Title: A Phase Ib/II trial to test the safety and efficacy of vaccination with HPV16-E711-19 nanomer for the treatment of incurable HPV16-related oropharyngeal, cervical and anal cancer in HLA-A*02 positive patients

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TITLE: A Phase Ib/II trial to test the safety and efficacy of vaccination with HPV16-$E7_{11-19}$ nanomer for the treatment of incurable HPV16-related oropharyngeal, cervical and anal cancer in HLA-A*02 positive patients

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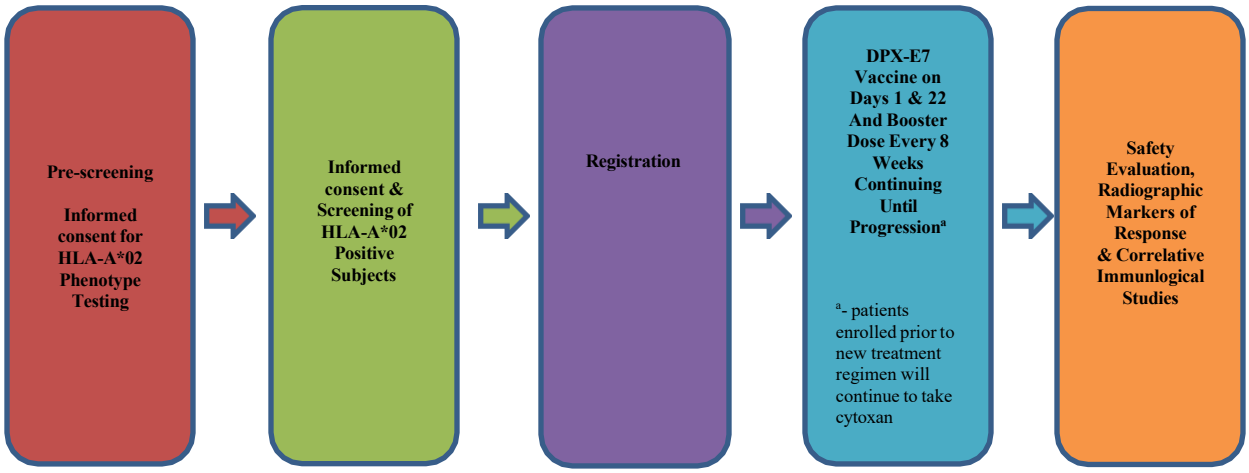
Agent: DPX-E7 vaccine, supplied by Immunovaccine Inc.

SCHEMA OF THE STUDY



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LIST OF ABBREVIATIONS

Abbreviation	Definition
β-hCG	Beta Human Chorionic Gonadotropin
AE	Adverse Event
ALT	Alanine Aminotransferase
ANC	Absolute Neutrophil Count
AST	Aspartate Aminotransferase
CBC	Complete Blood Count
CBCD	Complete Blood Count with Differential
cGMP	Certified Good Manufacturing Practice
CTCAE	Common Terminology Criteria for Adverse Events
DF/HCC	Dana-Faber/Harvard Cancer Center
DFS	Disease-free survival
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic Case Report Form
ELISpot	Enzyme-linked Immunosorbent Spot
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPV	Human Papilloma Virus
IB	Investigator's Brochure
IND	Investigational New Drug
IRB	Institutional Review Board
IV	Intravenous
MHC	Major Histocompatibility Complex
MS^3	Poisson detection Mass spectrometry
NCI	National Cancer Institute
NSAIDs	Non-steroidal Anti-inflammatory Drugs
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cell
PD	Progressive Disease
PFS	Progression Free Survival
PI	Principal Investigator
PS	Performance Status
SAE	Serious Adverse Event
QACT	Quality Assurance of Clinical Trials
RECIST	Response Evaluation Criteria in Solid Tumors
irRECIST	Immune-Related Response Evaluation Criteria in Solid Tumors

1. OBJECTIVES

1.1 Study Design

This is a single center, open label, non-randomized, phase Ib/II trial of HPV16-E7$_{11-19}$ nanomer vaccine (DPX-E7) for treatment of incurable HPV16-related oropharyngeal (HPVOC), cervical and anal cancer.

Safety and clinical efficacy of the DPX-E7 vaccine will be evaluated in up to n=44 patients (Considering that patients continue to be screened and at least n=7 patients have been entered and began protocol treatment at the original dose prior to approval of Amendment 10, and allowing for 1 or 2 patients to sign the main consent and to be registered to the main protocol but not begin protocol treatment or be ineligible, the overall accrual goal following approval of Amendment 10 is up to n=44).

The new treatment regimen (amendment 10) changed the treatment regimen, removing Cytoxan and doubling the vaccine dose. For patients enrolled on the trial before amendment 10's approval, they will continue to dose with Cytoxan and the original vaccine doses (0.25mL for priming, 0.1mL for boosting)

Potentially eligible patients with confirmed diagnosis of incurable oropharyngeal, cervical or anal cancer will be asked to sign a pre-screening consent that will allow us to use a blood sample for HLA testing.

HLA-A*02 positive subjects will sign the main consent and screened further. If eligible, will be registered and receive treatment with DPX-E7 vaccine.

Following approval of Amendment 10, the below stopping rule will also be followed for the first 6 patients who sign the main consent and begin protocol treatment at the new dose (of note, there have been no DLTs observed among the first 6 patients who have been treated at the dose originally outlined in the protocol)

As this is the first study testing the DPX-E7 vaccine in humans, the following monitoring will be used: The enrollment of first six subjects who begin protocol treatment will be concurrent and they will be observed for adverse events. If zero or one DLT (see section 6.2 for definition) is observed through the first month after the second priming dose in the first six subjects, then study enrollment into the first stage (n=16) will continue and may continue concurrently. If there are two DLT's observed through the first month after the second priming dose in the first six subjects, the dose of DPX-E7 vaccine will be de-escalated. If there are 3 or more DLTs observed through the first month after the second priming dose in the first 6 subjects, accrual will be suspended to further evaluate the events and decisions made regarding the overall status of the trial. If dose de-escalation is required, six new subjects will be enrolled and treated with DPX-E7 vaccine at the lower dose. If zero or one DLT is observed through the first month after the second priming dose in these six subjects, then study enrollment into the first stage (n=16) will continue. If there are two or more DLTs OR one death OR two grade 4 events observed through the first month after the second priming dose in these six subjects, then accrual will be suspended to further evaluate the events and decisions made regarding the overall status of the trial. Only those patients treated at the acceptable dose will be included in the phase II portion of the trial. Adverse events will be continuously

monitored throughout the trial by the study team and appropriate committees with decisions made accordingly re: the study status and patient entry throughout the duration of the trial.

As overall monthly accrual is estimated to be 6 pre-consented for HLA and HPV testing, with 33% of those HLA-A*02 positive, the above monitoring will be ongoing and not require accrual to be suspended for adverse event evaluation after the first 6 patients who begin protocol treatment, however, these estimates will be monitored and accrual will be suspended as needed. Two 0.50 mL priming doses of DPX-E7 will be given 3 weeks apart, followed by 0.2 mL booster doses every 8 weeks, until clinical progression. Subjects will be re-primed with a 0.50 mL vaccination if they are not exhibiting injection site induration, a hallmark of DepoVax vaccination, within four weeks of their next boost and/or their immune response is not being maintained.

DLT assessment period will last until day 50 (28 days after the second priming dose). Vaccine dose reduction would occur in the event of 2 DLTs. If a dose reduction is required then the new dose level will be 0.2 mL for the priming doses while the boosting doses will remain at 0.2 mL.

DPX-E7 vaccine dose de-escalation in Phase Ib[a]

Modified Priming Dose*	0.2 mL
Priming Dose 1 (starting)	0.50 mL
Priming Dose 2	0.50 mL
Booster Doses	0.2 mL

* If there are two DLTs observed through the first month after the second priming dose in the first six subjects, the dose of DPX-E7 vaccine will be de-escalated.
[a] Patients enrolled prior to amendment 10's approval will receive priming and boosting doses of 0.25mL and 0.1mL, respectively.

Cyclophosphamide dose reduction in Phase Ib/II[a]

Modified Dose*	50 mg once per day
Planned Dose	50 mg twice per day

* Cyclophosphamide dose can be reduced to 50 mg once per day. If ANC ≤ 1,000/uL or Platelet ≤ 100,000/ uL, then the cyclosphosphamide will be held. In order to restart administration of cyclophosphamide, the platelet and ANC levels should have resolved to ≤ Grade 1 or to the patient's baseline value. The maximum delay before treatment should be discontinued is 3 weeks. Once the cyclophosphamide is restarted, it can be dose reduced to 50mg per day per investigator's discretion. If the cyclophosphamide dose has been reduced, it can't be re-escalated.
[a] Only for patients enrolled before amendment 10's approval.

Visits for pre-screening, screening, treatment, safety evaluation and assessment of tumor response will occur as outlined in section 10.

All patients who begin treatment, will be followed for response until first disease progression and for survival until 2 years post study registration.

1.2 Primary Objectives

1. Evaluate changes in CD8+ T cells in peripheral blood and tumor tissue.
2. Evaluate the safety of DPX-E7 vaccination in HLA-A*02 positive patients with incurable HPVOC, cervical cancer, and anal cancer.

1.3 Secondary Objectives

1. Evaluate the overall response rate (ORR) of DPX-E7 vaccination in HLA-A*02 positive patients with recurrent and/or metastatic HPVOC, cervical cancer, and anal cancer using modified RECIST 1.1 and irRECIST (Appendix B & C).
2. To estimate progression free survival, time to progression, and overall survival.
3. To evaluate the biologic correlates of response to therapy by determining the antigen-specific CD8 T cells elicited by $E7_{11-19}$ pMHC multimer binding assay, their activation status, effector/memory phenotype, cytokine profile, quantitation per ml blood, perforin/granyme content, CD4 activation status, including Treg profiling.

2. BACKGROUND

2.1 Study Disease

Human papilloma viruses (HPV) are double-stranded DNA viruses that infect epithelial cells of the skin and mucosa[1]. Of the more than 200 known HPV types, 30-40 are transmitted through sexual contact, infecting the anogenital region and oropharynx. Among these, approximately 15 are designated "high-risk" (i.e. oncogenic) and have been linked to cervical cancer as well as anal, vulva, vaginal, penile and oropharyngeal cancers[2]. Worldwide, >5% of all new cancers are attributable to high-risk HPV infections[3]. HPV is the cause of virtually all cases of cervical cancer, the second leading cause of cancer deaths among women worldwide[4-6]. While the implementation of screening has greatly reduced the burden of this disease in the developed world, treatment options for persistent, recurrent, or metastatic cervical cancer remain limited and the prognosis is poor[7,8].

Anal cancer accounted for 7060 cases per year estimated for the U.S. in 2013 and are closely linked to HPV infection. A recent analysis of 96 patients from Montreal showed that 92% had detectable HPV and HPV 16 was by far the most common subtype at 90%, with multiple other subtypes seen in small numbers[9]. An earlier study, limited to men from the San Francisco area, found that in the setting of HIV infection 93% of anal cancer patients had evidence of HPV, whereas the rate of HPV involvement was 61% among HIV-negative men[10]. A large population-based study in Denmark and Sweden detected HPV in 84% of patients with anal cancer[11].

Overall, patients with anal cancer do quite well with the standard treatment of pelvic radiation given concurrently with two cycles of 5FU and mitomycin C[12]. While most patients with this disease can be cured, 880 deaths related to anal cancer are anticipated annually in the US alone[6]. Especially patients who present with locally advanced disease, i.e. tumors greater than 5 cm or involved lymph nodes, the prognosis is poor, both in terms of DFS and OS[13]. A high percentage of these patients will recur, typically with distant metastases. First line chemotherapy, often cisplatin and 5FU, can be

effective in treating recurrences, but virtually all patients will eventually progress. These patients with recurrent anal cancer and progression through first line chemotherapy are an appropriate group for testing the current vaccine.

HPV associated oropharyngeal cancer (HPVOC) is an emerging epidemic with currently over 10 000 new cases per year in the US alone and the incidence is projected to double every 5-10 years in the coming decades. While HPVOC responds well to treatment and is curable in the vast majority of patients when detected early, approximately 10% of patients will be diagnosed with or will eventually develop incurable disease and eventually die of their disease[14-16]. Metastatic HPVOC responds frequently to first line chemotherapy which typically consists of platinum based combination regimens but survival remains poor and better treatments both in terms of efficacy and tolerability are urgently needed.

The viral E6 and E7 encoded proteins have transforming activities through functional inactivation of the p53 and retinoblastoma (Rb) tumor suppressor proteins, respectively[17-20]. Not surprisingly, therefore, abrogation of activities of E6 and/or E7 experimentally terminates the malignant state of HPV-transformed cells *in vitro*[21].

A key advance in combating HPV infection and its causally related diseases has been based on virus-like particle prophylactic vaccine development[22]. This vaccine consists of recombinant HPV L1 capsid protein that self-assembles to create virus-like particles against which protective, high-titered anti-L1 neutralizing antibodies are elicited *in vivo*[22,23]. In both females and males, such vaccines comprising 2 high risk capsids effectively prevent development of anogenital diseases[24]. However, capsid-based therapeutic HPV vaccine strategies cannot be employed once infection/disease is established for at least two reasons. First, humoral protection is associated with antibody binding to the capsid L1 protein thereby blocking virion entry into the cell. The L1 protein is exclusively expressed late in the HPV replication cycle and only in differentiated keratinocytes within the upper layers of the epithelium to create infectious virions. Persistent infection in the host is maintained by low copy numbers of unencapsulated virus in basal stem cells[25]. As humoral immune responses against capsid proteins do not affect persistently infected basal cells, they are consequently unable to clear infection[24,26]. Second, high risk HPV-associated cancers arise with genomic integration of E6 and E7 viral oncogenes. Although L1 and L2 genes may also be integrated and capsid proteins expressed[27] these are intracellular and hence, antibody inaccessible.

During natural infection, HPV is cleared by the immune system in the vast majority of subjects. A few percent of persistent infections evolve into neoplasias that may progress to malignancy with high risk HPV types[28]. Although immune suppression is associated with chronic infection, most patients with persistent HPV infections do not show immune deficiencies in response to other pathogens[29]. Persistent infection with malignant or premalignant lesions is often associated with HPV-specific T cells whose significance for anti-tumor immunity is not always clear. For example, one study that detected HPV-responding T cells in peripheral blood of patients with high grade cervical intraepithelial neoplasia (CIN2/3) was unable to correlate IFN-γ levels or antigen specificity of T cell response with the 26% of the patients that showed spontaneous lesion regression[30]. Whereas the presence of circulating HPV-specific CD8+ cytotoxic T lymphocytes (CTL) did not correlate with disease prognosis in early stage cervical cancer, the degree of CD8+ tumor infiltration and balance (ratio) of CD8+ T cells to regulatory T cells were correlated[31,32]. Consistent with a critical role of inflammatory signals in inducing T cell responses, vulval intraepithelial lesions treated with the toll-like receptor 7 activator Imiquimod showed regression and viral clearance in association with

circulating HPV-specific T cells[33,34]. Recently, vaccination with synthetic long peptides covering the E6 and E7 oncoproteins plus conventional adjuvant treatment was reported to clear HPV-16 induced high-grade vulva intraepithelial neoplastic lesions in ~50% of patients[35].

While it is widely acknowledged that antitumor cellular immunity is influenced by regulatory mechanisms, tumor microenvironment and tumor escape mechanisms[36], the nature of T cell antigen specificity is less defined despite its critical role. *Ex vivo* T cell assays that mark HPV-specificity identify a broad priming display by professional antigen presenting cells (pAPCs) but do not interrogate antitumor CTL activity nor do they identify the breadth of HPV-specific MHC I display of the tumor per se[37]. Pointedly, in the context of a narrow tumor display, antitumor vaccines that prime a broad T cell response generate a small fraction of tumor lytic CD8+ T cells, resulting in low densities of useful tumor infiltrating T cells.

2.2 DepoVax™ Technology

Immunovaccine has developed a patented prophylactic/therapeutic vaccine delivery and enhancement platform called DepoVax™. DepoVax (DPX) is a lipid-based depot formulation able to enhance immune responses towards peptide antigens. DepoVax has been demonstrated to enhance peptide immunogenicity in animal models and to maintain an immune response to the antigens after repeated immunizations[38,39]. The platform also includes a synthetic adjuvant to enhance and sustain the immune response.

DepoVax formulations are flexible compositions that can be modified for specific indications by inclusion of specific peptide antigens. The formulations consists of: (1) lipids (2) an adjuvant, (3) specific antigen(s), and (4) a hydrophobic carrier. DepoVax formulations are oil based, which allows for practical delivery of peptide antigens without having to resort to cumbersome emulsification. The unique combination of lipids in a hydrophobic carrier such as mineral oil creates a very strong depot at the site of immunization, co-delivering and holding antigens and the adjuvant at the site for a prolonged period of time. Immunovaccine's hypothesis is that the immune enhancement properties of this unique formulation are a result of this co-delivery and depot effect.

DepoVax has been used as the basis for two therapeutic cancer vaccines currently under clinical development, DPX-0907 and DPX-Survivac, in the US and Canada. Immunovaccine's first immunotherapeutic vaccine, known as DPX-0907, is a candidate anti-cancer vaccine for the treatment of breast, ovarian and prostate cancer. DPX-0907 vaccine consists of 7 tumor-specific human leukocyte antigen (HLA-A*02) restricted peptides, a universal T helper epitope from tetanus toxoid, a synthetic polynucleotide adjuvant, and phospholipids and cholesterol. In 2011 Immunovaccine completed a first-in-human Phase 1 clinical study (protocol ONC-DPX-0907-01) in the US which demonstrated safety and immunogenicity of the vaccine. These findings were published in the Journal of Translational Medicine[40].

Immunovaccine is currently conducting, with its second immunotherapeutic vaccine candidate, a Phase 1b clinical trial (DPX-ONC-DPX-Survivac-03) in ovarian, fallopian tube, and peritoneal cancer patients in centers in both the US and Canada and a Phase 2 trial (ONC-PX-Survivac-05) in diffuse large B cell lymphoma (DLBCL) patients in Canada; subjects in both trials receive up to six doses of vaccine along with low dose cyclophosphamide (50 mg bid). DPX-Survivac is a therapeutic cancer vaccine containing one decapeptide and four nonapeptides with different HLA restrictions, a universal T helper epitope from tetanus toxoid, a synthetic polynucleotide adjuvant, and

phospholipids and cholesterol. DPX-Survivac is designed to target survivin, a member of the inhibitor of apoptosis protein (IAP) family. A first-in-human Phase 1 trial was completed in 2013 (protocol ONC-DPX-Survivac-01). The Phase 1 study findings have been published in Oncoimmunology[41].

2.3 IND Agent: DPX-E7 Vaccine

Recently, an $E7_{11-19}$ nanomer peptide as a dominant T cell epitope presented by HLA-A*02:01 on the majority of HPV-16 cervical squamous and adenocarcinomas before and post-adjuvant chemotherapy has been identified, opening the way to therapeutic vaccination strategies targeting disease specific epitopes that result in optimal immunogenicity. Similar clinical trials use metronomic, low dose oral cyclophosphamide to deplete regulatory T cells (Treg) and to enhance immunogenicity further.

In this trial, we will use the $E7_{11-19}$ nanomer as target for CTL vaccination of HLA-A*02patients, given that HPVOC and anal squamous cell cancer are epithelial tumors as are HPV cervical cancers. DPX-E7 is formulated with the same DepoVax platform technology as Immunovaccine's previous products DPX-0907 and DPX-Survivac. With the exception of the HPV16-$E7_{11-19}$ peptide, the remaining components of the vaccine are the same as DPX-0907 and DPX-Survivac, both of which have completed Phase 1 testing. Despite evidence supporting metronomic cyclophosphamide in previous studies, our group has not seen immunologic activity in a cohort of six patients, and we have even seen immunologic depletion after treatment with cyclophosphamide. These disappointing results prompted us to eliminate cyclophosphamide from the treatment regimen and double the vaccine dose. Including the cohort previously treated with low dose metronomic cyclophosphamide, a total of up to n=44 patients will receive the vaccine (Considering that patients continue to be screened and at least n=7 patients have been entered and began protocol treatment at the original dose prior to approval of Amendment 10, and allowing for 1 or 2 patients to sign the main consent and be registered to the main protocol but not begin protocol treatment or be ineligible, the overall accrual goal following approval of Amendment 10 is up to n=44).. Patients on the previous treatment regimen (0.25mL priming doses, 0.1mL booster doses, and metronomic cyclophosphamide (50mg BID, 7 days on, 7 days off) will continue to receive this treatment regimen. This will allow us to obtain information about safety of the approach and to gain preliminary insight into clinical efficacy of this strategy. Patients will undergo mandatory tumor biopsies to obtain fresh tumor tissue prior and also post vaccination (prior to first vaccination and at 13 (+/-1) weeks after the first injection).

The tissue will be used to confirm HPV16-$E7_{11-19}$ peptide display by MS.

Once established, DPX-E7 vaccination could become an attractive option for the adjuvant treatment of patients with persistent HPV infection who are cured of HPVOC as well as for healthy individuals with high risk HPV infection such as patient's spouses, who are at risk to develop oropharyngeal, cervical or anal cancer over time.

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

1. Each patient must be positive for HLA-A*02 and meet all of the following inclusion criteria to be enrolled in the study:

2. Histologically or cytologically proven HPVOC or cervical cancer or anal cancer, based on the presence of HPV type16, detected by immunohistochemistry with P16 staining followed by PCR of tumor tissue from the primary or metastatic lesions. Please see Appendix D for reference and methodology.
3. Incurable HPVOC, as defined by:
    a. Relapsed or progressive disease at the primary site and/or regional lymph nodes after initial treatment (e.g. Surgery, radiotherapy or chemoradiotherapy) with no potentially curative option (i.e. surgery or radiation); OR
    b. Distant metastasis
4. Incurable cervical or anal cancer, as defined by:
    a. Relapsed or progressive disease at the primary site and/or regional lymph nodes after initial treatment (e.g. systemic chemotherapy) with no potentially curative option (i.e. surgery or chemoradiotherapy). Chemotherapy administered in conjunction with primary radiation as a radiosensitizer will not be counted as a systemic chemotherapy regimen; OR
    b. Distant metastasis refractory to initial treatment (at least one prior chemotherapeutic regimen which can include a single chemotherapeutic, a combination of chemotherapeutics, or biologic drugs). Cervical cancer subjects with distant metastases will have received and failed Bevacizumab prior to enrollment onto the trial
5. Accessible tumors for sequential biopsies
6. Recovery from toxicity from any prior therapy to National Cancer Institute Common Terminology Criteria for Adverse Events Version 4.0 (CTCAE v4.03) to grade 1 or better (except for $\leq$ grade 2 neuropathy, alopecia, xerostomia, dysphagia, or mucositis);
7. Age $\geq$ 18 years;
8. Measurable disease, according to modified RECIST 1.1 and irRECIST **(**Appendix B & C);
9. Eastern Cooperative Oncology Group performance status (ECOG PS) $\leq$ 2 **(**Appendix A)
10. Adequate bone marrow, liver and renal function, defined by:
    a. Hemoglobin $\geq$ 10 g/dL;
    b. Absolute neutrophil count (ANC) $\geq$ 1000/μL;
    c. Absolute lymphocyte count $\geq$ 400/μL;
    d. Platelet count $\geq$ 100,000/μL;
    e. ALT and AST $\leq$ 2.5 X upper limit of normal (ULN);
    f. Total bilirubin $\leq$ 1.5 X ULN; and
    g. Serum creatinine $\leq$ 1.5 X ULN;
11. Women of child-bearing potential (WOCBP) must be willing to use acceptable means of birth control;
12. Men who could potentially father a child must also use birth control
13. Signed informed consent.

3.2 Exclusion Criteria

Patients meeting any of the following exclusion criteria are not to be enrolled in the study:

1. Radiotherapy for primary HPVOC within 8 weeks, or radiotherapy for any other reason within 3 weeks prior to the first dose of vaccine;
2. Chemotherapy or immunotherapy within 3 weeks prior to the first dose of vaccine;

3. Prior malignancy in the past 5 years, except for carcinoma in situ of the cervix or bladder, or non-melanomatous skin cancer;
4. Inaccessible tumor or lack of consent for sequential biopsies
5. Uncontrolled central nervous system (CNS) metastases (i.e. known CNS lesions that are radiographically unstable, symptomatic and/or requiring escalating doses of corticosteroids);
6. Active hepatitis, known HIV, or other condition that requires immunosuppressive therapy, including current use of high dose systemic corticosteroids;
7. Autoimmune disease, such as systemic lupus erythematosis or rheumatoid arthritis, that is active and requires current immunosuppressive therapy;
8. Active uncontrolled serious infection;
9. WOCBP who have a positive β-hCG test or are breastfeeding.
10. Acute or chronic skin disorders that would interfere with subcutaneous injection of the vaccine or subsequent assessment of potential skin reactions;
11. Allergies to any vaccine, that after discussion with Immunovaccine, are serious enough to warrant exclusion from this study

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 Pre-screening

Potentially eligible patients with confirmed diagnosis of incurable oropharyngeal, cervical or anal cancer will be asked to sign a pre-screening consent that will allow us to use a blood sample for HLA testing and to analyze archival tissue for confirmation of the HPV16 genotype by PCR.

Obtain written informed consent for pre-screening from the potential participants prior to HLA testing.

A de-identified whole blood sample will be sent to the lab for HLA analysis. It will take approximately one week to carry out the HLA analysis. If the patient has previously undergone HLA analysis at a certified laboratory and the documented results are available then typing does not have to be repeated for the study. Patients must test positive for the HLA-A*02 phenotype in order to be eligible for registration and study treatment.

Confirmation of the HPV16 genotype by PCR analysis will be completed by the Brigham and Women's hospital on archival tissue samples stored in-house or on samples requested from outside institutions. It will take approximately 2 weeks to carry out the HPV16 confirmation. If HPV Type 16 associated malignancy has already been confirmed by PCR analysis by a certified laboratory and the documented results are available, then confirmation does not have to be repeated for the study. The HPV16 genotype must be confirmed in archival tissue samples by PCR analysis in order to be eligible for registration and study treatment.

For patients interested in the vaccine trial with difficulties traveling to Boston, the pre-screen consent may be signed remotely in order to confirm their potential eligibility, pending approval by the Primary

Investigator (PI). Interested patients and their treating physicians would be required to contact the PI to assess whether they would be a potential candidate for the trial. Upon the PI's approval, the patient would be sent a copy of the consent form electronically, which would be explained by the PI, signed remotely, and sent back to DFCI. Once the PI receives and signs the pre-screening consent form, the patient— along with the assistance of their local institution— would have their blood drawn and shipped refrigerated and overnight back to DFCI for analysis in the lab Archival tissue may also be requested and analyzed for the HPV16 genotype by PCR or IHC upon receipt of the signed pre-screening consent.

4.2 General Guidelines for DFCI Registration Process

HLA-A*02 positive patients (through pre-screening) who also meet all eligibility criteria (through screening) will be registered in OnCore by the study team. Registration must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible.

The investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator (PI). If a participant does not receive protocol therapy within 14 days following registration, the participant's registration on the study must be canceled.

The following source documents are required for registering the patient in OnCore:

- Copy of screening lab results
- Signed participant consent form
- Eligibility Checklist

4.3 Recruitment Process

Subjects will be recruited from the patient populations of the DFCI Head and Neck Oncology Program, the DFCI Gynecologic Oncology Program, and the DFCI Gastrointestinal Oncology Program, through our clinical referral network and by internet advertising for the study, including the Dana-Farber/Partners CancerCare website, www.cancercare.harvard.edu, websites (e.g., www.headneckcancer.net), and the National Cancer Institute's clinical trials website, www.cancer.gov/clinicaltrials.

5. TREATMENT PLAN

5.1 Treatment Regimen

This is a single center, open label, non-randomized, phase Ib/II trial of HPV16-E7$_{11-19}$ nanomer vaccine DPX-E7 in treatment of HLA-A*02 positive patients with histologically confirmed HPVOC, cervical, or anal cancer that is metastatic or unresectable and for which standard curative measures do not exist or are no longer effective.

Up to n=44 patients will be treated (n= 7 to 10 at the original dose prior to approval of Amendment 10 and n=33 at the dose outlined in Amendment 10). and we anticipate that this will require HLA screening at least 100 patients following approval of Amendment 10.

HLA-A*02 positive subjects will receive two 0.50 mL priming doses of DPX-E7 3 weeks apart followed by 0.2 mL booster doses every 8 weeks until clinical progression. Subjects will be re-primed with a 0.50 mL vaccination if they are not exhibiting injection site induration, a hallmark of DepoVax vaccination, within four weeks of their next boost and/or their immune response is not being maintained. Because the correlation between the induration and the immune endpoint is still being investigated, immune responses in addition to the induration will be collected to better establish this potential efficacy parameter.

Former Treatment Regimen

Patients enrolled on the previous treatment regimen (0.25mL priming doses, 0.1mL booster doses, and 50mg cyclophosphamide BID for 7 days on, 7 days off) will continue this regimen until treatment discontinuation. Injection visits will remain the same (D1, D22, etc.), but cyclophosphamide will be taken starting one week prior to the first injection visit.

Low dose metronomic oral cyclophosphamide (50 mg twice per day) will start 7 days before the first vaccination, continue for 7 days on and then 7 days off, throughout the treatment period, until progression. Cyclophosphamide dose can be reduced to 50 mg, once a day. If ANC ≤ 1,000/uL or Platelet ≤ 100,000/ uL, then the cyclosphosphamide will be held. In order to restart administration of cyclophosphamide, the platelet and ANC levels should have resolved to ≤ Grade 1 or to the patient's baseline value. The maximum delay before treatment should be discontinued is 3 weeks. Once the cyclophosphamide is restarted, it can be dose reduced to 50mg per day per investigator's discretion.

Cylophosphamide should be taken with or immediately after meal. Cylophosphamide capsule should be swallowed whole, with a large glass of water. It should not be crushed or chewed. Missed or vomited doses of cylophosphamide should not be retaken to make up for the lost dose. Patient should complete the diary on a daily basis and bring it to the clinic at each visit. Missed or vomited doses should be recorded on the diary.

Visits for screening, treatment, safety evaluation and assessment of tumor response will occur as outlined in section 10.

5.2 Agent Administration

The vaccine will be injected deep subcutaneously using the guidelines below, on days 1 and 22 (+/-2) days, to allow for holidays or problems with transportation). The patient will be observed for acute hypersensitivity reactions on the nursing floor for one hour following the injection. Following the first two doses of vaccine, booster doses of vaccine will be administered every 8 weeks (+/-2 days), until progression.

All preparations of the reconstituted vaccine will be performed at room temperature on the day of immunization. Subjects will receive two 0.50mL or 0.25 mL priming three weeks apart followed by 0.2 or 0.1 mL boosting doses of DPX-Survivac every eight weeks, in alternating upper thigh regions.

Doses are dependent on when the patient enrolled on the trial. An alcohol swab will be used to clean the site prior to injection. If a subject experiences pain on the first injection then cooling of the injection site with ice packs 15 minutes prior to subsequent immunization is permitted.

It is very important to follow the administration instructions below to avoid exaggerated injection site reactions:

1. Inject the vaccine in the front and/or outer upper half of the thigh region closer to the inguinal lymph nodes so the vaccine can be processed by the immune system.
2. Inject the vaccine deep subcutaneously and NOT intracutaneously. The full length of the needle must be inserted and at the correct angle (45-90 degrees) based on the size of the subject's upper thigh region.
3. Whether or not an injection site reaction is present, vaccine should be administered approximately 10 cm to prior injection sites and the location of each injection should be recorded in the source documents.
4. The sponsor may be present on vaccinations days and/or a video may be provided to assist with the injection technique.
5. It is recommended that the same trained study staff inject all subjects at their sites.

If a subject experiences <u>any</u> grade 2 or greater injection site reaction(s) or any grade injection site ulceration within 4 weeks prior to a vaccination time point, then that vaccination will be omitted. All other study procedures scheduled for that clinic visit will still be conducted. This same evaluation will be repeated at each vaccination time point.

Subjects will be re-primed if they are not exhibiting injection site induration, a hallmark of DepoVax vaccination, within four weeks of their next boost and/or their immune response is not being maintained.

5.3 General Concomitant Medication and Supportive Care Guidelines

Possible immediate side effects from vaccine injections, including DPX-E7, may include allergic reactions such as fever, hives, or rash. For fever >101.5 ºF (>39 ºC), acetaminophen (650 mg) may be given orally. The induction of autoimmunity (manifests as arthritis, serositis, nephritis, thyroiditis, colitis, neutropenia, etc.) is theoretically possible. Acute allergic reactions may be treated with diphenhydramine, and/or epinephrine as needed. Delayed events such as rash or hives maybe treated with diphenhydramine (25-50 mg); topical steroids should not be given without consultation with the study's principle investigator.

To care for possible infected injection site reactions proper wound care is appropriate. Infected areas should be kept clean and exposed. Topical antibiotics such as fucidin or polysporin may be applied. Cultures should be sent for infections and treated promptly with oral antibiotics. Topical corticosteroid (hydrocortisone ointment) may be used to help reduce inflammation.

Possible side effects from Montanide ISA 51 VG are granuloma, abscess, and fever. Acetaminophen 650 mg every 4 hours may be given for fever after appropriate blood cultures are taken. Referral to a surgeon is encouraged for abscess.

<u>Cyclophosphamide (only for patients on former treatment regimen)</u>

Low dose metronomic oral cyclophosphamide (50 mg twice per day) will start 7 days before the first vaccination, continue for 7 days on and then 7 days off, throughout the treatment period. Cyclophosphamide dose can be reduced to 50 mg, once per day. If ANC ≤ 1,000/uL or Platelet ≤ 100,000/ uL, then the cyclosphosphamide will be held. In order to restart administration of cyclophosphamide, the platelet and ANC levels should have resolved to ≤ Grade 1 or to the patient's baseline value. The maximum delay before treatment should be discontinued is 3 weeks. Once the cyclophosphamide is restarted, it can be dose reduced to 50mg per day per investigator's discretion. If the cyclophosphamide dose has been reduced, it can't be re-escalated.. Cyclophosphamide will continue until clinical progression occurs.

Possible side effects from low doses cyclophosphamide are grade 1 nausea and/or vomiting, grade 1 and 2 anemia, neutropenia, leukopenia, and lymphopenia as well as low-grade fatigue. If platelet levels decrease below 100,000/μL or if absolute neutrophil count decreases below 1000/μL in any cycle then the dose of cyclophosphamide can be reduced to 50 mg once daily in that subject for the duration of their treatment. Following consultation with the PI, a subject's cyclophosphamide could be further modified by extending the interval between cycles of administration or held.

5.4 Criteria for Taking a Participant off Protocol Therapy

Because swelling and inflammation at the site of tumor may follow vaccination, increased size of already existing tumor found on clinical exam up to week 7 will not be considered PD. If, however, new sites of disease are found, the subjects will be considered to have PD. If first restaging CT scan shows PD and the physician and patient want to continue protocol therapy, progression will be confirmed with a second CT scan 4 weeks later.

Continuation of treatment with booster doses of vaccine after clinical progression:
If there are no other treatment options available, at the discretion of the PI, participants may continue receiving treatment with booster doses of vaccine after clinical progression. Treatment may continue until RECIST 1.1 defined progression, unacceptable toxicity, or withdrawal of consent.

In addition to withdrawal from protocol therapy for PD, subjects should be withdrawn from the protocol therapy if, in the opinion of the investigator, it is medically necessary, or if it is the wish of the patient. If a patient does not return for a scheduled visit, every effort should be made to contact the patient. In any circumstance, every effort should be made to document subject outcome, if possible**.**

Participants should also be removed from protocol therapy if any of the following occurs:

- Unacceptable adverse event(s), including a DLT
- Participant demonstrates an inability or unwillingness to comply
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Intercurrent illness that prevents further administration of treatment and/or would affect the assessment of the subject's clinical status to a significant degree
- Pregnancy

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

Information will be entered in OnCore when a participant is removed from protocol therapy.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the PI, Karthik Sehgal, MD at 617-632-3090.

5.5 Duration of Follow Up

All patients who begin protocol treatment will be followed until first disease progression and death for 2 years from study registration, whichever occurs first.

5.6 Criteria for Taking a Participant off Study

If progression is documented (except new lesions) on restaging scans patients may remain on study and be followed at the discretion of the PI and according to protocol and if no life threatening complications from progression in the immediate future are expected (see study calendar). In these cases, follow up scans will be obtained 4 weeks later and if progression continues, the patient will be considered to have PD.

Because swelling and inflammation at the site of tumor may follow vaccination, increased size of already existing tumor found on clinical exam up to week 7, will not be considered PD (i.e. possible pseudo-progression). If, however, new sites of disease are found, the subjects will be considered to have PD.

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the electronic case report form (eCRF).

Information will be entered in OnCore when a participant comes off study.

5.6.1 Treatment Beyond Disease Progression

Accumulating evidence indicates a minority of subjects treated with immunotherapy may deri*ve* clinical benefit despite initial evidence of progression of disease (PD).

Subjects will be permitted to continue on study treatment for treatment beyond initial RECIST 1.1 defined PD as long as they meet the following criteria:

- Investigator-assessed clinical benefit and no rapid disease progression

- Tolerance of study drug
- Stable performance status
- Treatment beyond progression will not delay an imminent intervention to prevent serious complications of disease progression (eg, CNS metastases)
- Subject provides written informed consent prior to receiving additional study treatment, using an ICF describing any reasonably foreseeable risks or discomforts, or other alternative treatment options. The decision to continue treatment beyond initial investigator-assessed progression should be discussed with the investigator and documented in the study records.

If the investigator feels that the subject continues to achieve clinical benefit by continuing treatment, the subject should remain on the trial and continue to receive monitoring according to the Study Calendar (section 10).

For the subjects who continue study therapy beyond progression, further progression is defined as an additional 10% increase in tumor burden from time of initial PD. This includes an increase in the sum of diameters of all target lesions and/ or the diameters of new measurable lesions compared to the time of initial PD. Study treatment should be discontinued permanently upon documentation of further progression.

New lesions are considered measurable at the time of initial progression if the longest diameter is at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm). Any new lesion considered non-measurable at the time of initial progression may become measurable and therefore included in the tumor burden if the longest diameter increases to at least 10 mm (except for pathological lymph nodes which must have a short axis of at least 15 mm).

In situations where the relative increase in total tumor burden by 10% is solely due to inclusion of new lesions which become measurable, these new lesions must demonstrate an absolute increase of at least 5 mm.

6. ANTICIPATED TOXICITIES

It is anticipated that there will be no significant toxicity associated with vaccination other than redness and discomfort at the injection site. Please refer to section 6.1 for an overview of the expected toxicities and the current Investigator's Brochure for details. Subjects will be monitored during the injections and in the immediate post-injection period for fevers and other signs of immune activation. Any evidence of reactions will be recorded. Reactions will be treated as clinically appropriate.

All toxicities occurring from the time of consent to within 30 days of the last vaccination will be recorded in eCRF and assessed for relationship to study therapy.

6.1 Risks associated with DPX-E7 vaccine

This is the first in human study for the DPX-E7 vaccine so we do not know everything that possibly

can happen, and there may be unforeseen risks associated with this study. The following side effects were experienced by subjects who participated in three research studies with two similar DPX vaccines.

Frequency	Side Effect
Frequent >10%	Injections Site Reactions: Hardness; Redness that may persist as a discoloration; Swelling; Discomfort; Itchiness; Warmth; Dryness; Ulceration*; Rashes
Occasional 1-10%	Temporary bleeding/bruising at the injection site; Discharge at the injection site; Skin infection at the injection site; Allergic reactions such as fever, hives; Myalgia (achy muscles); Joint pain; Flu-like illness
Rare <1%	Granuloma (lump of inflamed tissue); Abscess (pockets of pus that are likely to turn hard as they scar and heal); Blood clots; Autoimmune Disorders (inflammation of the joints, kidneys, thyroid, intestines, muscles, heart, pancreas, liver, spleen, lung, brain, eye and low blood counts)

* Grade 3 injection site reaction as per NCI CTC v.4.03 is defined as ulceration or necrosis; severe tissue damage, operative intervention indicated. As we are increasing the dose, AEs may be more frequent.

Some participants may experience one or more of the above side effects from DPX-E7. Most of the side effects do not have the potential of being permanent. It is not yet known how long effects like hardness, hyperpigmentation, or scarring from an ulceration may persist. It is possible that an injection site reaction may not occur until after treatment or may re-occur after you have stopped receiving study treatment. The side effects for DPX-E7 listed as rare are theoretically possible but have not been seen in any of the human participants receiving the similar DPX vaccines.

6.2 Definition of a Dose Limiting Toxicity

Toxicity will be assessed using the NCI Common Toxicity Criteria for Adverse Events, version 4.03 http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

A dose limiting toxicity (DLT) is defined in this study as any grade 3 or greater adverse event at least possibly related to the study agent including injection site reactions; or grade 2 or greater allergic reactions which occur in a subject prior to day 50 (monitoring for DLT to be continued up to 1 month (28 days) after the second priming dose), will trigger a DLT. In addition, to be considered a DLT in this study the adverse event must be considered at least possibly related to study treatment. At the investigator's discretion, grade 3 or greater abnormal lab values lasting $\leq$ 72 hours may be excluded as DLTs if there are no accompanying clinical signs or symptoms.

Vaccine dose reduction would occur in the event of 2 DLTs. If a dose reduction is required then the new dose level will be 0.2 or 0.1 mL for the priming doses while the boosting doses will remain at 0.2 or 0.1 mL (depending on treatment regimen).

6.3 Risks Associated with Cyclophosphamide (former treatment regimen)

Frequency	Side Effect
Frequent >10-100%	Fatigue
Occasional 1-10%	Decrease in White Blood Cells.
Rare** <1%	Low Platelet Counts (increases risk of bruising and bleeding); Low Red Blood Cell Counts (may cause shortness of breath); Nausea; Vomiting; Diarrhea; Stomach pain; Mouth sores; Dehydration; Diminished fertility

**The side effects for cyclophosphamide listed as rare were not observed in the previous studies with a similar DPX vaccine but were very common in studies conducted by other investigators researching different dosing schedules of cyclophosphamide and various cancers.

7. ADVERSE EVENTS LIST AND REPORTING REQUIREMENTS

7.1 Definition of an Adverse Event

The following definition of an AE will be used for this study:

Any untoward medical occurrence in an enrolled subject who was administered the clinical trial material; the occurrence does not have to have a causal relationship with treatment to be considered an AE.

An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of the investigational product, whether or not it is considered to be related to the investigational product.

AEs may include pre- or post-treatment events that occur as a result of protocol mandated procedures (i.e. invasive procedures, modification of the subject's previous therapeutic regimen).

7.2 Reporting Period

The AE reporting period for this study begins at the time the patient signs consent and ends at 30 days after the last dose of vaccine.

In addition, any known untoward event that occurs subsequent to the AE reporting period that the investigator assesses as possibly related to the investigational product should also be reported to the study sponsor.

All AEs will be followed until: resolution, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up. However, once the subject is on new treatment no pre-existing AEs should be followed and no new AEs reported.

7.3 Definition of a Serious Adverse Event

The definition of a SAE is an AE that meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires hospitalization or a prolongation of an existing hospitalization (for reason(s) other than scheduled tumor biopsy)
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly or birth defect
- Other important medical event that may jeopardize the subject or may require medical intervention to prevent one of the outcomes listed above.

Any events or hospitalizations that are unequivocally due to progression of disease will not be reported as an SAE. In many cases only the symptoms of the disease progression will be listed as AEs.

A SAE requires additional detailed reports and follow-up. The content of these detailed reports must address the PI's estimate of causality. The PI will review the SAE to determine expectedness (whether or not the AE is identified in nature, severity, and frequency in the IB).

7.4 Recording AEs and SAEs

It is the responsibility of the investigator to perform periodic and special assessments for AEs. The investigator and clinical staff will note all AEs described by the subject from the time of ICF signature through to 30 days after the last dose of the vaccine. Each subject will be questioned about AEs at each clinic visit. The question asked will be: "*Since your last clinic visit or since you began taking the investigational medication, have you had any health problems?"* All clinical complaints volunteered by or elicited from the subject during the study will be recorded on the appropriate forms of the eCRF for the study period indicated.

When an AE or SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory, and diagnostic reports) relative to the event(s). The PI is to record all relevant information regarding any AE (including SAEs) on the AE form of the eCRF and for SAEs, must also enter on an SAE Report Form.

The PI will also attempt to establish a diagnosis of the event based on the signs, symptoms, or other clinical information. In such cases, the diagnosis, not the individual signs and symptoms, should be documented on the appropriate eCRF as the AE or SAE.

7.5 Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study. The intensity will be graded according to the criteria in the NCI CTCAE version 4.03.

7.6 Assessment of Causality

The investigator is obligated to estimate the relationship between the investigational product and the occurrence of each AE or SAE using their best clinical judgment. Other causes, such as the history of the underlying disease, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational product will be considered and investigated. The PI will also consult the IB, or product labeling information for marketed products, in the determination of the assessment.

There may be situations when a SAE has occurred and the PI has minimal information to include in the initial report. However, it is very important that the PI always makes an assessment of causality for every event prior to the transmission of the SAE Report Form. The PI may change his opinion of the causality in light of follow-up information, amending the SAE report. The causality assessment is one of the criteria used to determine regulatory reporting requirements and should not be left blank.

Table 1: Assessment of Causality/Relatedness of AEs

Relatedness	Definition
Definitely related	The adverse event *is clearly related* to the investigational agent(s) or research intervention: the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a known pattern of response, and no alternative cause is present.
Probably related	The adverse event is *likely related* to the investigational agent(s) or intervention: the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a known or suspected pattern of response, but an alternative cause may be present.
Possibly related	The adverse event *may be related* to the investigational agent(s) or intervention: the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, follows a suspected pattern of response, but an alternative cause is present.
Unlikely to be related	The adverse event is *doubtfully related* to the investigational agent(s) or intervention: the adverse event has a temporal relationship to the administration of the investigational agent(s) or research intervention, but follows no known or suspected pattern of response, and an alternative cause is present.
Unrelated (or not related)	The adverse event is *clearly NOT* related to the investigational agent(s) or intervention: the adverse event has no temporal relationship to the administration of the investigational agent(s) or research intervention, follows no known or suspected pattern of response, and an alternative cause is present.

7.7 Expectedness of AEs

An expected AE is one that is consistent with the known risk information described in current IB.

An unexpected AE is defined as any AE where the specificity or severity of which is not consistent with the known risk information described in current IB.

Expectedness will be determined by the PI.

7.8 Reporting of SAEs

It is the responsibility of each participating investigator to report adverse events within 48 hours to the PI, Kartik Sehgal, MD, or representative personnel; and submitted to DFCI IRB within 10 working days. All events should be communicated with the Head and Neck Oncology prior to submission.

The DFCI IRB requires the following Adverse Events (AE) be reported for all subjects enrolled and actively participating in the trial or when the AE occurs within 30 days of the last study intervention (e.g. drug administration):

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are Unexpected and Possibly, Probably or Definitely Related / Associated with the Intervention.

- ALL Grade 4 (life threatening or disabling) Events – Unless expected AND specifically listed in protocol as not requiring reporting.

- ALL Grade 5 (fatal) Events

Notes:
- If subject is in Long Term Follow Up, death is reported at continuing review.
- See protocol for additional reporting requirements (to sponsor, FDA, etc.).
- Grade 2 and Grade 3 laboratory abnormalities that are considered by the investigator to be clinically insignificant and do not require therapy, or adjustment in prior therapy, do not need to be reported to the DFCI IRB.

In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event.

The PI, as study sponsor, will be responsible for all communications with the FDA. Unexpected, fatal or life-threatening experiences, associated with the use of the investigational vaccine, will be reported to FDA according to FDA reporting requirements. The Head and Neck Oncology Group will forward all adverse events meeting the requirements listed below to the appropriate staff members in the DFCI CTO High Risk IND Program within 2 working days from notification for evaluation and submission to the FDA.

- Toxicity
    - Grade ≥ 3 non-hematologic and hematologic and all Grade 5 events
- Known Correlation
    - Any (Expected or Unexpected)

- Attribution to Investigational Vaccine
  - Any

All events must be emailed using a Medwatch 3500A form. Medwatch 3500A downloadable form at http://www.fda.gov/medwatch/getforms.htm

MedWatch forms should be mailed to the FDA by the appropriate staff members in the DFCI CTO High Risk IND Program at the address below:

7.9 Reporting to Immunovaccine

On behalf of the Sponsor, the appropriate staff members in the DFCI CTO High Risk IND Program will notify IMV of the occurrence of any significant safety finding observed during the course of the study, including, without limitation:
(a) Any Adverse Event (as defined above) that warrants expedited (7 or 15-day) reporting to the FDA;
(b) Any significant, safety-related finding, concern, or observation identified by either the PI or the governing IRB during the course of the study;
(c) Any patient who experiences an outcome of death, regardless of perceived relatedness to a IMV product; or
(d) Any FDA-initiated safety related inquiry directed to study personnel.

Table 2: Timeline for Expedited Reporting SAEs to FDA and Immunovaccine Inc.

Initial SAE Report		Follow-up SAE Report	
Time Frame	Documents	Time Frame	Documents
7 days	SAE Report Form	15 days	Updated SAE Report Form

7.10 Follow-up of SAEs

After the initial SAE report, the investigator is required to follow the subject and provide further information in regards to the subject's condition. All SAE(s) will be followed until:

- Resolution
- The condition stabilizes
- The event is otherwise explained
- The subject is lost to follow-up

Once the event is resolved, the SAE Report Form and the eCRF will be updated. The PI will also ensure that the follow-up includes any supplemental information, excluding source documents, that may explain the causality of the event(s).

New or updated information will be recorded on the originally completed SAE Report Form, with all changes signed and dated by the investigator or designee. The updated SAE Report Form will then be signed by the PI and resubmitted.

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 6.

8.1 DPX-E7 vaccine

8.1.2 **Form**

The antigen/adjuvant/lipid mixture is formulated in a phosphate buffer, filled into vials and lyophilized to a dry cake. In the clinic, the cake is re-suspended in the hydrophobic carrier Montanide ISA51 VG before injection.

8.1.3 **Packaging, Labeling, Storage, and Handling of Study Products**

A bulk supply of DPX-E7 Vial 1 will be provided in a box to be stored at -20°C in the pharmacy with a 24 hour temperature monitoring and alarm system. A temperature log will be maintained by the pharmacy to ensure the product is correctly stored. Vial 2 (oil diluent) and the ancillary components will be provided in bags to be stored at ambient temperature in a secure and 24 hour temperature monitored room.

The DPX-E7 labels will comply with FDA requirements for investigational products. Detailed reconstitution instructions will be provided by Immunovaccine in a Pharmacy Manual. Training of the site pharmacists will also be provided by Immunovaccine if required.

8.1.4 **Availability**

DPX-E7 vaccine will be manufactured and supplied by:

Immunovaccine Inc.

8.1.5 **Preparation**

The proposed vaccine components will be supplied by the Immunovaccine(R) in two vials. Vial 1 will contain the freeze dried adjuvant system and antigen. Vial 2 will contain the oil component (Montanide ISA51 VG) alone which will be used as a diluent. The reconstituted vaccine will be prepared at the pharmacy by re-suspending the contents fo Vial 1 using the oil diluent of Vial 2. Detailed reconstitution instructions will be provided by Immunovaccine Inc. The final vaccine will be a transluscent oily solution (not an emulsion) that is syringable and easy to inject. The appropriate volume of the reconstituted vaccine will be injected subcutaneously following the instructions provided in "Reconstitution Instructions" (embedded below and also posted on Oncpro).

DPX-E7 Reconstitution Instructions - v1.0 (17

It is important to note the following instructions:

Once the vaccine is reconstituted, the time until administration should NOT exceed 4 hours. If time has exceeded 4 hours then the vaccine should NOT be used.

Once the pharmacy has drawn the vaccine into the provided Medallion syringe, the time until administration should not exceed 60 minutes.

8.1.6 **Administration**

All subjects will be treated in an outpatient unit equipped with emergency equipment. Subjects will be monitored with blood pressure, pulse, and temperature assessments at pre-injection, and at approximately (i.e. within 5 minutes) 15 minutes after injection. The patient will be observed for acute hypersensitivity reactions on the nursing floor for one hour following the injection. Diphendydramine, solumedrol , and epinephrine must be available and used per institutional policy at the bedside. The clinic must have a code cart available for emergency use. If hypotension (systolic blood pressure (SBP) <90 mmHg for subjects with a baseline SBP >110 mmHg or >20 mmHg decrease for those with a baseline SBP <110 mmHg), urticaria, orofacial or laryngeal edema, or bronchospasm occur, an intravenous catheter will be placed and the diphendydramine and solumedrol will be administered. The physician in charge will be notified and the epinephrine will be

administered for reactions that do not begin to resolve within 10 minutes or continue to become more severe. In this event, subject will be transported immediately to the emergency room if stabilized, or the code team will be contacted if subject continues to have progression of symptoms or worsening hypotension.

The vaccine will be injected deep subcutaneously, using the guidelines provided in section 5.2, on days 1 and 22 (+/-2) to allow for holidays or problems with transportation). The patient will be observed for acute hypersensitivity reactions on the nursing floor for one hour following the injection. Following the first two doses of vaccine, booster doses of vaccine will be administered every 8 weeks (+/-2 days), until progression. For patients continuing the former treatment regimen, every effort should be made to ensure a full 7 days of cyclophosphamide is administered before the first vaccine is delivered.

8.1.7 **Ordering**

Immunovaccine Inc. will supply DPX-E7 vaccine to DFCI research pharmacy in two shipments, one at the beginning of the trial and the second one almost half way during the trial.

8.1.8 **Accountability**

Accountability for DPX-E7 vaccine at the study site is the responsibility of the sponsor-investigator. Study vaccine will be dispensed only to eligible patients by Dana-Farber Research Pharmacy. The appropriate study personnel will maintain records of study vaccine receipt and dispensing at Dana-Farber Research Pharmacy. A careful record of the inventory and disposition of the vaccine will be maintained, using the appropriate accountability log, per institutional policy.

8.1.9 **Destruction and Return**

Unused supplies and any expired supplies of the vaccine will be destroyed on site, by the Dana-Farber Research Pharmacy.

8.2 Cyclophosphamide

Commercial supply of cyclophosphamide will be ordered by the Dana-Farber Research Pharmacy. Cyclophosphamide will be administered per protocol. It will be stored according to the instructions in the package insert. Accountability and destruction will be per institutional policy.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

Correlative studies will be performed in the DFCI flow cytometry core. Before any material is transferred from the clinic to the lab, the sample will be de-identified and a booklet with the key to the numeric code will be kept in a secure location in the Clinical Research Office.

9.1 Primary Immunological Endpoints

In ONC-DPX-Survivac-01 and -03 studies, which used a similar vaccine and treatment plan, research subject's flow cytometry results were considered positive if the measurement is greater than the standard level of sensitivity (approximately > 0.05% positive cells) and the post treatment value is at least two times greater than the pre-treatment value.

Blood

1. Induction of HPV16-E711-19 (pMHC) specific T cells as revealed by ELISpot IFN-γ at least 2 fold over background (pre-immunization)
2. Induction of HPV16-E711-19 specific T cells as revealed by HPV16-E711-19 dextramer or tetramer analysis
3. Functional T cell avidity as determined by peptide titration to determine the sensitivity of vaccine-engendered T cells with respect to pMHC recognition. pMHC copy number on APCs required for activation of cytokine production shall be determined by MS when possible
4. Determine the nature of immune subsets as defined in the sample panel below

Blood Sample Panel

Peripheral blood mononuclear cells (PBMC) will be isolated from vaccine recipient's blood samples at the indicated time points. Samples will be banked for further biological testing of functional correlatives of immune responses to the vaccine.

Blood samples will be collected at screening, Day 1, 22 and 78 and stained with multi-color antibody panels to test T, B, NK and antigen specific T cell immune responses to the vaccine. These optimized antibody panels will be analyzed by flow cytometer.

Whole blood samples will also be collected at screening and Day 22. DNA analysis on the samples will be correlated with tumor DNA content and oncologic response.

Tumor biopsy

Patients will undergo mandatory tumor biopsies to obtain fresh tumor tissue prior and also post vaccination, at 13 (+/-1) weeks after the first injection. Part of the material will be used to confirm the presence of the HPV-16 $E7_{11-19}$ peptide prior to vaccination and post-vaccination. To this end, Poisson MS detection will be used as described previously[42].

The remaining portion of the tumor biopsies will be used for immune monitoring at the Specialized Histopathology core at the Brigham and Women's hospital and Johns Hopkins University will include analysis of inflammatory infiltrate.

The pre-treatment tissue will be used to observe the tumor microenvironment and T cell repertoire prior to therapy.

The material will be used for IHC and other correlative study methods according to the table below as previously described[43] and as sample size permits.

Table 4: Analysis of patient biopsy samples

Specimen Type	Method	Analyses
Formalin-fixed paraffin embedded tissue (FFPE)	Quantitative immuno-histochemistry (IHC)	Determine resident cell subsets and colocalization in epithelial and stromal cell compartments
Flash-frozen tissue	IMMUNO-LCM	mRNA transcriptomes, RNAseq, TCR sequencing

Primary tissue explant	Expand and isolate T cells	Multiple functional analyses

10. STUDY CALENDAR

Procedures	Screening[1]	Day 1	Day 22	Day 29	Day 50	Day 78	Day 91	Day 106	Day 134	Day 162	Day 190	Day 218	Day 246	Day 270	Day 302	Day 358 and beyond[7]	End of treatment[9]
Informed consent	X																
Demographics	X																
Medical history	X																
Height	X																
Weight	X	X	X			X			X		X		X		X	X	X
Physical exam	X	X	X			X			X		X		X		X	X	X
ECOG PS	X	X	X			X			X		X		X		X	X	X
Vital signs	X	X	X			X			X		X		X		X	X	X
Concurrent meds	X	X	X			X			X		X		X		X	X	X
Performance status	X	X	X			X			X		X		X		X	X	X
CBC w/diff, plts	X	X	X			X			X		X		X		X	X	X
β-hCG (WOCBP) [2]	X																
Serum chemistry must include alkaline phospatase, AST, ALT, bilirubin and creatine	X	X	X			X			X		X		X		X	X	X
Immune Bloods[3]	X	X	X	X		X			X		X		X		X	X	X
DNA Bloods	X	X	X														
Tumor assessment by CT, PET/CT, MRI of neck, chest, abdomen/pelvis[4]	X*					X			X		X		X		X	X	X
Tumor biopsy for MS analysis and immunohistology[5]	X						X										
Adverse event evaluation[6]		X	X		X[10]	X	X	X[10]	X	X[10]	X	X[10]	X	X[10]	X	X	X
DPX-E7 Vaccination[7]		X	X			X			X		X		X		X	X	
Cyclophosphamide[8]		X															

1. HLA-A*02 positivity should be confirmed prior to screening. Screening assessments should be done within 3 weeks prior to study registration.

2. Must be obtained in women of childbearing potential during screening
3. Unless otherwise specified, approximately 60ml of whole blood will be obtained for immune phenotyping, ELISpot assays, Cytokine flow cytometry and banking. Plasma will be saved for luminex analysis. Blood for immune responses in addition to the induration will also be collected.
4. There is a +/- 1 week window for scans. *within 6 weeks of study registration. The first restaging scan will be on Day 78, 8 weeks post second vaccine. If first restaging CT scan shows PD, progression will be confirmed with a second CT scan 4 weeks later. All scans are every 8 weeks, unless medically indicated otherwise. Details in Table 6
5. Tumor biopsy will be obtained prior to the vaccination and at 13 (+/-1) weeks post vaccination
6. Patients will be followed throughout the study for the side effect of vaccine such as injection site reactions and fever
7. After priming doses on Day 1 and 22, booster doses will start on Day 78 and continue every 56 days, until progression. There is a +/- 2 days window for vaccine administration. Also section 5.6.1 for Treatment beyond Progression.
8. Applies to patients already on the cyclophosphamide treatment regimen. Cyclophosphamide will start 7 days before the first vaccination, continue for 7 days on and then 7 days off, throughout the treatment period. *The first dose of cyclophosphamide should only be dispensed after screening is complete and subject is enrolled in study.
9. Post end of protocol treatment, tumor assessments are to continue every 3 months until first progression, death, or 24 months from study registration, whichever occurs first. After first progression, participants will be followed by phone only, every 3 months (+/- 2 weeks) for 24 months from study registration or until death whichever occurs first.
10. Research Nurse will complete an over-the-phone AE assessment.

Table 5: Details of Study assessments

After the pre-screening consent, one blood sample will be collected for HLA typing. Subjects who are positive for HLA-A*02 will be consented for the main study and undergo screening, as shown in table below.

Pre-Screening and Screening		**Timing prior to registration**
Informed consents	To be obtained at pre-screening and screening	Within 21 days
Medical history and physical examination (PE)	Including weight, height, ECOG PS, vital signs (heart rate/blood pressure/temperature)	Within 21 days
Hematology	Complete blood count and differential (CBCD)	Within 21 days
Serum chemistries and other baseline bloods	Must include alkaline phosphatase, AST, ALT, bilirubin, creatinine, β-hCG for WOCBP.	Within 21 days
Infectious Diseases Markers	Hepatitis B and C, HIV	Within 21 days

Peripheral Blood for correlative science	60 ml should be drawn for full HLA typing using RNA prep for 454 sequencing and 30ml standard phenotyping material for frozen PBMC.	Prior to vaccination day 1
Safety and Tumor assessment	Physical examination	Within 21 days
	CT scan and/or MRI of the tumor[a]	Within 6 weeks
	Chest CT scan [b]	Within 6 weeks
	PET or PET/CT scan [b], if clinically indicated	Within 6 weeks
	Abdominal/pelvic CT scan or MRI in case of serum liver abnormalities or known abdominal/pelvic metastasis	Within 6 weeks
	Bone scan, if clinically indicated	Within 6 weeks
Tumor block	Tumor block for correlative studies will be requested after informed consent is signed.	No time restriction applies
Mandatory Tumor biopsy[c]	Fresh tumor tissue biopsy in all patients	Prior to first injection
[a] To ensure comparability, initial and subsequent radiographic imaging to assess response should be performed using identical techniques. **However for the purposes of screening local scans can be used** [b] Chest CT is not required if subject's baseline testing includes a PET/CT that covers the chest and does NOT show intrathoracic metastasis. [c] All patients		

Table 6: Details of Study treatment and follow up

Table below outlines the required evaluations during the vaccine administrations and follow up.

Required Data During and After Vaccination		**Timing**
Medical history and PE	Including weight, height, ECOG PS, vital signs	On days 1, 22 & 78, then every 56 days until progression
Hematology	CBCD	Same as above
Serum chemistries	Must include alkaline phosphatase, AST, ALT, bilirubin, and creatinine.	Same as above
Peripheral blood for correlative science	60 mL for immune monitoring and blood banking at days 1, 22, 29, 78, 134, 190, 246, 270, and 358.	Days 1, 22, 29, 78, 134, 190, 246, 270, and 358.
Whole blood for correlative science	10 mL for DNA analysis at screening, day 1, and day 22	Screening, Day 1, and22
Response assessment	Restaging CT scan and/or MRI of the neck,[b] if clinically indicated	8 wks post second injection[c], then every 2 months until progression[e]
	Restaging chest CT scan[d]	Same as above
	Restaging PET or PET/CT scan, if clinically indicated	Same as above

	Restaging abdominal/pelvic CT scan or MRI in case of KNOWN abdominal/pelvic metastasis [b]	Same as above
Mandatory tumor biopsy	Fresh tumor tissue biopsy	~13 wks post first injection AFTER first restaging scans

[b] To ensure comparability, baseline and subsequent radiographic imaging to assess response should be performed using identical techniques. **Every effort will be made to follow each lesion with the same technique from baseline through follow-up.**
[c] First restaging scan will be done at 8 (+/-1) weeks post second injection. If first restaging CT scan shows PD, progression will be confirmed with a second CT scan 4 weeks later.
[d] Chest CT is not required if subject's baseline testing includes a PET/CT or CT scan that covers the chest and does NOT show intrathoracic metastasis.
[e] Patients may remain on study if progression on scans is not likely to cause life threatening complications to allow for pseudoprogresson. In these cases, follow up scans will be obtained 4 weeks later and if progression continues, the patient will be considered to have PD.

10.1 Contraindicated Medications

Subjects should be taken off trial if they initate any chemotherapeutic agents, systemic corticosteroids or other immunosuppressives, or other immunotherapy while enrolled on study. Medications that result in immunosuppression should also be avoided while on study unless medically necessary due to unforeseen circumstances. Examples include systemically absorbed corticosteroids, NSAIDs and aspirin. No adjuvanted vaccine or live attenuated vaccine (such as Flumist) should be given while on this study. Non-adjuvanted vaccines (such as most influenza vaccines) can be given. For subjects receiving the flu vaccine, Immunovaccine strongly recommends the shot be given at least one week before immunological assessments.

11. MEASUREMENT OF EFFECT

Measurable disease will be defined, according to modified RECIST 1.1 as the presence of at least one measurable or evaluable lesion and according to irRECIST (Appendix B & C). If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology, if clinically feasible.

Measurable lesions will be defined as lesions that can be accurately measured in at least one dimension with the longest diameter ≥2.0 cm by conventional techniques. With a spiral CT scan, the lesion must be ≥1.0 cm in at least one dimension. If no other measurable lesions can be found outside of a previously irradiated field, (i.e. as may be seen with locoregional recurrence), and the lesion in the irradiated area meets all other conditions for being measurable, and the lesion is new or has increased in size by at least 20% in longest diameter, taking as reference the smallest longest diameter since radiation, and the previous radiation was completed at least 6 months prior to registration, it can be chosen. Non-measurable lesions include all other lesions, such as small lesions (longest diameter <2.0 cm with conventional techniques or <1.0 cm with spiral CT scans) bone lesions without significant extraosseous component, leptomeningeal disease, ascites, pleural/pericardial effusion, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules, palpable lymph nodes). For the case of skin lesions, documentation by color photography including a ruler to estimate the size of the lesions is recommended.

As outlined above, all subjects will be evaluated for response to vaccination by clinical examination, and CT scans of the known disease. CT scans (and/or MRI, if applicable) of the neck, chest or pelvis will be used to follow local recurrence or intrathoracic metastasis. Subjects with known abdominal or pelvic metastasis will also have ct scans (or MRI, if applicable). Radiographic response is determined as per modified RECIST 1.1 and irRECIST at 12 weeks. A confirmatory scan may be performed for subjects with response no earlier than 4 weeks from the documented response. Restaging studies will continue, as noted above. If progression is documented (except new lesions) on scans at 12 weeks, patients may remain on study at the discretion of the PI and if there is no life threatening complications from progression in the immediate future are expected. In these cases, follow up scans will be obtained 4 weeks later and if progression continues, the patient will be considered to have PD.

Scans will be evaluated for best overall response to vaccine therapy.

Because swelling and inflammation at the site of tumor may follow vaccination, increased size of already existing tumor found on clinical exam up to week 7, will not be considered PD. If, however, new sites of disease are found, the subjects will be considered to have PD.

11.1 Baseline Documentation of Target Lesions and Response Criteria

All measurable lesions, up to a maximum of 10 lesions representative of all involved organs, will be identified as target lesions, recorded and measured at baseline, according to RECIST 1.1 and irRECIST. The sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference to further characterize the objective tumor response for determination of ORR. Evaluation of target and non-target lesions will be performed and response determined according to RECIST 1.1 and irRECIST (Appendix B & C).

12. DATA REPORTING / REGULATORY REQUIREMENTS

The QACT will collect, manage, and perform quality checks on the data for this study. Adverse event lists, guidelines, and instructions for AE reporting can be found in Sections 6 and 7.

12.1 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the PI and study team.

The DSMC will review each protocol at least once or up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention; audit

results; and a summary will be provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

Following approval of Amendment 10, the below stopping rule will also be followed for the first 6 patients who sign the main consent and begin protocol treatment at the new dose (of note, there have been no DLTs observed among the first 6 patients who have been treated at the dose originally outlined in the protocol).

As this is the first study testing the DPX-E7 vaccine in humans, the following monitoring will be used: If zero or one DLT (see section 6.2 for definition) is observed through the first month after the second priming dose in the first six subjects who begin protocol treatment, then study enrollment into the first stage (n=16) will continue. If there are two DLT's observed through the first month after the second priming dose in the first six subjects, the dose of DPX-E7 vaccine will be de-escalated. If there are 3 or more DLTs observed through the first month after the second priming dose in the first 6 subjects, accrual will be suspended to further evaluate the events and decisions made regarding the overall status of the trial. If dose de-escalation is required, six new subjects will be enrolled and treated with DPX-E7 vaccine at the lower dose. If zero or one DLT is observed through the first month after the second priming dose in these six subjects, then study enrollment into the first stage (n=16) will continue. If there are two or more DLTs OR one death OR two grade 4 events observed through the first month after the second priming dose in these six subjects, then accrual will be suspended to further evaluate the events and decisions made regarding the overall status of the trial. Only those patients treated at the acceptable dose will be included in the phase II portion of the trial. Adverse events will be continuously monitored throughout the trial by the study team and appropriate committees with decisions made accordingly re: the study status and patient entry throughout the duration of the trial.

As overall monthly accrual is estimated to be 6 pre-consented for HLA and HPV testing, with 33% of those HLA-A*02 positive, the above monitoring will be ongoing and not require accrual to be suspended for adverse event evaluation after the first 6 patients who begin protocol treatment, however, these estimates will be monitored and accrual will be suspended as needed.

One of the primary objectives of this trial is to evaluate changes in CD8+ T cells in the peripheral blood and tumor tissue in patients positive for HLA-A*02 with advanced incurable HPVOC, cervical , or anal cancer. 'Responders' will be defined as patients with at least a two-fold increase in number of CD8+ T cells (dextramer, ELISpot or both methods) in the peripheral blood and tissue at the final analysis. In ONC-DPX-Survivac-01 and -03 studies, which used a similar vaccine and treatment plan, research subject's flow cytometry results were considered positive if the measurement is greater than the standard level of sensitivity (approximately > 0.05% positive cells) and the post treatment value is at least two times greater than the pre-treatment value).

A two-stage design will be used to minimize the number of patients exposed to this regimen. There are no preliminary efficacy data for this vaccine. The null hypothesis is that the 'response rate' (percentage of patients with at least a two-fold increase in number of CD8+ T cells in the peripheral blood and tissue at the final analysis) is not clinically meaningful (<10%). The alternative hypothesis is that the response rate is clinically meaningful (>10%).

Original Statistical Design: Efficacy

A two-stage design (Simon Minimax) was used where if ≥7 patients' disease was in 'response' among 40 evaluable (eligible and began protocol treatment) patients (assuming that disease in ≥3 patients 'responded' among 27 evaluable patients accrued in the first stage), further testing of this regimen was to be considered. The probability of concluding the regimen as effective was 90% if the true 'response rate' was 25%. The probability of concluding the regimen as effective was 10% if the true 'response rate' was 10%.

Revised Statistical Design: Efficacy (Amendment 10)

During review of Amendment 10, the statistical design for efficacy has been changed per request of SRC in order to revise the stopping rule to prevent larger numbers of subjects being enrolled on a futile trial. Operating characteristics used in the following design also take into account the poor prognosis of these patients.

Note that given the observed lack of activity in patients who began protocol treatment prior to approval of Amendment 10, accrual mentioned below refers to patients who sign the main consent and begin protocol treatment at the new dose outlined in Amendment 10.

A two-stage design (Simon Optimal) is used. In the first stage, accrual will continue until 16 evaluable patients (eligible and begin protocol treatment) are entered. If there is ≤1 patient with a two-fold increase in number of CD8+ T cells, the trial and data will be evaluated and accrual may close with the conclusion that there is little evidence that the 'response rate' would reach 25%. The probability that the trial will close early is 51% if the true proportion with a two-fold increase in number of CD8+ T cells is 10%.

If there are ≥2 patients among 16 evaluable patients with a two-fold increase in number of CD8+ T cells, the trial will continue to accrue patients until a total of 33 evaluable patients are entered. If there are ≥6 patients with a two-fold increase in number of CD8+ T cells among 33 evaluable patients, further testing of this vaccine will be considered. The probability of concluding the vaccine is effective is 84% if the true proportion with a two-fold increase in CD8+ T cells is 25%. The probability of concluding the regimen is effective is 10% if the true proportion with a two-fold increase in number of CD8+ T cells 10%.
Considering that patients continue to be screened and at least n=7 patients have been entered and began protocol treatment at the original dose prior to approval of Amendment 10, and allowing for 1 or 2 patients to sign the main consent and to be registered to the main protocol but not begin protocol treatment or be ineligible, the overall accrual goal following approval of Amendment 10 is up to n=44. Since the prevalence of the HLA-A*02 phenotype in the population is approximately 33%, we assume that we will screen at least 100 patients to be able to treat and follow n=33 evaluable HLAA* 02 positive patients following approval of Amendment 10. We anticipate that among the n=33, that approximately n=20 patients will have HPVOC, n=10 will have cervical cancer and n=3 patients will have anal cancer in the treatment cohort.

The primary efficacy population includes all eligible patients who begin protocol treatment at the new dose outlined in Amendment 10. Outcome data for the first group of patients who began protocol treatment prior to approval of Amendment 10 will be summarized in a secondary analysis. Response will be summarized as a proportion with a corresponding exact confidence interval.

Safety will be assessed via the CTCAE and frequencies of adverse events will be summarized and will include all patients who begin protocol treatment (those starting protocol treatment before and after approval of Amendment 10). Time to event endpoints will be estimated using the Kaplan-Meier method and 95% confidence intervals for the median or time-specific event time will be summarized.

With a monthly accrual rate of approximately 6 patients with advanced incurable HPVOC, cervical, or anal cancer patients who pre-consent for HLA and HPV testing, accrual to 100 patients for screening following approval of Amendment 10 is estimated to last at least 17 months. The requirement for mandatory fresh tumor tissue biopsies in all treated patients could also impact the accrual rate and time to complete accrual. As is customary with this type of design, accrual will be suspended after the first stage (n=16 evaluable patients) in order to assess outcome, however, this suspension is also dependent on the actual observed accrual rate and the number of patients with a two-fold increase in CD8+ T cells while the first stage of the trial is accruing.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section 9. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. Manuscripts, abstracts, or reports will be prepared by the Principle Investigator and at minimum reviewed by all sub-investigators and by Immunovaccine before submission or release.

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16. APPENDICES

APPENDIX A: Performance Status Criteria

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B: Modified Response Evaluation Criteria in Solid Tumors (RECIST 1.1) Quick Reference
http://ctep.cancer.gov/protocolDevelopment/docs/Recist_Guideline.pdf

Eligibility

Only patients with measurable disease at baseline should be included in protocols where objective tumor response is the primary endpoint.

- **Measurable disease** - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology when feasible.
- **Measurable lesions** - lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan. If no other measurable lesions can be found **outside of a previously irradiated field, (i.e. as may be seen with locoregional recurrence), and** the lesion in the irradiated area meets all other conditions for being measurable, **and** the lesion is new or has increased in size by at least 20% in longest diameter, taking as reference the smallest longest diameter since radiation, **and** the previous radiation was completed at least 6 months prior to registration, it can be chosen.
- **Non-measurable lesions** - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques.
- All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.

- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline documentation of "Target" and "Non-Target" lesions

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).
- A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.
- All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

Evaluation of target lesions

• Complete Response (CR):	Disappearance of all target lesions
• Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
• Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of non-target lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
- Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits
- Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1)

Although a clear progression of "non target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

Target lesions	Non-Target lesions	New Lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended

that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

Duration of overall response

- The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.

The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

APPENDIX C: Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria (irRECIST) Quick Reference

http://clincancerres.aacrjournals.org/content/15/23/7412.long

Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria

Table 1.

Comparison between WHO criteria and the irRC

	WHO	irRC
New, measurable lesions (i.e., ≥5 × 5 mm)	Always represent PD	Incorporated into tumor burden
New, nonmeasurable lesions (i.e., <5 × 5 mm)	Always represent PD	Do not define progression (but preclude irCR)
Non-index lesions	Changes contribute to defining BOR of CR, PR, SD, and PD	Contribute to defining irCR (complete disappearance required)
CR	Disappearance of all lesions in two consecutive observations not less than 4 wk apart	Disappearance of all lesions in two consecutive observations not less than 4 wk apart
PR	≥50% decrease in SPD of all index lesions compared with baseline in two observations at least 4 wk apart, in absence of new lesions or unequivocal progression of non-index lesions	≥50% decrease in tumor burden compared with baseline in two observations at least 4 wk apart
SD	50% decrease in SPD compared with baseline cannot be established nor 25% increase compared with nadir, in absence of new lesions or unequivocal progression of non-index lesions	50% decrease in tumor burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in SPD compared with nadir and/or unequivocal progression of non-index lesions and/or appearance of new lesions (at any single time point)	At least 25% increase in tumor burden compared with nadir (at any single time point) in two consecutive observations at least 4 wk apart

Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumors: Immune-Related Response Criteria

Table 2.

Derivation of irRC overall responses

Measurable response	Nonmeasurable response		Overall response
Index and new, measurable lesions (tumor burden),* %	Non-index lesions	New, nonmeasurable lesions	Using irRC
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent/Stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent/Stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25?	Any	Any	irPD†

*Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).

†Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart.