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Immune modulating effects and safety of Vvax001, a therapeutic Semliki Forest Virus based cancer vaccine, in patients with a history of (pre) malignant cervical lesions: Study protocol

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ABSTRACT

Background: Currently, high-grade premalignant lesions of the cervix are treated with large loop excision of the transformation zone (LLETZ). This procedure has potential complications, and does not necessarily eradicate the underlying HPV infection, causative agent for (pre) malignant neoplasia of the cervix. Therefore, therapeutic vaccination is a potential non-invasive alternative for the treatment of these premalignant cervical lesions. We developed a new therapeutic vaccine, called Vvax001. Vvax001 aims at inducing long-lasting immune responses against HPV16-infected cells. In preclinical studies, Vvax001 was shown to be an effective and well-tolerated treatment for HPV16-induced tumors. These preclinical results supported the application for a first-in-human phase I clinical trial. The aim of this study is to assess the immunological activity, safety and tolerability of Vvax001 in human.

Methods: In this first-in-human phase 1 clinical trial, four dose levels of Vvax001 will be tested. Vvax001 is a therapeutic viral vector vaccine consisting of replication-incompetent Semliki Forest Virus (rSFV) replicon particles encoding the HPV16 derived tumor antigens E6 and E7. Patients will receive three consecutive vaccinations, with an interval of 3 weeks. Cohorts of 3 patients will be treated per dose level.

Discussion: Based on preclinical results, Vvax001 represents a promising therapeutic vaccine for the treatment of (pre)malignant lesions of the cervix caused by HPV16. All results on immunogenicity and tolerability of Vvax001 in human are expected to be available mid-2018.

Trials registration: NCT03141463; NL56680.000.16
BACKGROUND

Human Papillomavirus (HPV) is the causative agent for cervical cancer\cite{1}, the second most common cause of cancer death among women worldwide.\cite{2,3} In addition, persistent infection with high-risk HPV types has been linked to the development of other genital cancers in men and women such as anal cancer, vulvar cancer and penile cancer, but also in the development of oropharyngeal cancer.\cite{4,5} Overall, HPV is therefore estimated to be responsible for ~5% of the worldwide cancer burden. Of all HPV subtypes, HPV16 is most commonly associated with (pre)malignant disease of the cervix.\cite{6}

The risk of developing malignancies after an HPV infection is largely due to the capacity of high-risk HPVs to transform epithelial cells by integrating DNA encoding the viral early proteins E6 and E7 into the host cell genome. This integration leads to constitutive overexpression of E6 and E7 proteins, mediating transformation of the infected cells through premalignant conditions towards a malignant phenotype.\cite{7,8}

The premalignant condition of cervical cancer is called cervical intraepithelial neoplasia (CIN) and, due to population-based screening programs, most patients are diagnosed at this stage of the disease. Currently, high-grade CIN lesions are treated with surgical excision, a so-called large loop excision of the transformation zone (LLETZ). This procedure has potential complications, such as bleeding, infection, cervical stenosis and preterm birth, probably due to cervical insufficiency.\cite{9-11} Above all, surgical excision does not necessarily eradicate the underlying HPV infection. Therefore, there is a need for novel non-invasive therapeutic approaches.

Since the continued production of E6 and E7 is required for the maintenance of the transformed phenotype, E6 and E7 in fact represent tumor-specific antigens in HPV-associated carcinoma and premalignant HPV-transformed cells.\cite{7,8} Therefore, E6 and E7 are potential targets for immunotherapeutic intervention strategies involving induction or stimulation of cytotoxic T lymphocyte (CTL) activity against HPV-transformed cells.\cite{4}

Here, we report the study protocol of our first-in-human clinical trial using Vvax001, a therapeutic viral vector vaccine consisting of recombinant Semliki Forest Virus replicon particles (rSFV) encoding the HPV16 derived tumor antigens E6 and E7 (Vvax001; rSFVeE6,7). Remarkably, the strong booster effect of rSFV makes Vvax001 a viral vector vaccination strategy with exquisite potency.\cite{12} In line with this, we previously demonstrated that immunization of mice with rSFVeE6,7 induces strong CTL activity and eradication of established HPV-transformed tumors.\cite{13-19} In this phase I study, immunogenicity, safety and tolerability of Vvax001 will be evaluated.
METHODS

Investigational product

The therapeutic vaccine Vvax001 is a rSFV vector encoding a fusion protein of HPV-16 E6 and E7 (rSFV/E6,7). A translational enhancer has been inserted upstream of the gene encoding the E6,7 fusion protein to increase recombinant gene expression. Vvax001 induces production of a fusion protein of HPV-16 E6 and E7 in the cytoplasm of infected cells. Between 48 and 72 hours after infection, infected cells die. Dying cells, containing large amounts of the E6,7 fusion protein or fragments thereof, are taken up by dendritic cells, which present the E6,7 antigens to CTL thereby resulting in the induction of an immune response against HPV-infected tumor cells and consequently regression/eradication of the tumor.

Design

In this phase I immunization study, four dose levels of Vvax001 will be tested. Patients will receive three consecutive vaccinations, with an interval of 3 weeks. Cohorts of 3 patients will be treated per dose level. Although no limiting toxicities are anticipated based on previous experience with similar viral vector vaccines, enrollment of subsequent patients will proceed with a minimum interval of 48 hours. In this phase I trial, there will be no restriction based on HPV-status in order to expedite accrual, given that immune responses are anticipated irrespective of HPV-status.

Results will be analyzed and the optimal dose will be determined based on level of systemic cellular immune response and by monitoring limiting toxicity.

**Figure 1:** Phase I trial design. Bold arrows represent the VVAX001 immunizations at day 0, 21 and 42. Thin arrows represent the peripheral blood mononuclear cells (PBMC) collection for immunomonitoring at baseline, day 28-31 and day 49-52.
Participants

The study population consists of adult female patients with a history of cervical intraepithelial neoplasia (CIN) II and III, and patients with a history of cervical cancer, both minimally 12 weeks after completion of treatment. Patients will be accrued from the outpatient clinic of the University Medical center Groningen (UMCG).

Additional inclusion criteria are: Adequate bone marrow functions; HIV- and HBV-negative; 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; written informed consent according to local guidelines.

A potential subject who meets any of the following criteria will be excluded from participation in this study: Prior treatment with immunotherapeutic agents against HPV; history of an autoimmune disease or other systemic intercurrent disease that might affect the immunocompetence of the patient; current or prior use of high dose immunosuppressive therapy (4 weeks before start of the study); participation in a study with another investigational drug within 30 days prior to the enrolment in this study.

Intervention

Patients will receive three consecutive vaccinations of Vvax001, with an interval of 3 weeks. Each dose will be given as two injections; 1 injection in each leg. The injections will be administered intramuscularly in the upper legs, preferably in the m. vastus lateralis. Patient evaluation will be performed before, during and after vaccination, including history, physical examination and toxicity scoring using common toxicity criteria grades. Biochemistry will be performed at baseline, prior to each vaccination, and at follow up including full blood count, urea, electrolytes and liver function tests. Urine dipstick, ECG, HIV and HBV tests will be performed at baseline. In case of child bearing potential, a pregnancy test will be performed prior to each vaccination. Peripheral blood mononuclear cells (PBMC) and serum will be collected at baseline, 7-10 days after the second vaccination, and 7-10 days after the third vaccination, to monitor HPV-specific immune responses and anti-SFV antibodies.

Outcome

The main study endpoint is the immunogenicity of Vvax001. HPV-16 E6,7-specific T cell immune responses as measured by IFN-γ-ELISPOT. The secondary parameters are side effects/ adverse events related to Vvax001. Toxicity will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0.
**Immunomonitoring**

In order to assess the systemic changes in immunity induced by vaccination, venous blood samples obtained at baseline, and 7-10 days after the second and third vaccination, will be examined by using peripheral blood mononuclear cells (PBMCs). PBMCs will be tested for: 

i. Antigen-specific responses by IFN-γ-ELISPOT;

ii. Proliferation (Ki67+ T cells) and cytokine production (e.g. IFNγ, TNFα, IL-4, IL-5, IL-10, and IL-2);

iii. Intracellular cytokine production (by CD4+ and/or CD8+ cells) after one round of in vitro stimulation;

iv. Phenotypic analysis by flowcytometry. Serum will be tested for antibodies against SFV by Elisa. The IFN-γ-ELISPOT (i) is the parameter for the primary immunogenicity endpoint. The other immunomonitoring parameters are exploratory.

**Sample size**

Cohorts of 3 patients will be treated per dose level using a standard 3+3 dose escalation design. Four dose levels of Vvax001 will be tested. A minimum total of 12 subjects will be included. In case dose limiting toxicity occurs, a maximum of 24 patients will be included.

**Statistical analysis**

There will be 3-6 subjects per dose level, 12-24 subjects in total. Descriptive statistics will be used to summarise the data. In order for patients to be included in the evaluation of the HPV-16 E6,7-specific T cell responses (IFN-γ-ELISPOT) they have to fulfil all of the below listed criteria: Patients should have received at least two doses of Vvax001; The pre-vaccination blood sample should have sufficient numbers of PBMCs (i.e. 10x10^6); The patients should have given at least one blood sample after the second vaccination (at visit 4 or 6) and this blood sample should have sufficient numbers of PBMCs (i.e. 10x10^6). All patients receiving at least one dose of Vvax001 will be included in the evaluation of safety.

**DISCUSSION**

Within this Phase I first-in-human clinical study, Vvax001, a therapeutic viral vector vaccine consisting of a replication-incompetent Semliki Forest Virus (rSFV) encoding the HPV16 derived tumor antigens E6 and E7, will be evaluated for immunogenicity and tolerability. Preclinical studies revealed exquisite efficacy by demonstrating that immunization of mice induced strong CTL activity and eradication of established HPV-transformed tumors. These preclinical results have led to the development of a clinical batch, named Vvax001, and the initiation of this Phase I clinical trial.

Although prophylactic vaccines have been successful in the prevention of HPV infections, they are not capable of curing pre-existing HPV-infections and HPV-related diseases. In addition,
current vaccine rates hover at about only 50% of the young adult population.\textsuperscript{23} Therefore, there remains a need for therapeutic vaccination for the treatment of pre-existing as well as new HPV-infections and HPV-related diseases.

Thus far, various immunotherapeutic strategies, including therapeutic vaccination strategies, have been explored in early phase clinical trials. These immunotherapeutic strategies aim to stimulate CTL activity against HPV-transformed cells and thereby target HPV-related (pre) malignancies. Vaccination with therapeutic HPV16 overlapping synthetic long peptides (HPV16-SLP) resulted in regression of HPV16-induced premalignant lesions of the vulva.\textsuperscript{24-26} Furthermore, HPV16-SLP vaccination in patients with advanced or recurrent HPV16-related cervical cancer partly installed specific T cell reactivity, although no clinical effect was observed on tumor growth.\textsuperscript{27} Therapeutic vaccination using a DNA-vaccine showed clearance of HPV infection and regression of CIN3 lesions in 7 out of 9 patients.\textsuperscript{28} In addition, pembrolizumab (anti-PD1 antibody) treatment resulted in a 20% response rate in patients with HPV-related head and neck squamous cell carcinoma (HNSCC),\textsuperscript{29-32} and adoptive cellular transfer therapy using HPV-targeted tumor-infiltrating T cells resulted in complete regression of disease in metastatic cervical cancer.\textsuperscript{33}

In contrast to the above described SLP- and DNA-based vaccines, Vvax001 is a therapeutic vaccine based on a viral vector. Viral vector vaccines, and Vvax001 in particular, have specific advantages over other vaccination platforms. First, cells are very efficiently infected after which large amounts of the encoded antigens are produced. This enhanced presentation of tumor antigens to the immune system leads to an increase in the frequency of CTL that target tumor cells expressing the tumor antigen(s) encoded in the vaccine vector. Secondly, the virus infection leads to a ‘danger’-signal by the infected cells and the subsequent accumulation and activation of dendritic cells.\textsuperscript{34,35} Due to this pro-inflammatory environment produced by the expression of viral proteins, no adjuvant is needed to enhance delivery of the tumor antigens. On the contrary, a disadvantage of some vectors is the development of host-induced neutralizing antibodies to the vector itself, thus limiting its continued use.\textsuperscript{34} However, preclinical studies with rSFV revealed that neither SFV-specific antibodies nor T cells directed against rSFV-infected cells affect the boosting activity of this vector system. In line with this, the strong booster effect of rSFV makes Vvax001 a viral vector vaccination strategy with exquisite potency.\textsuperscript{12}

We previously demonstrated that immunization of mice with rSFVe6,7 induces strong CTL activity and eradication of established HPV-transformed tumors. Furthermore, mice that underwent a second tumor-challenge, 6 months after initial immunization with rSFVe6,7, were protected for tumor outgrowth.\textsuperscript{13-19} These results implicate that vaccination with rSFVe6,7 induces long-term immune memory and that therapeutic vaccination might also protect for recurrence of (pre)malignant cervical lesions.
Within this phase I first-in-human clinical study, Vvax001 will be evaluated for immunogenicity and tolerability. When successfully completed, Vvax001 will be evaluated in a phase II clinical trial for clinical efficacy in terms of HPV clearance and regression of high-grade CIN lesions. Once Vvax001 is proven to be well tolerated, immunogenic and therapeutically effective in these phase I and II clinical trials, SFV can be further expanded as an immunization platform. This could be for the treatment of other HPV types, for the treatment of other HPV-related malignancies such as head and neck squamous cell carcinoma, or against a variety of other cancer antigens, including e.g. neo-antigens.

In conclusion, based on preclinical results, Vvax001 represents a promising therapeutic vaccine for the treatment of (pre)malignant lesions of the cervix caused by HPV16. The clinical results on immunogenicity and tolerability of Vvax001 in human are expected to be available mid-2018.

CONFLICT OF INTEREST

Toos Daemen and Hans Nijman are stock holders/founders of ViciniVax BV, a spin-off company of the UMCG, developing therapeutic cancer vaccines.

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REFERENCES


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