CANCER

Strong vaccine responses during chemotherapy are associated with prolonged cancer survival

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Therapeutic cancer vaccines have effectively induced durable regressions of premalignant oncogenic human papilloma virus type 16 (HPV16)–induced anogenital lesions. However, the treatment of HPV16-induced cancers requires appropriate countermeasures to overcome cancer-induced immune suppression. We previously showed that standard-of-care carboplatin/paclitaxel chemotherapy can reduce abnormally high numbers of immunosuppressive myeloid cells in patients, allowing the development of much stronger therapeutic HPV16 vaccine (ISA101)–induced tumor immunity. We now show the clinical effects of ISA101 vaccination during chemotherapy in 77 patients with advanced, recurrent, or metastatic cervical cancer in a dose assessment study of ISA101. Tumor regressions were observed in 43% of 72 evaluable patients. The depletion of myeloid suppressive cells by carboplatin/paclitaxel was associated with detection of low frequency of spontaneous HPV16-specific immunity in 21 of 62 tested patients. Patients mounted type 1 T cell responses to the vaccine across all doses. The group of patients with higher than median vaccine-induced immune responses lived longer, with a flat tail on the survival curve. This demonstrates that chemoimmunotherapy can be exploited to the benefit of patients with advanced cancer based on a defined mode of action.

INTRODUCTION

T cells recognizing tumor-specific antigens created by DNA mutations or oncogenic viruses are key to the success of cancer immunotherapies (1, 2). Therapeutic vaccines can selectively enlarge the pool of tumorspecific T cells. However, cancer treatment requires appropriate countermeasures to overcome the systemic and local suppression of T cell activation, expansion, and effector function imposed by suppressive myeloid cell populations, regulatory T cells (Tregs), and coinhibitory molecule expression (3–6).

Human papillomavirus type 16 (HPV16) is a major HPV type causing anogenital cancers and the predominant HPV type causing oropharyngeal cancers. Studies in healthy individuals and patients have assigned a major role for HPV16 E6/E7 oncoprotein-specific type 1 T cell immunity in the protection against progressive premalignant disease and a better response to standard therapy at the stage of cancer (7, 8). Despite all patients with progressive disease being infected with HPV16, such an immune response is usually not demonstrable or insufficient to have clinical impact (7–10). Raising the numbers of HPV-specific T cells by therapeutic vaccine monotherapy is effective in a high proportion of patients with HPV16-induced cervical or vulvar premalignant lesions (11–13). Clinical benefit in these studies was associated with the strength of the vaccine-induced type 1 T cell response (4, 11–14). However, vaccination alone of patients with late-stage HPV16-induced cervical cancer elicited weak T cell immunity and showed no clinical benefit (15). Potential explanations included generalized and local immune suppression by myeloid cells, Treg and immune checkpoint inhibitor expression (3–6). To overcome such mechanisms, we have explored several chemotherapeutics and demonstrated synergistic effects with HPV16 synthetic long peptide (SLP) vaccination in two mouse models of HPV-induced cancer (16, 17). Careful analysis of the immunomodulatory effects of chemotherapy revealed that the combination of carboplatin and paclitaxel elicited a systemic and local reduction in the cancer-driven abnormal numbers of immunosuppressive myeloid cells (18). A pilot experiment in patients with late-stage cervical cancer showed the optimal time point to start vaccination with the therapeutic HPV16-SLP vaccine ISA101 to be 2 weeks after the second cycle of carboplatin/paclitaxel. At that time point, the...
systemic abnormally high numbers of immunosuppressive myeloid cells had declined to the numbers seen in healthy donors. This coincided with decreased immunosuppression and a stronger T cell response to a single timed ISA101 vaccine injection (18).

These findings led to the design of the current study, in which patients with late-stage HPV16-positive cervical cancer were vaccinated with ISA101 in a timed manner during chemotherapy. Successive patient cohorts were vaccinated with increasing ISA101 doses, and half of the patients in each cohort also received pegylated type 1 interferon (IFN) (PegIntron). This was tried because IFNα is a strong promoter of cross-presentation of proteins by dendritic cells (DCs) (19) and is responsible for the up-regulation of costimulatory molecule expression and costimulatory cytokine production by DCs (20) and also promotes more efficient processing and presentation of long peptides (21). In addition, our previous study suggested a positive effect of this agent on the vaccine-induced type 1 immune response (22). Safety and immunogenicity of the ISA101 vaccine combined with carboplatin/paclitaxel were the primary endpoints. A secondary endpoint was the clinical benefit of the combined treatment.

**RESULTS**

**Patients and treatment**

Patients (n = 79) with advanced [Fédération Internationale de Gynécologie et d’Obstétrique (FIGO) stage IIIb/IVa with involvement of lymph nodes beyond the renal vein], metastatic (FIGO stage IVb), or recurrent HPV16+ cervical cancer were enrolled in Gynécologie et d’Obstétrique (FIGO) stage IIIb/IVa with involvement.

Patients with late-stage HPV16-positive cervical cancer were vaccinated (22). Safety and immunogenicity of the ISA101 vaccine combined with carboplatin/paclitaxel were the primary endpoints. A secondary endpoint was the clinical benefit of the combined treatment.

**Safety and toxicity**

The safety analysis was performed for the safety population (SAF) in the full ISA101 cohort (n = 77) and was descriptive. A summary of the treatment-emergent adverse events (TEAEs) related to any treatment component is shown in table S3. Most every patient (98.9%) reported chemotherapy-related adverse events (AEs). In addition, more than 80% of the patients reported an AE probably/possibly/definitely related to the trial medication (ISA101 and/or IFNα). Less than 16% of all patients withdrew to an AE. Special attention was given to TEAEs possibly related to ISA101. For TEAEs that were considered by the investigator to be at least possibly related to ISA101 vaccination (n = 72), there were 63 (87.5%) patients with TEAEs, 16 (22.2%) patients with treatment-emergent serious AEs (SAEs), 20 (27.8%) patients with National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade 3 or 4 TEAEs, and 4 (5.7%) patients with TEAEs that led to withdrawal (table S4). From a comparison of tables S3 and S4, it follows that most of the serious TEAEs in the current study were expected toxicities related to chemotheraphy or to complications associated with progression of cervical cancer. Previous trials documented treatment-emergent injection site reactions (TEISRs, defined as vaccination site or ISRs) and treatment-emergent systemic allergic reactions (TESARs, defined as drug hypersensitivity, hypersensitivity, injection-related reaction, systemic inflammatory response syndrome, vaccination complication, or cytokine release syndrome) as TEAEs that are likely induced by vaccination and thus regarded as AEs of special interest. TEISRs were reported in 50 (69.4%) of 72 patients receiving at least one vaccination with ISA101 (57). We were typically more than one event per patient. The incidence of TEISRs appears to be dose related (fig. S2) and characterized by mostly NCI-CTCAE grade 1 or 2 event. None was graded serious, and the TEISR of only one patient was classified as NCI-CTCAE grade 3, which was observed at the highest dose level of 300 μg per peptide of ISA101. The events are potentially related to the amount of Montanide administered together with the vaccine ISA101. The amount of Montanide is proportional to the vaccine dose, and known typical AEs for Montanide are local pain and/or induration and/or redness at the injection site. TESARs related to ISA101 were reported in 11 (15.3%) of the 72 patients receiving at least one vaccination (table S5). Five (6.9%) patients were reported to have serious TESARs that were considered to be possibly related to ISA101, and all but one were at a dose level of 300 μg per peptide. The highest numbers of patients with TESARs, serious TESARs, and TESARs of grade 3 or 4 were reported for the 300 μg per peptide group. Two (2.8%) patients experienced TESARs that led to withdrawal from the study drug. One patient had a grade 3 TESAR reported at the 100 μg per peptide dose level. Although the event was determined to have occurred within 30 min after receiving intravenous contrast material but 2 to 3 hours after ISA101 (with a clear temporal relationship to intravenous contrast material), a relation to ISA101 could not be absolutely excluded, so the reaction was reported to be possibly related to ISA101. In addition, one patient at the 300 μg per peptide dose withdrew from ISA101 vaccination because of a TESAR after the second vaccination. Together, ISA101 was generally safe and well tolerated in that its safety profile was not different from chemotherapy alone. The addition of ISA101 (with or without IFNα) was not different from paclitaxel/carboplatin chemotherapy alone, with the exception of mild to moderate injection site reactions in 69.4% of the patients and systemic allergic reactions in 15.3% of the 77 patients. These reactions were most prominent at the 300-μg dose. Grade 3 or 4 AEs due to any cause led to drug withdrawal in 12 of 77 patients.

**Immunogenicity**

Carboplatin/paclitaxel reduced the absolute number of circulating leukocytes, whereas the lymphocyte counts remained stable (Fig. 1B and fig. S3). This resulted in a shift of the proportions of lymphocytes and myeloid cells measured by flow cytometry (Fig. 1C and fig. S3), with a specific decline in CD14+ monocyte myeloid-derived suppressor cells (mMDSCs) (24) but not CD14+ HLA-DR+ myeloid...
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Nondepleted PBMCs
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by in E samples as in (C) (see fig. S3B for the gating strategy). (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. (G64 immunologically evaluable SAF90 patients. 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cells (Fig. 1D and fig. S3), and a concomitant relief of immune suppression demonstrated by an increased T cell response to a mixture of common microbial antigens [memory response mix (MRM)] and the increased detection of spontaneous low-frequency T cell reactivity against the HPV16 E6/E7 oncoproteins in 21 of 62 tested patients (Fig. 1, E and F) who were evaluable for this analysis because it required measurable HPV and MRM responses at V4 (fig. S1). The in vitro depletion of CD14+ myeloid cells before any therapy recapitulated the chemotherapy effect with detection of spontaneous HPV-specific immunity (Fig. 1G). The frequency of T cells expressing checkpoints programmed cell death protein 1 (PD-1), T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), and natural killer group 2 member A (NKG2A) did not indicate an alternative mechanism for enhanced T cell reactivity (fig. S4A). In all 64 evaluable patients, ISA101 vaccination induced HPV16 E6/E7-specific T cell responses to one or more peptide pools (fig. S1 and table S6). The response after the ISA101 vaccinations was much higher than before vaccination, which was at the time that carboplatin/paclitaxel had alleviated myeloid cell suppression of T cell reactivity (V4, 2 weeks after the second cycle of chemotherapy). The presence

![Fig. 2. ISA101-induced T cell reactivity in relation to PegIntron coinjection and Treg.](image-url)

(A) Cumulative specific spot counts for HPV16 E6/E7-specific T cells against six peptide pools per patient (circles), measured by validated IFNγ-ELISPOT, at indicated doses, for the indicated number of patients and time points (SAF90 population). Median and interquartile range are shown. MRM responses are of all patients at that time, irrespective of dose. V8/V10 values were compared with V4 (asterisks) or V0 (all P < 0.0001). (B) Specific production of IFNγ (n = 40 of 50) and TNFα (n = 21 of 50) in supernatants of 6-day HPV16 E6/E7 peptide pools or of MRM-stimulated PBMCs, before and after one ISA101 dose, analyzed by cytometric bead array. The sum of the HPV16-specific cytokine production (left y axis) for each patient (n = 50) irrespective of dose and MRM-specific cytokine production (right y axis) shown by boxes and whiskers with minimum and maximum, all data points. (C) The frequency of HPV16 E6/E7-specific CD4+Tbet+ and CD8+Tbet+ T cells (median and interquartile range) in available PBMCs at V8 or V10 of n = 32 patients from the SAF90 population after 8 days of in vitro stimulation measured by flow cytometry. Patients were divided into tertiles according to their median of MRM (MoM) in the IFNγ-ELISPOT. (D) T cell reactivity (boxes and whiskers with minimum and maximum) against medium control (Ctr), HPV16 E6/E7 peptides (HPV), or MRM, measured by IFNγ-ELISPOT irrespective of vaccine dose (SAF90 population). (E) The frequency of HPV16 E6/E7-specific Tbet+CD4+ T cells, Tbet+CD8+ T cells, and Treg in nine strong (left; MoM > 130) and eight weak (right; MoM < 40) IFNγ-ELISPOT responders from the 100- to 300-µg dose groups at V8/10. Asterisks indicate significance [**P < 0.01, ***P < 0.001, ****P < 0.0001]; Mann-Whitney (A, C, E) or paired Student’s t test (B).
of a preexisting HPV16-specific immune response at V4 did not affect the strength of the vaccine-induced response (fig. 5A), nor did the V4 serum concentrations of the cytokines transforming growth factor–β, interleukin-4 (IL-4), and vascular endothelial growth factor, which are known to be associated with immune suppression (fig. S4C) (25). The recall antigen (MRM)–specific T cell response remained constant (Fig. 2A), and therefore, the substantially increased HPV16-specific T cell response in all dose cohorts, measured by a validated IFNγ–ELISPOT (enzyme-linked immune absorbent spot) assay, indicated a specific effect consistent with vaccination and not a general effect of enhanced immune function. However, there were no clear differences in vaccine reactivity between the different dose cohorts (Fig. 2A), indicating a broad dose–response optimum. T cell reactivity to unrelated recall antigens was unaffected by ISA101 (Fig. 2A). T cell proliferation after the first vaccination was associated with the production of high concentrations of IFNγ, followed by IL-5, IL-10, and tumor necrosis factor–α (TNFα) (Fig. 2B and fig. S4D), the production of these four cytokines was especially found among the strong responders in the IFNγ-ELISPOT assay (fig. S4E).

The composition of the HPV16 E6/E7–specific T cell response was analyzed in 32 patients with a weak, intermediate, or strong response in the IFNγ-ELISPOT assay, for whom sufficient peripheral blood mononuclear cells (PBMCs) had been cryopreserved. We observed a correlation between the increased frequencies of Tbet-expressing CD4+ and CD8+ T cells after HPV-specific stimulation and the vaccine-induced response as measured by IFNγ-ELISPOT (Fig. 2C and figs. S4F and S5), showing the concordance between the percentage of Tbet-expressing T cells and the number of IFNγ-producing cells in two different assays. Moreover, increased CD8+ T cell frequencies were predominantly found among the strong responders (Fig. 2C). No differences in the proportion of Tbet+ T cells were found between patients vaccinated with or without PegIntron (fig. S4F), and there were no differences in IFNγ-ELISPOT responses between these two patient groups (Fig. 2D). In all subsequent analyses of the vaccine response as measured by IFNγ-ELISPOT, the data of patients with or without PegIntron were therefore combined. Patients with HPV16-induced (pre-) malignancies may display circulating and tumor-infiltrating Treg that are reactive to the HPV16 E6/E7 oncoproteins (3, 10). Increased numbers of these Treg in the blood correlated with a less successful immune and clinical response to HPV16-SLP vaccination (4, 26). We therefore assessed the presence of circulating HPV-specific Treg in a group of 17 patients who mounted a relatively strong or weak response to the two highest vaccine doses and for whom sufficient PBMCs were available for testing. HPV-specific Treg were present in seven of eight cultures of patients with a low vaccine response, and this coincided with the outgrowth of lower percentages of Tbet+CD4+ and Tbet+CD8+ T cells, whereas the cultured cells from nine strong T cell responders to the vaccine displayed no outgrowth of HPV-specific Treg (Fig. 2E and fig. S5).

**Efficacy**

Chemoimmunotherapy resulted in regressions (43%) and stable disease (43%) in 62 of 72 patients with an available assessment according to response evaluation criteria in solid tumors (RECIST) 1.1 (table S7), and this included regression of the target lesion in 29 of 59 patients with a measurable target lesion (Fig. 3 and fig. S1). Because all patients received chemotherapy, known to be active in cervical cancer (27), in addition to ISA101, it is difficult to interpret short-term clinical outcomes as being due to chemotherapy alone or to the combination. PegIntron did not appear to provide any additional clinical benefit in this trial (table S8). In addition, the groups of patients treated with different ISA101 vaccine doses showed no apparent differences in tumor shrinkage (Fig. 3) or in survival of the patients (Fig. 4A). However, when the effect of the immune response on overall survival (OS) was compared in the 64 evaluable patients (fig. S1), the median OS of the group of 32 patients with a relatively strong (higher than median) vaccine-induced HPV16-specific immune response, as measured by a validated IFNγ-ELISPOT, was significantly longer (16.8 months) compared to the 32 patients with a low (less than median) vaccine-induced HPV-specific immune response [11.2 months; log-rank P = 0.012; hazard ratio (HR), 0.491] (Fig. 4B). Eleven of 14 patients still alive at the end of the study displayed a strong vaccine-induced response and included 9 patients with FIGO stage IVa/IVb who had a mean OS of 3 years. This effect was not overtly different when considering changes in immune status independent of vaccination, as measured by alterations in reactivity to the recall antigen mixture (MRM) (Fig. 4C). The OS was also not influenced by a preexisting HPV16-specific T cell response (fig. S6A). Overall, the group of chemotherapy-naïve patients entered in this trial did not display better survival than the group of patients who had received prior therapy (fig. S6B and table S9). However, in both categories, a strong vaccine-induced HPV-specific immune response was correlated with a better OS (fig. S6C).

**DISCUSSION**

This study reports a favorable outcome of ISA101 HPV16 therapeutic vaccination during standard-of-care chemotherapy in a subgroup of patients with advanced, recurrent, or metastatic cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101. We could confirm the reduction of myeloid cell numbers, but not lymphocyte cervical cancer in a dose assessment study of ISA101.
strong type 1 HPV16-specific T cell response to the vaccine across all doses. The group of 32 patients with higher than median vaccine-induced immune responses lived longer, with a flat tail on the survival curve, when compared to the 32 patients with lower than median induced HPV-specific immune responses. This difference was not explained by preexisting HPV16-specific immunity or the general immunocompetence of T cells because the reactivity to unrelated microbial antigens was similar in both groups.

In general, the immune system of patients with late-stage cancer is compromised to such an extent that vaccine monotherapy generally fails (28). Carboplatin/paclitaxel has a direct cytoreductive effect on tumors but also acts through various nonoverlapping immune mechanisms. First, we confirmed that carboplatin/paclitaxel transiently decreases systemic immunosuppression by immature mononuclear myeloid cells. According to our animal studies, this also occurs within the tumor (18). To obtain this effect, carboplatin/paclitaxel may be used in the treatment of several types of cancer because cancer-related leukocytosis is commonly observed in patients with immunogenic tumors (29, 30). Furthermore, we have previously shown that chemoinmunotherapy with a platinum compound synergizes with TNFα released from vaccine-induced T cells to induce tumor cell apoptosis (17) and enhances immune infiltration into the tumor, as shown for cisplatin in mice (16). Our earlier study showed that carboplatin/paclitaxel does not alter the numbers of Tregs in patients (18), explaining why the presence of HPV-specific Tregs can be associated with a lower vaccine-induced T cell response in some of the patients in this study. Tumor-infiltrating Tregs, including HPV-specific Tregs, are frequently detected in cervical cancer (3, 31, 32). In addition, the great majority of advanced and metastatic cervical cancers display down-regulation of human leukocyte antigen (HLA) class I and II molecules (33). These factors are independently associated with worse clinical outcome (31) and may also curtail the clinical response to vaccination.

In a recent study, patients with late-stage HPV16+ oropharyngeal cancer who failed to spontaneously mount a strong
HPV-specific T cell response (8) appeared to benefit from ISA101 vaccination when combined with nivolumab (34). Patients treated with this combination had about twice the overall response rate and twice the median survival time compared to those treated in the registration study of nivolumab monotherapy for this disease indication (35). ISA101 reinstated a tumor-specific immune response, which presumably was locally suppressed by the adaptive expression of PD-1 and programmed death-ligand 1 (PD-L1) (8, 36). A better clinical response to checkpoint blockade when the HPV16-specific T cell response is increased is consistent with the good clinical response of checkpoint blockade-treated Merkel cell virus–induced cancers, which are known to spontaneously induce strong antitumor responses (1). The current study shows that patients with recurrent or metastatic HPV16+ cervical cancer may also benefit from ISA101 treatment. We treated a heterogeneous population of patients with advanced/metastatic cervical cancer, of whom 45.4% were previously treated with chemotherapy, and 13% with more than one regimen. A systematic review on the use of cisplatin- and carboplatin-based chemotherapy regimens for first-line advanced/ metastatic disease (stages IVa/IVb) reported an objective response rate (ORR) of 48.5% for cisplatin and 49.3% for carboplatin and an OS of 12.9 and 10 months, respectively (37). In a phase 3 study, the ORR in patients with stage IVB (not IVA) recurrent or persistent cervical carcinoma ranged from 23.4 to 29.1%, depending on the doublet chemotherapy regimens used (38). A recent study of first-line chemotherapy in a mixed population of patients with advanced metastatic and recurrent cervical cancer revealed an ORR of 46% (39) and an OS of 13.3 months (40). Thus, the ORR and OS in our trial are well within the ranges that can be expected in this type of population, whereby it is clear that the patients experiencing a higher than median vaccine-induced HPV16-specific immune response have a meaningfully longer median OS (16.8 months), regardless of the number of previous lines of treatment. The median OS of the entire group of first-line–treated patients was 16.1 months, high and low HPV vaccine responders combined, and was close to the outcome of the study in which a mixed population of patients with advanced metastatic and recurrent cervical cancer was treated first line with the combination of chemotherapy and bevacizumab (39, 40). The flat tail end in the survival plot of strong ISA101 responders is encouraging and consistent with that of patients with melanoma treated with ipilimumab to broaden the tumor-reactive T cell response (2, 41). The nonredundant nature of the mechanisms that play a role when ISA101 vaccine is combined with either anti–PD-1 or chemotherapy calls for future studies in which the benefit of triple therapy will be investigated in a randomized fashion. This will also overcome the limitation of the current open-label single-arm study, which was not randomized for ISA101 vaccine in combination with carboplatin/paclitaxel versus standard-of-care chemotherapy alone.

This study comes with two important limitations. We were not able to collect pre- and post-vaccination tumor material, the study of which may have allowed us to determine the myeloid cell–depleting effect in the tumor microenvironment as well as the presence of T cell infiltration and particular therapy-induced changes in that compartment. Such a study may have revealed under which conditions the applied combination therapy works best. Furthermore, the study design did not include an arm in which patients received chemotherapy only, which would have allowed us to make a firmer statement on the effects of ISA101 treatment.

In conclusion, standard-of-care carboplatin and paclitaxel chemotherapy of late cervical cancer depletes suppressive myeloid cells, but not lymphocytes, allowing a robust T cell immune response to timed administration of an SLA-based therapeutic vaccine against HPV16 E6/E7. Patients with a stronger than median vaccine-induced HPV16-specific T cell response lived longer. This type of chemoinmunotherapy approach may be generally applicable if the vaccine ingredients are changed to tumor-associated or neoantigen-containing SLP instead of HPV-SLP.

**MATERIALS AND METHODS**

**Study design**

This was a multicenter, open-label nonrandomized phase 1/2 study with expansion cohorts to determine the safety and immunomodulating effects of the therapeutic HPV16 E6/E7 SLP vaccine (ISA101) as immunotherapy in combination with standard-of-care chemotherapy (carboplatin and paclitaxel) in women with HPV16-positive advanced (FIGO stage IIIb-IVa with involvement of lymph nodes beyond the renal vein), metastatic (FIGO stage IVb), or recurrent cervical cancer. All individuals who participated in this trial provided written informed consent before any trial-related procedure being performed. The trial was overseen by a data monitoring committee that reviewed the safety data and study conduct at specified time points during the trial. The trial was registered under NCT02128126 and EudraCT 2013-1804-12 and consisted of two stages. In the first stage of the trial, eight cohorts of six patients each were planned to be enrolled. Four doses of ISA101 (20, 40, 100, and 300 μg per peptide) were evaluated without and with pegylated IFNα (1 μg/kg) in combination with fixed doses of standard-of-care chemotherapy: carboplatin at an area under the curve (AUC) of 6 mg/ml per minute and paclitaxel at a dose of 175 mg/m². In the second stage, the expansion stage, six additional patients were planned to be enrolled each at 40 and 100 μg per peptide dose of ISA101, without IFNα. ISA101 consists of nine overlapping E6 peptides (five 32-mer and four 25-mer E6 peptides) and four 35-mer E7 peptides (table S10). Patients were evaluable for immunogenicity if they received at least one vaccination with ISA101 and had a prevaccination blood sample and at least one postvaccination blood sample (all with sufficient PBMCs).

**Inclusion and exclusion criteria**

**Inclusion criteria**

On the basis of the protocol version 9.0, to be eligible to participate in this study, candidates must have met the following eligibility criteria: (i) women ≥18 years of age; (ii) cervical cancer confirmed by histology; (iii) advanced (FIGO stage IIIb/IVa with para-aortic lymph node involvement beyond the renal vein) or metastatic (FIGO stage IVb) or recurrent cervical cancer confirmed by clinical and/or radiological proof with no curative treatment options; (iv) HPV16-positive tumor (determined on archival tumor tissue ≤10 years old; if that was not available, a pretreatment biopsy was required); (v) patients eligible for chemotherapy with carboplatin and paclitaxel and having consented to chemotherapy with carboplatin and paclitaxel before the start of the informed consent procedure for the study; (vi) performance status [World Health Organization scale/Eastern Cooperative Oncology Group (ECOG)] ≤1; (vii) written informed consent according to local guidelines.
Exclusion criteria
Patients fulfilling any of the following criteria were not to be enrolled in the study (protocol v 9.0): (i) prior treatment with anti-HPV agents; (ii) chronic systemic steroid use (local application of stable doses of topical or inhaled corticosteroids was allowed); (iii) less than 4 weeks since the last treatment with other cancer therapies (endocrine therapy, immunotherapy, radiotherapy, and chemotherapy), less than 8 weeks after cranial radiotherapy, and less than 6 weeks after nitrosourea or mitomycin C; (iv) toxicities resulting from previous anticancer therapy (radiation, chemotherapy, or surgery) must have resolved to grade ≤2 as defined by CTCAE version 4.03; (v) recent treatment (within 30 days of first study treatment) with another investigational drug; (vi) patients with known hypersensitivity to any component of the Investigational Medicinal Product (ISA101, Montanide, and dimethyl sulfoxide (DMSO) or IFN α (DMSO) or IFN α; (vii) any contraindication to the use of authorized applied products (paclitaxel or carboplatin); (viii) inadequate bone marrow function: absolute neutrophil count (ANC) <1.5 × 10^9/liter, platelet count <100 × 10^9/liter, or hemoglobin <6 mM; (ix) inadequate liver function, defined as serum (total) bilirubin >2× upper normal limit (ULN), aspartate aminotransferase or alanine aminotransferase >2.5 × ULN (>5× ULN in patients with liver metastases), or alkaline phosphatase >2.5 × ULN (>5× ULN in patients with liver metastases or >10× ULN in patients with bone metastases); (x) clinical suspicion or radiological evidence of brain or leptomeningeal metastases [a computed tomography (CT) or magnetic resonance imaging (MRI) scan should be performed if there was any clinical evidence of brain metastases]; (xi) previous or current malignancies at other sites, with the exception of basal or squamous cell carcinoma of the skin and with the exception of other malignancies from which the patient may be considered cured as evidenced by complete regression of all lesions >10 years ago; (xii) active HIV, chronic hepatitis B, or hepatitis C infection; (xiii) patients of childbearing potential (defined as <2 years after last menstruation and having an intact reproductive system) not willing to consistently and correctly use a contraceptive method resulting in low failure rate of less than 1% per year such as oral contraceptives; (xiv) pregnancy or lactation (serum pregnancy test was to be performed within 7 days before study treatment start in patients of childbearing potential); (xv) major surgical procedure within 28 days before the first study treatment; (xvi) uncontrolled sustained hypertension (systolic >180 mmHg and/or diastolic >110 mmHg); (xvii) clinically relevant (active) cardiovascular disease defined as: stroke within ≤6 months before day 1, transient ischemic attack within ≤6 months before day 1, myocardial infarction within ≤6 months before day 1, unstable angina, New York Heart Association grade II or greater congestive heart failure (42), or serious cardiac arrhythmia requiring medication; (xviii) history of severe bronchial asthma and/or severe allergy; and (xix) evidence of any other medical conditions (such as psychiatric illness, infectious diseases, and autoimmune diseases) that would interfere with the planned treatment (the possibility of receiving all six cycles of planned chemotherapy including the concomitant vaccinations), affect patient adherence to the study treatment, or place the patient at high risk of treatment-related complications.

Study treatment
The ISA101 vaccine contains nine HPV16 E6 and four HPV16 E7 SLPs (table S10). The ISA101 peptides were dissolved in DMSO and subsequently diluted in water for injection (WFI) and emulsified with Montanide ISA51 (SEPPIC). The final ratio of DMSO/WFI/Montanide was 20/30/50 as described previously (I1). The subcutaneous route of administration of ISA101 was dictated by the use of Montanide. The ISA101 vaccine was administered via two subcutaneous injections in two different limbs. It was recommended to rotate the vaccine injections between the limbs, for example, first vaccination in the upper leg, second vaccination in the upper arm, and third vaccination in the other upper leg. This procedure assured more uniformity of ISA101 administration and allowed for a better systematic assessment of ISRs.

Pegylated IFNα (PegIntron) was used as an immune modulator in specified even-numbered cohorts (two, four, six, and eight) and was injected subcutaneously within 10 cm of each vaccination site at a dose of 1 μg/kg bodyweight in two equally divided doses. Hence, the total dose per vaccine day equaled the standard dose used for treatment of chronic hepatitis B virus infection, namely, 1 μg/kg body weight. Chemotherapy was administered intravenously, as specified in the product labeling for carboplatin and paclitaxel, given at fixed doses of carboplatin at an AUC of 6 mg/ml per minute and paclitaxel at a dose of 175 mg/m^2.

Endpoints
Primary endpoints
Safety (according to NCI-CTCAE version 4.03) was determined by the incidence rate at each dose on the basis of the following safety parameters: AEs and SAEs, changes in hematology and chemistry values (including those associated with hepatic and renal function), as well as assessment of physical examinations, vital signs, and performance status. HPV-specific immune responses to the ISA101 vaccine with or without IFNα in combination with carboplatin/paclitaxel were determined by the quality, breadth, and magnitude of the HPV16 E6/E7-specific T cell responses as measured by a validated assay (IFNγ-ELISPOT) after injection of different doses of the ISA101 vaccine.

Secondary endpoints
Antitumor efficacy (according to RECIST 1.1) was studied by the following parameters: ORR (the proportion of patients with a best overall response of confirmed complete response or partial response), tumor shrinkage as measured by the sum of longest dimensions of target lesions, duration of the response (the time from objective response to documented progression or death from any cause), PFS (the time from start of carboplatin/paclitaxel treatment to documented progression or death from any cause), and OS (the time from start of carboplatin/paclitaxel treatment to death).

Study duration
From September 2013 to October 2016, 79 patients were enrolled in 11 hospitals in The Netherlands and Belgium. The trial included 3 weeks of screening, 18 weeks of treatment (six cycles of chemotherapy), a 30-day follow-up after the end of the last cycle of chemotherapy (up to 10 weeks after the last dose of ISA101 for safety), and an additional 34-week follow-up after treatment for assessment of clinical endpoints. CT/MRI scans were performed at screening, after the third cycle of chemotherapy, at the end of therapy, and at weeks 30, 42, and 52 during follow-up. The total trial duration was up to 55 weeks per patient. Per amendment, patients were to be followed for progression-free survival (PFS) and OS for an additional period of up to 3 years or until death, whichever was first. Patients who had already completed all visits and who were still alive were asked for reconsent to collect the follow-up information.
Data reporting
The study was an open-label nonrandomized dose-finding study. At each dose of ISA101, patients in odd-numbered cohorts received chemotherapy and ISA101 vaccine alone followed by even-numbered cohorts that received chemotherapy and ISA101 with the immune modulator pegylated IFNα. The standard 3 + 3 patients per cohort design was used, which was consistent with the cancer vaccine clinical trial working group guidelines for proof-of-principle trials. No statistical method was used to predetermine the sample size. Data from different predefined groups of patients as explained in Fig. 1A were used for reporting. The safety data were reviewed by the data monitoring committee at each dose of ISA101 before dose escalation was allowed.

Antigens for immunomonitoring
The HPV16 peptides used for the immunomonitoring were the same as used in the ISA101 vaccine. Peptide pools consisting of two to three peptides were prepared as follows: E6.1 covering amino acids 1 to 50 (E61–125; E61–69; E61–50), E6.2 (E641–651; E655–801; E671–95), E6.3 (E685–1096; E681–1222), E6.4 (E609–1401; E627–1548), E7.1 (E71–352; E722–56), and E7.2 (E743–75; E756–80) (11). The exact sequences are given in the table S10. The mix of common microbial antigens in the MRM included tetanus toxoid, tuberculin purified protein derivative, and Candida albicans (4, 11, 18).

Immunomonitoring
Peripheral blood samples were taken at baseline [visit 0 (V0)], immediately before the first (V4), second (V6), and third vaccination (V8), 3 weeks after the third vaccination (V10), and after chemotherapy was completed (FU). Sodium-heparinized blood was subjected to Ficol gradient centrifugation within 6 hours after withdrawal to isolate the PBMCs, which were cryopreserved by controlled freezing and stored in the vapor phase of liquid nitrogen until needed. Fresh PBMCs (V4 and V6) were stimulated with HPV16 E6 and E7 peptide pools (table S10) in the lymphocyte stimulation test (LST), from which the conditioned medium was harvested at day 6 and analyzed for cytokines by Becton Dickinson. The IFN-γ ELISPOT cryopreserved PBMCs were thawed, counted, and plated in 1 ml of medium in one well in a 24-well plate. The PBMCs were stimulated by adding different peptide pools (10 μg/ml). At day 4, the cells were harvested, counted, and prepared in X-VIVO 15 medium (Lonza) at the indicated cell concentration, and transferred onto an IFN-γ-capture antibody (Mabtech)—coated nitrocellulose-backed 96-well ELISPOT plate (Millipore) in quadruplicate test wells. After an overnight incubation, the ELISPOT plates were developed, and the numbers of spots per well were counted using an ELISPOT reader.

The linear cell density range for this 4-day IFN-γ-ELISPOT assay was 25,000 to 100,000 cells per well.

Spots were only detected under the condition where both the capture and the detection antibody (both from Mabtech) were used.

The preset acceptance criteria for the intra-assay and intraoperator coefficient of variation (CV) were ≤40% for responses with an average of >30 spots per 100,000 cells and ≤60% for responses between 10 and 30 spots per 100,000 cells.

The observed intra-assay variation was well within the preset acceptance criteria, with >90% of responses (84 of 93) with a spot count >30 displaying a CV equal to or less than 25%, and all others were below CV of 35%. Moreover, for 86% (13 of 15) responses with a spot count between 10 and 30, the CV was <40%, whereas for the remaining 2, the CV was <60%.

The intraoperator variability was analyzed by calculation of the CV using the averaged mean and averaged SD from the three different means and SDs of each donor/Ag combination obtained from three plates simultaneously tested on the same day. For all 31 datasets of responses with >30 spot counts, the intraoperator CV was below 30%. For the five datasets of responses with a spot count between 10 and 30, the intraoperator percent CV never exceeded 41%. Thus, the observed intraoperator variation was well within the preset acceptance criteria.

The interassay variability was determined by calculation of the CV using the averaged mean and averaged SD from the three different means and SDs of each donor/Ag combination tested on three different days. For 28 of 32 datasets of responses with >30 spot counts, the interassay CV was below 20%, whereas for the remaining 4 datasets, the CV was below 30%. For the four datasets of responses with a spot count between 10 and 30, the interassay CV never exceeded 40%. Thus, the observed interassay variation was well within the preset acceptance criteria.

The accuracy of the test ranged from 96 to 184% within the range of 2- to 32-fold dilutions. An accuracy of 184% indicates that twofold differences measured in outcomes should not be considered to be a different result. Thus, a greater than twofold difference in spot counts between different samples was defined as a true different response.

The lower limit of detection (LLOD) was determined, and this ranged between 5 and 9 spots per 100,000 cells per well. As a safe estimate, the LLOD of the reader spot counts was set to 10, and responses below the LLOD were not taken into account as measurement of positive responses.
The maximally detected number of spots was 323, and therefore, the upper limit of detection (ULOD) of the ELISPOT reader was 323. The lower limit of quantification for this assay was defined as the lowest measurable response over background, or LLOD, which was 10. The upper limit of quantification was defined by the maximum positive measurable response for the assay, or ULOD, which was 323. The optimal detection range for the ELISPOT reader, therefore, was 10 to 323 spots per well.

**Phenotyping by flow cytometry**

Thawed PBMCs (1.5 × 10⁶) were stimulated with HPV16 E6/E7 peptide mix (5 μg/ml) or cultured in medium only for 8 days to determine HPV16 E6/E7-specific Tregs and effector T cells as described before (24, 43). The frequency of Tregs (activated Tregs and naïve Tregs) and Tbet⁺ non-Treg effector cells was determined by staining the harvested cells using the anti-human antibodies (company, clone) directed against CD3 (BD, UCHT1), CD4 (BD, RPA-T4), CD8 (BD, HIT8a), CD25 (BD, 2A3), CD127 (BioLegend, A019D5), FoxP3 (BD, 259D/C7), CD45RA (BD, HI100), and Tbet (eBiosciences, HIT8a), CD25 (BD, 2A3), CD127 (BioLegend, HCD56). Myeloid cells were identified as CD3⁻ CD19⁻ CD56⁻ cells using the anti-human antibodies (company, clone) directed against CD3 (BD, UCHT1), CD19 (BD, SJ25C1), and CD56 (BioLegend, HCD56). Myeloid cells were identified as CD3⁺ CD20⁻ cells, and T lymphocytes as CD3⁺ CD19⁻ cells, both within the CD56 population. Acquisition of the cells was done on a BD LSRSporta as the flow core facility of Leiden University Medical Center (LUMC), and flow data were analyzed using BD FACSDiva (version 8.0.2).

**CD14 depletion assay**

Stimulation of PBMCs in vitro with or without depletion of suppressive myeloid cells was performed as described earlier (18). Briefly, the CD14⁺ myeloid cells in V0 PBMCs of one patient with cervical cancer were depleted by magnetic cell sorting (Miltenyi) as described earlier. Depleted and nondepleted PBMCs were stimulated for 11 days with autologous monocytes (V4) pulsed with either MRM, a mix of the HPV16 E6/E7 SLP, or a mix of p53 SLP (control) and then tested in a proliferation test (triplicate wells) in which nonpulsed autologous monocytes served as a negative control and phytohemagglutinin (PHA) stimulation was a positive control.

**Statistics**

The trial was analyzed as per the Statistical Analysis Plan Version 1.0, and output was produced by the Statistics Department of Data Investigation Company Europe (DICE), Brussels, Belgium, using the Statistical Analysis System (SAS) version 9.2. The change in immune status between the blood samples isolated at V4 and V8/10 was defined as the absolute response to MRM at V4 minus the absolute response to MRM at V8 or V10 (depending on the highest reaction at either V8 or V10). The sum of the vaccine-induced HPV16 response was calculated on the basis of the HPV-specific T cell immune response against all six pools of peptides as measured in the validated ELISPOT assay. The strength of the vaccine-induced T cell response in a patient was defined as the median of the maximum (MoM) response to each of the six peptide pools at either V8 or V10 in the ELISPOT assay. Paired Student’s t test was performed when two blood samples of the same patient were analyzed. Mann-Whitney test was used to compare the results between two groups. For the Kaplan-Meier survival curves, log-rank test and Cox model giving the HR were used. The differences in clinical response according to RECIST 1.1 were determined by a two-sided Fisher’s exact test. P values below 0.05 were considered significant.

**SUPPLEMENTARY MATERIALS**

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Fig. 51. Flowchart of patient numbers for different analyses.

Fig. 52. Summary of TEBRs.

Fig. 53. Decreased myeloid cell and intact lymphocyte numbers upon chemotherapy.

Fig. 54. ISA101-specific induction of T cell responses.

Fig. 55. Gating strategy to detect HPV-specific Treg and Tbet⁺ effector cells.

Fig. 56. OS of patients when stratified for prior chemotherapy and immune response.

Table S1. Patient characteristics.

Table S2. Treatment history of the patients.

Table S3. Summary of TEARs per dose in ISA101 cohort.

Table S4. Summary of possible ISA101-related TEARs per dose in ISA101 cohort.

Table S5. Summary of TESRs per dose in vaccinated ISA101 cohort.

Table S6. T cell responses determined by 4-day IFNγ measurement.

Table S7. Clinical outcome per dose in vaccinated ISA101 cohort.

Table S8. Duration of objective responses based on prior systemic treatments.

Table S9. Duration of objective responses based on prior systemic treatments.

**REFERENCES AND NOTES**

1. P. T. Nghiem, S. Bhatia, E. J. Lipson, R. R. Kudchadkar, N. J. Miller, L. Annamalai, S. Berry, E. K. Chakraborty, A. Daud, S. P. Fling, P. A. Friedlander, H. M. Kluger, H. E. Kohrt, L. Lundgren, K. Margolin, A. Mitchell, T. Olenczyk, D. M. Pardoll, S. A. Reddy, E. H. Santha, W. H. Sharman, E. Sharon, L. R. Shershani, M. M. Shinozaka, J. C. Sunshine, J. M. Taube, J. A. Thompson, S. M. Twomloon, J. H. Yearley, S. L. Topalian, M. A. Cheever, P. S. Llorente, J. V. Mroz, and P. H. Yang, “Dendritic cells loaded with HPV16 E6/E7 peptides induce antitumor immunity in patients with HPV-positive cancer,” J. Exp. Med. 210, 1435–1436 (2013).

2. J. D. Wolchok, V. Chiarion-Sileni, R. Gonzalez, P. Rutkowski, J.-J. Grob, C. L. Cowey, C. D. Lao, J. Wagstaff, S. Schadendorf, P. F. Ferrucci, M. Smylie, R. Dummer, A. Hill, D. Hogg, J. Haenen, M. S. Carlingo, O. Bechther, M. Maio, I. Marquez-Rodas, M. Guidoboni, G. McArthur, P. B. Leppe, P. A. Asciero, G. V. Long, J. Geisboun, J. C. Sosman, M. A. Postow, M. K. Callahan, D. Walker, L. Rollin, R. Bhore, F. S. Hodj, J. Larkin, Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N. Engl. J. Med. 374, 2542–2552 (2016).

3. S. H. van der Burg, S. J. Piersma, A. de Jong, J. M. van der Hulst, K. M. Kwappenberg, M. van der Hende, M. J. Welters, J. V. Van Rood, G. J. Fleurens, C. J. Meijel, G. G. Kenter, R. Offringa, Association of cervical cancer with the presence of CD4⁺ regulatory T cells specific for human papillomavirus antigens. Proc. Natl. Acad. Sci. U.S.A. 104, 12087–12092 (2007).

4. M. J. Welters, G. G. Kenter, P. J. De Zoys, S. Smits, M. J. Löffler, D. M. Berendts-van der Meer, E. Essah, L. F. Stynenbosch, A. P. Woon, H. P. L. A. Ramwadhdoebe, C. A. Hulst, R. E. Dijkhuis, K. L. Franken, J. K. Jong, R. Schadendorf, P. Rood, C. Grob, C. A. van der Ham, S. Santegoets, R. C. E. Ehsan, E. Goedemans, I. J. van Egmond, T. H. van der Kuster, S. E. J. Jordanova, and J. S. van der Wel, Critical role of T-cell receptor cell fate in response to HPV16-positive vulvar lesions correlates with kinetics and phenotype of induced T-cell responses. Proc. Natl. Acad. Sci. U.S.A. 107, 11895–11899 (2010).

5. D. I. Gabrilovich, S. Nagaraj, Myeloid-derived suppressor cells as regulators of the immune system. Nat. Rev. Immunol. 9, 162–174 (2009).

6. M. J. Smyth, S. F. Ngijow, A. Ribas, M. W. Teng, Combination cancer immunotherapies tailored to the tumour microenvironment. Nat. Rev. Clin. Oncol. 13, 143–158 (2016).

7. A. de Jong, M. I. van Poelgeest, J. M. van der Hulst, J. W. Dijkhuis, G. J. Fleurens, C. J. Meijel, G. G. Kenter, R. Offringa, S. H. van der Burg, Failure or success of vaccination for HPV16-positive vulvar lesions: Correlations between viral load and phenotype of induced T-cell responses. Proc. Natl. Acad. Sci. U.S.A. 107, 11895–11899 (2010).

8. M. J. Welters, W. Ma, S. J. A. M. Santegoerts, R. Goedemans, L. Ehsan, E. S. Jordanova, V. J. van Ham, V. van Unen, F. Koning, S. I. van Egmond, P. Charoentong, Z. Trajanoski, L.-A. van der Velden, S. H. van der Burg, Intratumoral HPV16-specific T cells constitute a type I–oriented tumor microenvironment to improve survival in HPV16-driven oropharyngeal cancer. Clin. Cancer Res. 24, 634–647 (2018).
M. E. van der Steenwijk, S. J. Piersma, M. J. W.elters, J. M. van der Hulst, G. J. Fleuren, B. W. Hellebrekers, G. K. Genter, S. H. van der Burg. Surgery followed by persistence of high-grade squamous intraepithelial lesions is associated with the induction of a dysfunctional HPV16-specific T-cell response. Clin. Cancer Res. 2014, 19, 1626–1636 (2009).
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Data and materials availability: The materials used and the Statistical Analysis Plan for this study are available upon request. All data associated with this study are present in the paper or the Supplementary Materials. ISA Pharmaceuticals provided the vaccine, and any request concerning this vaccine should be directed to ISA Pharmaceuticals.

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Vaccinating a cancer away

Despite advances in prevention and early detection, cervical cancer remains prevalent worldwide and continues to cause mortality, thus requiring improved therapeutic interventions. One approach being developed for the treatment of cervical cancer is therapeutic vaccination targeting human papillomavirus 16 (HPV16), a key virus associated with the pathogenesis of this cancer. Melief et al. tested this therapeutic vaccine approach in 77 patients with advanced, recurrent, or metastatic cervical cancer who were also receiving standard chemotherapy with carboplatin and paclitaxel. Almost half of the tumors regressed with the combination therapy. The authors characterized the patients' immune responses and correlated them with the likelihood of successful treatment.