, before any study specific study procedures. The informed consent process may be conducted by non-medically qualified persons, provided that any such person is adequately trained to do so, and a written delegation from the investigator is in place. Potential patients will be given sufficient time to consider if they wish to participate.

10.3. Benefits and risks assessment, group relatedness

Potential risks with the current trial are mainly linked to the toxicity related to the treatment compounds utilized, the Hespecta. Although the rabbit toxicity testing of Hespecta (see IMPD brochure) did not show any severe side effects, we expect from previous results with HPV16-SLP, that the most important AEs of Hespecta may consist of fever, chills, nausea, malaise, fatigue and local reactions at the vaccination site including pain, redness, swelling and itching. Generally the AEs after vaccination with HPV16-SLP did not exceed grade 2 according to the CTCAE criteria. Almost all patients, with advanced CxCa as well as patients with CIN or VIN, vaccinated with HPV16-SLP formulated in Montanide ISA51 experienced local injection site reactions (grade 2). After vaccination, ulceration/abscess formation occasionally occurred, estimated to be in between 1 and 5% of vaccinations and in the order of 5.5% of patients, due to the use of this Montanide as adjuvant. The current vaccine is administered without Montanide, and therefore, it is expected that these local reaction will be less severe.

Allergic reactions have occurred in a few patients after HPV16-SLP vaccinations, controlled with antihistamines. Vaccinations should therefore only be administered in a clinic where immediate treatment of severe allergic reactions is possible. Thus, after vaccinations, patients will be closely monitored during one hour after injection to provide means for immediate treatment of allergic reactions if that would be necessary (precautions include availability of staff well-trained in resuscitation, intravenous access for administration of fluids, antihistamines and corticosteroids, and epinephrine for intramuscular injection).

All persons participating in this trial will receive the standard treatment they would have been offered outside of this clinical trial, allowing them to benefit from the normal standard of care as any patient with an HPV16+ tumor or premalignant lesion in the specified settings. At least on month following the standard of care, the patients participating in this trial will receive three vaccinations with Hespecta for

the induction of a strong and broad Th and CTL response against HPV16 E6. The development of HPV16+ tumors or premalignant lesions might be associated with a weak systemic and local immune response to HPV. Injecting patients with Hespecta might enhance this weak tumor-specific immune response in favor of a Th1/CTL response, which in high-grade premalignant lesions (VIN3) was correlated with clinical complete clearance of the lesions. Similarly in cancer patients it might be associated with an improved prognosis and decreased number of recurrences of HPV16+ tumors.

10.4. Compensation for injury

The sponsor will obtain an insurance to cover the participants in this trial according to local regulations.

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.

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.

10.5. Incentives

Study patients will not be paid for their participation. Any study-related travel expenses made by patient or the accompanying person will be reimbursed on the basis of actual cost as proven by original receipts, or an allowance per km travelled.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1. Handling and storage of materials, data and documents

The PI will maintain adequate records, including flow sheets, laboratory reports, signed patient consent forms, drug disposition records, and information on AEs, patient treatment discontinuation and reasons for treatment discontinuation. All records will be signed and dated by the Investigators. All records are to be retained for a period of 15 years following the date the entire clinical investigation is completed, terminated or discontinued. Cryopreserved material will be stored for a period of 15 years following the date the entire clinical investigation is completed, terminated or discontinued.

Data will be handled confidentially and anonymously. All included patients will get a code that is not retrievable to individuals. Where it is necessary to be able to trace data to an individual subject, a subject identification code list will be used to link the data to the subject. The key to the code is safeguarded by the investigator.

11.2. Recording of Data

All required data must be recorded in the CRF designed and provided by the Sponsor. All missing data will be explained. All entries must be in permanent, black ink. Errors will have single lines drawn through them with the correct data, recorder's initials, and date entered above the error. Erasion or obliteration of errors is strictly prohibited so that all errors remain legible.

11.3. Data Quality Assurance

As deemed necessary, the Sponsor may perform a comprehensive audit of the study site and study records to ensure compliance with GCP and adherence to the study protocol. At a time during or after completion of the study, it may be necessary for the investigator to comply with a regulatory inspection by local authorities.

11.4. Amendments

Amendments are changes made to the research after a favorable opinion by the CCMO has been given.

A 'substantial amendment' is defined as an amendment to the terms of the CCMO application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the CCMO and to the competent authority. The amendment may only be implemented after a positive opinion of the CCMO and confirmation of no grounds for non-acceptance from the competent authorities.
Non-substantial amendments will not be notified to the CCMO, but will be recorded and filed by the sponsor.

11.5. Annual progress report

The sponsor/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, SAE / serious adverse reactions, other problems, and amendments.

11.6. End of study report

The sponsor will notify the CCMO and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the sponsor will notify the CCMO and the competent authority within 15 days, 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 and the Competent Authority.

11.7. Public disclosure and publication policy

The results of this study are intended to be published in peer reviewed medical journals. The data will be presented in such way that individual patients cannot be recognized or identified.
The following arrangements have been made between ISA Pharmaceuticals B.V. (a company that supports this study) and the LUMC concerning publication. The LUMC is free to publish the trial results but in order to protect the business and obligations of ISA Pharmaceuticals B.V., publications may be requested to be delayed for a maximum of 90 days.
Anonymized data from this study may be used by ISA Pharmaceuticals B.V. for purposes of obtaining marketing authorization of the IMP or follow-on products. Data from this trial may therefore be transferred in paper or electronic form to authorities both within and outside the European Union.

12. STRUCTURED RISK ANALYSIS

12.1. Potential issues of concern

a. Level of knowledge about mechanism of action

By vaccinating patients with two long E6-derived peptides covering multiple potential CTL and T helper epitopes both coupled to Amplivant®, a TLR2 ligand, we hope to induce a strong type 1 T cell response that is able to eliminate HPV-infected cells/HPV-induced malignant cells.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

This is a new vaccine. The peptides have been selected from our experience with previous studies using synthetic long peptides vaccines as described in paragraph 1.6. Our previous experience has been published and summarized in paragraph 1.3

c. Can the primary or secondary mechanism be induced in animals and/or in *ex vivo* human cell material?

This has been studied and the peptides conjugated to the TLR ligand Amplivant® have been tested for toxicity, see paragraph 1.7.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

The vaccine induces only HPV-specific immune responses and is meant to induce T cells which specifically recognize HPV16 E6 peptides in the context of HLA molecules. Therefore, it is expected that only HPV16-infected cells/HPV induced malignant cells will be targeted by the vaccine-induced T-cell population.

e. Analysis of potential effect

During this study adverse events (AEs) will be collected according to CTCAE v4.0. Furthermore immunogenicity will be determined using an array of complementary immunological assays (HPV16-specific proliferation assay, IFNγ-ELISPOT, cytokine bead array and multiparametric flow cytometry assay). Clinical efficacy will not be studied during this trial.

f. Pharmacokinetic considerations

This vaccine should induce a long lasting immune response, normal pharmacokinetic considerations do not hold for vaccines.

g. Study population

The study population consists of 24 patients treated for HPV16+ tumors or premalignant lesions. Most healthy persons are able to get rid of the virus direct after infection and only in a minority the viral infection persists. Previous experience showed that the induced immune response after vaccination in the general population differs from individuals who are not able to eradicate the HPV16 virus and have

been treated for HPV16+ tumors, they show a weaker immune response after vaccination, compared to healthy individuals. Also, healthy individuals have more side effects after vaccination. As the targeted population of this therapeutic vaccine consists of patients with HPV16+ tumors or premalignant lesions we chose to vaccinate patients in good general health who have recently received standard treatment for HPV16+ tumors or their premalignant lesion and not healthy individuals without a history of such a HPV induced lesions.

h. Interaction with other products

The desired immune response may be blunted by subsequent use of immunosuppressive medications such as glucocorticoids. Also other immunomodulating substances such as antibodies against TNF-alfa, CTLA-4 and PD1, might affect the induced immune response.

i. Predictability of effect

At the moment there are no hall-marked biomarkers to predict the effect of the vaccine on killing HPV infected cells/HPV-induced malignant cells and on survival for cancer patients, however, we will analyze the induced T-cell response after vaccination and correlate these results with safety results. In our previous HPV16 SLP trial in patients with VIN3 lesions the proposed T-cell immune parameters were correlated with clinical outcome [17,18,32].

j. Can effects be managed?

This is the first-in-man study. However, in previous trials using the HPV16-SLP vaccine, allergic reactions have occurred in a few patients after vaccinations, controlled with antihistamines. For relief of fever or pain of the local injection site, paracetamol can be used.

12.2. Synthesis

Potential risks with the current trial are mainly linked to the toxicity related to the treatment compounds utilized, the Hespecta. Although the rabbit toxicity testing of Hespecta (see IMPD brochure) did not show any severe side effects, it is expected from previous results with HPV16-SLP, that the most important adverse events (AEs) of Hespecta may consist of fever, chills, nausea, malaise, fatigue and local reactions at the vaccination site including pain, redness, swelling and itching. Generally the AEs after vaccination with HPV16-SLP did not exceed grade 2 according to the CTCAE criteria. Almost all patients, vaccinated with HPV16-SLP formulated in Montanide ISA51 experienced local injection site reactions (grade 2). After vaccination, ulceration/abscess formation occasionally occurred, due to the use of this Montanide as adjuvant. The current vaccine is administered without Montanide, and therefore, it is expected that these local reaction will be less severe. Allergic reactions have occurred in a few patients after HPV16-SLP vaccinations, controlled with antihistamines. Vaccinations should therefore only be administered in a clinic where immediate treatment of severe allergic reactions is possible. Thus, after vaccinations, patients will be closely monitored during one hour after injection to provide means for immediate treatment of allergic reactions. In addition to the standard of care, the

patients participating in the trial will receive three vaccinations with Hespecta for the induction of a strong and broad CTL response against HPV16 E6.

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14. Appendix

14.1. Appendix 1: ECOG performance status scale

ECOG-ZUBROD-WHO	
Scale	**Status**
0	Normal activity
1	Symptoms, but nearly ambulatory
2	Symptomatic, but in bed <50% of the day
3	Needs to be in bed >50% of the day, but not bedridden
4	Unable to get out of bed
5	Dead

Adapted from Oken *et al*., 1982 [54].

14.2. Appendix 2: Independent Data Monitoring Committee (IDMC) CHARTER

14.2.1. Introduction of Clinical Trial and Purpose of Document

Title:

A Phase I Study of Hespecta vaccination in HPV+ lesions

Identification numbers:

ISA Clinical Trial Study Number HPV16HH01

ABR NL48274.000.14

EUDRACT nr. 2014-000658-12

Sponsor:

Leiden University Medical Center (LUMC)

Albinusdreef 2

2333 CH Leiden

The Netherlands

Principal Investigator (for the clinical trial, i.e. the PI referred to in this Charter):

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Objectives:

The primary objective of the clinical trial is:

Primary objective: to determine the biological activity of Hespecta that is able to induce HPV16 E6-specific T-cell immunity within a proposed dose escalation of 1, 5, 20 or 0 µg/peptide - conjugate in patients treated for HPV16+ tumors and premalignant lesions.

The secondary objectives of the clinical trial is:

To study safety of Hespecta by collecting all adverse events according to Common Terminology Criteria for Adverse Events v4.0 (CTCAE)

Purpose of this document

The purpose of this document is to describe the roles and responsibilities of the Independent Data Monitoring Committee (IDMC) for the clinical trial, including the timing of meetings, methods of

providing information to and from the IDMC, frequency and format of meetings, and relationships with other committees.

14.2.2. Roles and responsibilities

Aims of the IDMC:

To protect and serve clinical trial participants especially regarding safety assessments and to assist and advise the Sponsor and the Principal Investigator so as to protect the validity and credibility of the clinical trial.

Main task of the IIDMC:

The IDMC will receive and review the progress and accruing data of this clinical trial and provide advice on the conduct of the clinical trial to the Sponsor and the PI, in particular concerning safety.

Specific tasks of IDMC:

There are two main specific tasks for the IDMC:

- Review the clinical trial's progress especially regarding safety data, but also regarding recruitment, and data quality (including losses to follow-up). To facilitate this task the IDMC will receive quarterly progress reports provided by the Sponsor (see below regarding base content of quarterly progress reports and process to handle them).
- Assess and comment on the written summary from the Sponsor that recruitment at the next (higher) dose level can start (see below regarding process).

Quarterly progress reports and process to handle them

The intended base content of the quarterly progress reports to be sent to the IDMC include (the content might change during study conduct due to e.g. remarks from the IDMC):

1. Enrollment Status
2. Central Data Management (CDM)
3. Safety Reporting
    a. SAEs – till date of this Progress Report
    b. SUSARs - till date of this Progress Report
    c. Other items
4. Project Deviations

After receiving a quarterly progress report the IDMC members should individually review the material and within no longer than 1 week of receipt send an e-mail to the PI (cc all IDMC members) detailing if there are any concerns from the IDMC member, and if the clinical trial can continue as planned, or not, in the view of the individual IDMC member. In the same response the IDMC member should also make clear if he/she sees a necessity for calling on a IDMC meeting (TC or face-to-face). A meeting

must be held if a IDMC member would raise concerns regarding the continued conduct of the study for the whole IDMC to assess the situation and come to a recommendation for the Sponsor and PI.

Process for decision to approve recruitment at the next (higher) dose level

The important task of the IDMC is to comment on the written summary from the Sponsor that recruitment at the next (higher) dose level can start.

Note, decisions to allow escalation of dose-levels will only be taken in light of accumulated safety data since immunological and efficacy outcome data will need to be generated from all planned cohorts (provided they can be run in light of safety outcomes) before an adequate assessment can be made.

According to the clinical trial protocol Section 3 Study Design, the process to start enrollment at the next (higher) dose level include:

1. The patients will be vaccinated with a fixed dose of Hespecta every three weeks for a total of three rounds of vaccination.

2. Four dose levels of Hespecta will be tested as noted in Protocol Section 3 (i.e. dose-levels of 1, 5, 20 and 50 µg/peptide.

3. The first 6 patients will be enrolled in cohort 1, the next 6 patients in cohort 2, and so on until completion of all 4 cohorts.

4. The decision to start enrollment at the next dose level will be made by assessing the safety after at least 4 out of 6 patients have completed the first follow up visit after the third vaccination with Hespecta.

5. The IDMC will receive from the PI a brief summary as well as listings of individual patient data related to baseline characteristics (fulfillment of inclusion/exclusion criteria, patient demographics, past medical and surgical history, prior cervical cancer treatment, HPV16 typing), safety data (e.g. concurrent diseases/therapy, outcome of assessments of pregnancy test when applicable, physical examinations, vital signs, ECOG performance status, hematology tests, biochemistry tests, urinalysis, ECG, recording of adverse events and serious adverse events), and study medication administration for the patients necessary to assess per above before decision to start enrollment at the next dose level.

6. The IDMC will assess information per above for relevant patients, summarize findings, and give a recommendation to the PI whether or not he/she endorses the start of enrollment at the next dose level. If there is a consensus in the IDMC to endorse the start of enrolment at the next dose level it is not necessary to hold a IDMC meeting, but the procedure can run via e-

mail. It there are divergent opinions within the IDMC, a meeting (TC or face-to-face) must be held as soon as possible for the IDMC to conclude on a recommendation to the PI regarding enrolment at the next dose level.

7. After having received the opinion of the IDMC, the PI can issue a written approval (or disapproval) regarding the start of enrollment at the next dose level.

Further tasks of the IDMC include:

- Decide whether to recommend that the clinical trial continues to recruit participants or whether recruitment should be terminated either for everyone or for some treatment groups and/or some participant subgroups.

- Advise on protocol modifications suggested by the Sponsor or PI (e.g. to inclusion criteria, clinical trial endpoints, or sample size).

- Considering the ethical implications of any recommendations made by the IDMC.

- Assess the impact and relevance of external evidence and advice.

14.2.3. IDMC membership

As a main responsibility of the IDMC is the review of data regarding safety in order to ensure the safety of the study participants, it is essential to have an independent committee with clinical expertise in the respective indication being able to also cover ethical aspects. Accordingly, the IDMC for this clinical trial will consist of at least three voting members who are not involved in the conduct of the study, but do have experience in either the indication, the study drug or in the area of safety aspects.

All potential IDMC members should have reviewed the protocol before agreeing to join the committee. Therefore, if a potential IDMC member has major reservations about the clinical trial (e.g. the protocol or the logistics) they should report these to the Sponsor and may decide not to accept the invitation to join.

IDMC members will formally register their assent by confirming (1) that they agree to be on the IDMC and (2) that they agree with the contents of this Charter. This assent will be provided in writing (e.g. by e-mail) to the Sponsor who will also inform the PI.

Members of the IDMC will each also complete a Sponsor confidentiality disclosure agreement before confidential study documents are distributed to them study data is distributed. At no time may any information about the IDMC proceedings be disclosed to any third party.

Any member of the IDMC resigning, voluntary or involuntary, or in the event of a member's irregular attendance at IDMC meetings, will need to be replaced. In the event that a member is to be removed involuntarily, at the discretion of the Chair of the IDMC and with the consent of the other IDMC member, the Chair will provide that member with 15 day's notice in advance of the termination. The Sponsor and the Chair will be jointly responsible for finding a suitable replacement. The Sponsor may not dismiss individual members of the IDMC without the majority vote by the IDMC.

14.2.4. Composition of the IDMC

The proposed members of the IDMC for this clinical trial are:

Chair: prof. dr. Winald Gerritsen, medical oncologist
prof. dr. Dick Richel, medical oncologist
prof. dr. John Haanen, medical oncologist

The Chair is expected to lead, facilitate and summarize discussions during meetings. For further responsibilities of the Chair see Section 14.2.6 (Organization of IDMC meetings) below.

14.2.5. Relationships

There are no other Committees, beside the IDMC, associated with the current clinical trial.

The members of the IDMC will be reimbursed for travel and accommodation necessary for face-to-face meetings, and when applicable for telephone costs when meetings are held as telephone conferences. No other payments or rewards will be given to IDMC members. However, in case of face-to-face meetings the Sponsor is allowed to provide beverages and food as necessary at a non-luxurious level.

Competing interests of IDMC members should be disclosed per **Annex 1** to this Charter. IDMC members will not use interim results to inform trading in pharmaceutical shares, and careful consideration should be given to trading in stock of companies with competing products.

14.2.6. Organization of IDMC meetings including reporting of meeting outcomes

The IDMC is to meet at least once early in the course of the clinical trial, to review the protocol and all related documents, to discuss general items, including future meetings, and to have the opportunity to clarify any aspects with the Sponsor and PI.

The IDMC should also meet at least at one further time point, around 9 months after recruitment commencing (total recruitment time is projected to be approximately 15 months assuming 8 sites would participate in the clinical trial).

In addition, IDMC members, the Sponsor, or the PI can at any time call for a IDMC meeting due to the perceived necessity to discuss a specific item.

The IDMC meetings can be scheduled as face-to-face meetings or telephone conferences (TCs), with a preference for face-to-face meetings. However, the known complexities to arrange face-to-face meetings should not hinder the function of the IDMC to discuss and act within appropriate time lines.

The Chair of the IDMC is responsible for invitations to IDMC meetings, a task which the Chair may delegate to the Sponsor. If at all possible, meeting invitations including adequate meeting material should be sent out at least one week in advance of meetings. However, it should be acknowledged that meetings might need to be commenced on a short notice preventing such a timeline.

Necessary meeting material beyond the quarterly progress reports described in Section 2 above will be agreed between the Chair of the IDMC, the Sponsor, and the PI, and provided by the Sponsor.

At least one representative of the PI should, when deemed necessary, be available to attend open sessions of the IDMC meetings.

The format of the meetings will be:

1. Open session: Introduction and clarifications, PI representatives
2. Closed session: only IDMC member attendance for discussions and decision on recommendation(s)

The Chair of the IDMC is responsible for the documentation of IDMC meetings, but the task can be delegated to other IDMC members if so decided and agreed by the IDMC. Documentation of the open sessions might also be delegated to the PI.

Meetings of the IDMC must be documented in writing (meeting minutes), including especially recommendations (can be separately documented from the meeting minutes) of the IDMC.

Minutes of meetings and recommendations will be agreed upon and signed off (acceptable via e-mail) by all IDMC members participating, and kept by the Chair.

Recommendations from the IDMC (or a statement that no recommendations were made), with meeting minutes if appropriate (might be shortened version of full minutes) should be provided to the Sponsor and the PI within one week after a IDMC meeting.

A template possible to use as a report from a IDMC meeting where no recommendations were made can be found in **Annex 2** to this Charter.

14.2.7. Decision making

It is recommended that every effort should be made for the IDMC to reach a unanimous decision. If the IDMC cannot achieve this, a vote should be taken.

The IDMC can take decisions and make recommendations provided 2 out of the 3 IDMC members are in agreement, i.e. for a meeting to be functional at least 2 IDMC members must participate.

Possible recommendations from the IDMC could include:

- No action needed, clinical trial continues as planned.
- Early stopping of the clinical trial due to harm of a treatment.
- Stopping recruitment within a subgroup due to safety concerns within this special patient population.
- Extending follow-up.
- Sanctioning and/or proposing protocol changes

It is important that the implications (e.g. ethical, statistical, practical, and financial) for the clinical trial be considered before any recommendation is made. The emphasis of the recommendations made by the IDMC should regard safety.

14.2.8. After the clinical trial

IDMC members will be named and their affiliations listed in the main Clinical Study Report (CSR). A brief summary of the timings and conclusions of IDMC meetings will be included in the CSR.

The minutes of the IDMC meetings and their recommendations will remain confidential also after clinical trial finalization, unless otherwise decided by the Sponsor.

14.3. Annex 1: Competing interests form

Clinical trial:

Clinical trial: NL48274.000.14

A Phase I Study of Hespecta vaccination in HPV+ head and neck cancer

The avoidance of any perception that members of a IDMC may be biased in some fashion is important for the credibility of the decisions made by the IDMC and for the integrity of the clinical trial.

Possible competing interest should be disclosed via this form. In many cases simple disclosure up front should be sufficient. Otherwise, the (potential) IDMC member should remove the conflict or not accept (or stop) participating in the IDMC. Table 1 lists potential competing interests.

Table 1: Potential competing interests

• Stock ownership in any commercial companies involved • Stock transaction in any commercial company involved (if previously holding stock) • Consulting arrangements with the sponsor • Frequent speaking engagements on behalf of the intervention • Career tied up in a product or technique assessed by clinical trial • Hands-on participation in the clinical trial • Involvement in the running of the clinical trial • Emotional involvement in the clinical trial • Intellectual conflict e.g. strong prior belief in the clinical trial's experimental arm • Involvement in regulatory issues relevant to the clinical trial procedures • Investment (financial or intellectual) in competing products • Involvement in the publication

Please complete the following section and return to the Sponsor.

Do you have any competing interests to declare?

☐ **No**
☐ **Yes** (please detail below)

Please provide details of any competing interests: _____

_____

_____

Name: _____

Signed: _____ Date: _____

14.3.1. Annex 2: Suggested report from IDMC where no recommendations are made

[Insert date]

To: The Principal Investigator of Clinical Trial: NL48274.000.14
A Phase I Study of Hespecta vaccination in HPV+ lesions

Albinusdreef 2, 2333 CH Leiden, The Netherlands
E-mail address (contact person): f.m.speetjens@lumc.nl

Dear Sponsor of study NL48274.000.14,

The Independent Data Monitoring Committee (IDMC) for clinical trial NL48274.000.14 met on *[meeting date]* to review its progress and interim accumulating data. *[List members]* attended the meeting and reviewed the information.

We conclude that the trial question remains important and, on the basis of the data reviewed at this stage, we recommend continuation of the trial according to the current version of the protocol *[specify protocol version number and date]* with no changes.

We shall next review the progress and data *[provide approximate timing]*.

Yours sincerely,

*[*Name of meeting Chair*]*
Chair of the IDMC

On behalf of the IDMC (all members listed below)

IDMC members:
(1) [*Insert name and role*]
(2) [*Insert name and role*]
(3) [Insert name and role]