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Treatment of High-Grade Pre-Neoplastic Cervical Lesions (CIN 2/3) Using a Novel "Prime and Pull" Strategy

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SCHEMA

Women with high-grade (CIN 2/3) cervical dysplasia

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Subjects must have histologically-confirmed pre-invasive HPV lesion(s), cervical intraepithelial neoplasia grades 2 or 3 (CIN 2/3), as determined by colposcopy-guided cervical biopsies

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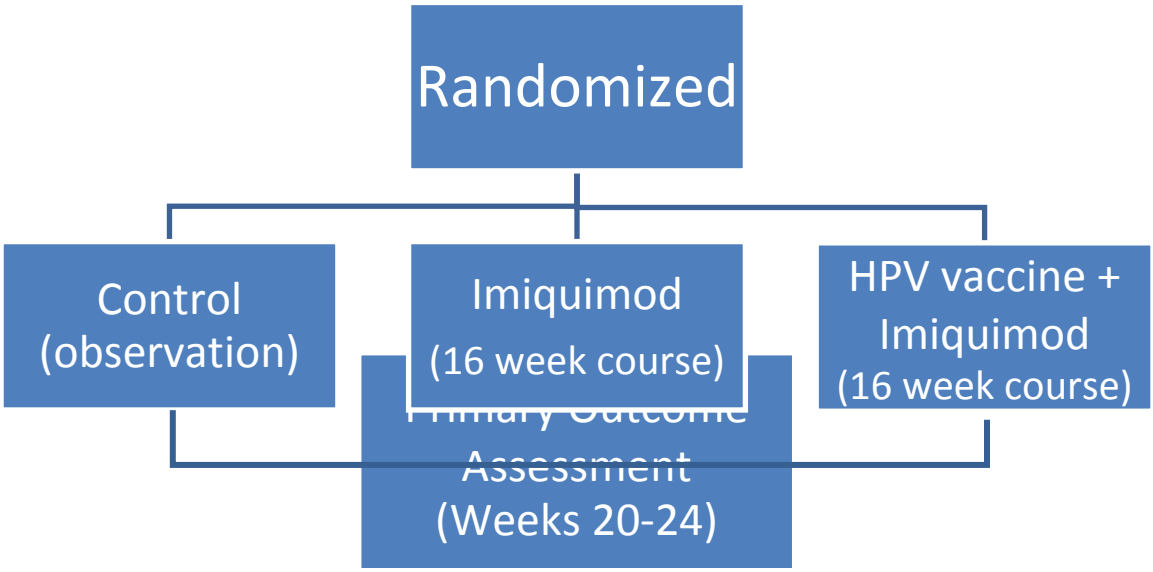

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1.1.2 To determine treatment efficacy defined as histologic regression to CIN 1 or less at weeks 20-24 (4 to 8 weeks after the end of imiquimod treatment) in the Imiquimod group compared to control

1.2 Secondary objectives

1.2.1 To assess complete regression (i.e., histologic remission) at weeks 20-24 (4 to 8 weeks after the end of imiquimod treatment) in each group

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1.3 Exploratory/correlative objectives

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1.3.2 To assess HPV16 E7 immunity in CD4/CD8 T cells

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2.0 BACKGROUND AND RATIONALE

Human papillomavirus (HPV) infection, which is sexually transmitted, leads to a wide range of conditions in women from genital warts and precancerous cervical dysplasia to cancers of the genital tract and elsewhere. Globally, 9 million cases of precancerous high-grade "cervical intraepithelial neoplasia (CIN 2/3) are detected annually through cervical cancer screening programs; 330,000 in the U.S. alone.[1] Persistent infection with oncogenic ("high-risk") HPV types is one of the most important risk factors for development of CIN 2/3 which poses significant risk of progression to invasive cervical cancer.[2-4] In order to prevent this progression to invasive cancer, CIN 2 and 3 lesions require frequent surveillance and management through serial pelvic exams, cytology, colposcopy-directed biopsies, and various invasive procedures, including cervical ablation or excision that can negatively impact future pregnancies. Surveillance is often complicated by poor compliance, significant stress and anxiety, and low tolerance for serial pelvic exams and procedures. In addition, the direct medical costs in the U.S. associated with both screening for and treating HPV-associated diseases is estimated to be $8.0 billion dollars, over 80% of which is for routine cervical cancer screening and follow-up.[5]

Current FDA-approved prophylactic HPV vaccines have not shown therapeutic effect in patients with pre-existing HPV-associated cervical lesions.[6-8] Treatment options for CIN 2-3 involve a variety of destructive or excisional procedures, although in the U.S. excision is generally favored in these women at higher risk of invasive cervical cancer as it allows for pathologic evaluation of the specimen for diagnosis and for evaluation of surgical margins. Ablation techniques typically utilize cryotherapy or laser while excision techniques include scalpel, electrosurgery or laser.

A major concern with the destructive or excisional treatment modalities currently available for CIN 2-3 include future obstetric risk (pregnancy loss, preterm premature rupture of membranes, and preterm delivery) and cervical stenosis, or narrowing of the cervical canal, which can result from scarring after treatment. The risk of cervical stenosis after an excision procedure was shown to be 7% in one study and this can have several negative outcomes including difficulty with normal cervical dilation during labor, difficulty with common office procedures (e.g., endometrial biopsy), and might impede normal menstrual flow.[9] One large population-based study found that women who gave birth after a cervical excision procedure were significantly more likely to have a preterm delivery and had a significantly higher risk of a late abortion (< 24 weeks gestation) compared with women who gave birth prior to the surgical excision.[10]

Given these circumstances, precancerous cervical dysplasia is an ideal candidate for therapeutic vaccination to clear CIN 2-3 without destroying cervical tissue. Previous attempts at developing vaccines that mount systemic cellular immunity responses against other viral sexually transmitted infections have been unsuccessful.[11,12] We believe that one of the problems in current therapeutic vaccine approaches is the lack of effector T cell migration to the sites of infection. Although systemic memory T cells can gain entry to certain organs, other anatomic locations including the vagina are immunologically restrictive and prevent entry of memory T cells.[13] Inflammation or infection is often needed to permit entry of systemically circulating activated T cells into restrictive tissues to establish tissue-resident memory T cells.[14-17]

Our group has recently reported a highly effective vaccination strategy ("prime and pull") to establish tissue-resident memory T cells in the genital tract.[18] An initial systemic T cell response is triggered ("prime") by parenteral vaccination. Activated T cells are then recruited into the genital mucosa ("pull") through the application of specific chemokines to the restrictive genital tract and then establish a long-term tissue-resident memory T cell (T_{RM}) pool and mediate protective immunity.[14] In

mice, this strategy reduces spread of infectious viruses and prevents clinical disease. These results strongly suggest the possibility of inducing T cell responses against oncogenic HPV genotypes through immunization with a prophylactic HPV vaccine, followed by recruitment of HPV-activated T cells into cervical lesions using a local immune-response modulator.

Imiquimod, a topical immune response modulator, has antiviral and antitumor activity, induces cytokines including interferon, tumor necrosis factor, and interleukins 1, 6, and 8, and activates T cells triggering an immune response associated with HPV clearance.[19,20] Imiquimod is approved for treatment of genital warts, 90% of which are caused by non-oncogenic HPV types 6 and 11.[21,22] One group has studied the use of imiquimod in the weeks prior to a planned surgical cervical excision procedure as a potential method for reducing recurrence of disease.[23] They found application of imiquimod to the cervix to be safe and tolerable. Only one prior study has evaluated the efficacy of imiquimod for therapeutic purposes in high grade cervical dysplasia. This Austrian study on the efficacy of imiquimod for treatment of CIN 2/3, found significantly higher rates of histologic regression (73% vs 39%) and complete remission (47% vs 14%) in the imiquimod group.[24] We are not aware, however, of any published study evaluating a "prime and pull" vaccination strategy for the potential treatment of CIN 2/3 in women. In the case of imiquimod treatment alone, this relies on the ability of T cell responses to endogenous antigens to elicit clearance of the HPV infected cells.

We believe that "prime and pull" will provide further protection to imiquimod treatment alone, by eliciting robust T cell responses that can be pulled into the genital mucosa using chemokines induced by imiquimod application. Currently, a 9-valent prophylactic HPV vaccine (9vHPV) is commercially available and provides protection against 7 high-risk HPV types that cause 90% of all cervical cancers (and protects against 2 additional HPV types that cause 90% of all genital warts).[22] 9vHPV is a sub-unit protein vaccine; vectors expressing the papillomavirus *L1* gene have been used to generate virus like particles (VLPs) that mimic the wild type virus capsid morphologically and antigenically but contain no DNA and are therefore noninfectious. This vaccine is highly immunogenic, inducing a robust serum neutralizing antibody response which prevents incident infection.[25,26] Previous studies have demonstrated induction of antigen-specific T cell responses by prophylactic HPV vaccines as well.[27-30]

Published treatment efficacy with imiquimod[24] and the preclinical in vitro data presented above and fully reported in *Nature*[18] clearly suggest that a "prime and pull" vaccination strategy may provide significant therapeutic effect for HPV-associated CIN 2/3 lesions. Because the development of a novel, effective treatment modality without the need of destructive interventions on the cervix in CIN 2/3 remains an unmet medical need, in this study we are planning a multi-arm randomized clinical trial to determine the therapeutic clinical effect and resulting immune response of a "prime and pull" method of treatment in women with CIN 2-3 lesions using imiquimod vaginal suppository in conjunction with the commercially available 9-valent HPV vaccine.

3.0 SUBJECT ELIGIBILITY AND EXCLUSIONS

3.1 Eligible Subjects:

3.1.1 Subjects must have untreated cervical biopsy-proven, CIN 2/3 ectocervical lesion(s).

3.1.2 Subjects must have satisfactory colposcopy with visualization of the entire transformation zone or a negative endocervical curettage if colposcopy is unsatisfactory.

3.1.3 Subjects must be high-risk HPV+ as determined by commercially available DNA hybridization test which tests for 13 high-risk HPV types.

3.1.4 All Subjects must have a histologic diagnosis of CIN 2,3 cervical lesion(s) confirmed by a study pathologist.

3.1.5 Subjects must have signed an approved informed consent.

3.1.6 Subjects of childbearing potential must have a negative urine pregnancy test within 7 days prior to the study entry and be practicing an effective form of contraception.

3.1.7 Subjects must be at least 18 years of age based on previous and current cervical cancer screening guidelines.

3.1.8 Subjects must be fluent in speaking English or Spanish.

3.2 Ineligible Subjects:

3.2.1 Subjects with unsatisfactory colposcopy* (unable to visualize entire transformation zone) or evidence of endocervical disease defined as CIN 2/3 diagnosed on endocervical curettage. *Subjects with unsatisfactory colposcopy but negative endocervical curettage are eligible (see 3.1.2).

3.2.2 Subjects with a history of invasive cervical cancer

3.2.3 Subjects with a history of other invasive malignancies, with the exception of non-melanoma skin cancers are excluded if there is any evidence of other malignancy being present within the last five years. Subjects are also excluded if their previous cancer treatment contraindicates this protocol therapy.

3.2.4 Subjects with any unstable medical issue (including cardiac issues as above, active treatment for pulmonary embolism, CVA, renal or hepatic insufficiency, active infection/sepsis requiring IV antibiotics).

3.2.5 Subjects who have an uncontrolled seizure disorder, or active neurological disease.

3.2.6 Subjects known to be seropositive for HIV and active hepatitis, even if liver function studies are in the normal range. Subjects otherwise immunocompromised will also be excluded (chronic steroid use, taking immunosuppressive medications).

3.2.7 Pregnant or breastfeeding subjects.

3.2.8 Subjects who have had a total hysterectomy (removal of uterus and cervix) or trachelectomy (removal of cervix).

3.2.9 Subjects with a known hypersensitivity to imiquimod. Subjects with a known hypersensitivity to any prophylactic HPV vaccine or severe allergic reactions to yeast (vaccine component).

3.2.10 Subjects who have received their first dose of HPV vaccine < 4 weeks ago or their second dose < 12 weeks ago.

3.2.11 Known hypersensitivity or prior intravaginal treatment with Imiquimod

3.3 Inclusion of Minorities:

3.3.1 As men do not have uteri, only women will be enrolled in this trial. Women fluent in English or Spanish will be eligible for study enrollment based on limited resources in providing appropriate informed consent and research follow up to subjects only fluent in other non-English or Spanish languages. We will not exclude potential subjects from participating in this or any study solely on the basis of socioeconomic status. Every attempt will be made to enter all eligible subjects into this protocol and therefore address the study objectives in a broad population of women with high-grade cervical dysplasia.

4.0 STUDY MODALITIES

4.1 Imiquimod

4.1.1 Packaging, labelling, and storage

Medication numbers will be unique to each box and will be used for tracking purposes only.

4.1.2 Imiquimod

Imiquimod will be supplied as vaginal suppositories. Available dosage strengths will be 6.25mg and 3.125 mg. Suppositories will be supplied in boxes.

Handling instructions will include washing hands before and after vaginal suppository is placed. Subjects will be advised not to have sexual intercourse during the nights in which they applied vaginal suppositories and to perform a vaginal douche in the morning. Subjects will be instructed to suspend the application of vaginal suppositories during the first 3 days of their menses. In addition, subjects may use a tampon following insertion to keep the suppository in place and apply Vaseline to skin surrounding the insertion area to prevent irritation. A 24-hour phone number is provided should subjects have any issues or concerns.

Boxes will be labelled according to local regulations and will include the following as a minimum:

- Study number.
- Product name (Imiquimod)
- Contents of the boxes (18 and 24 vaginal suppositories)
- Suppository strength (mg)
- Batch number
- Medication number
- Use-by date
- Storage information
- Instructions for use
- Sponsor name and address
- A statement that the medication is for clinical study use only
- A caution statement

A new box of medication will be dispensed on day 1 (day of enrollment). The subject will initially receive one box of 6.25 mg tablets and in the event that dose reduction is necessary the subject will return to the clinic and new medication will be dispensed.

4.1.3 Storage conditions

Imiquimod must be stored refrigerated in the original package. Suppositories must be stored according to label instructions.

4.1.4 Drug accountability

Drug supplies, which will be purchased from Apex Pharmacy (Hamden, CT), will be kept in a secure, limited access storage area under the storage conditions. Where necessary, a temperature log must be maintained to make certain that the drug supplies are stored at the correct temperature.

The responsible person must maintain records of the product's delivery to the study site, the inventory at the site, the use by each subject, and the return to Apex Pharmacy or alternative disposition of unused product(s).

These records will include dates, quantities, batch/serial numbers, expiry ('use by') dates, and the unique code numbers assigned to the investigational product(s) and study subjects. The responsible person will maintain records that document adequately that the subjects were provided the doses specified by the CSP

and reconcile all investigational product(s) received from Apex Pharmacy. The responsible person must verify that all unused or partially used drug supplies have been returned by the clinical study subject.

4.2 9-valent Human Papillomavirus Vaccine (*Gardasil 9)*

4.2.1 Packaging, labelling, and storage

Commercially available vaccine (GARDASIL®9- Merck) will be purchased by the Investigational Drug Service at Yale New Haven Hospital and stored under appropriate conditions in its original container until dispensed to study participants.

4.2.2 9-valent Human Papillomavirus Vaccine

9-valent HPV vaccine will be administered in the clinic by a nurse. The vaccine will be supplied in single-dose vials and is administered as 0.5 mL per dose intramuscularly in the deltoid region of the upper arm or in the anterolateral area of the thigh. Thorough agitation immediately prior to administration is necessary to maintain suspension of the vaccine.

Subjects randomized to the Imiquimod + HPV vaccine arm of the study will receive a dose of the vaccine on day 1 (day of enrollment). Subjects meeting criteria for a booster dose will return to the clinic to receive a second dose of the vaccine in week 8.

4.2.3 Storage conditions

HPV vaccine must be stored in the original package to protect from light and refrigerated at 2^{o}C to 8^{o}C (not to be frozen) and administered as soon as possible after being removed from refrigeration.

4.2.4 Drug accountability

9-valent HPV vaccine will be supplied, stored, and maintained by Yale-New Haven Hospital's Investigational Pharmacy (oversight by Sam Abdelghany). Per their protocol, the vaccine supply will be kept in a secure, limited access refrigerated storage area under the storage conditions. A temperature log must be maintained to make certain that the drug supplies are stored at the correct temperature.

The responsible person must maintain records of the product's delivery to the study site, the inventory at the site, the use by each subject, and the return to YNHH Investigational Pharmacy or alternative disposition of unused product(s).

These records will include dates, quantities, and batch/serial numbers, expiry ('use by') dates. The responsible person will maintain records that document adequately that the subjects were provided the doses specified by the CSP and reconcile all product(s) received from YNHH Investigational Pharmacy. The responsible person must verify that all unused or partially used drug supplies have been returned by the clinical study subject.

5.0 CORRELATIVE STUDIES

5.1 Histopathology: Cervical biopsies will be collected at the time of initial colposcopic evaluation based on clinical guidelines for management of abnormal pap tests. Cervical biopsies will be processed

in the surgical pathology department. Cervical biopsies with high-grade (CIN 2/3) dysplasia will be centrally reviewed and diagnosis confirmed by study pathologists, in the Pathology Department at Yale University. Subjects with confirmed CIN 2/3 are eligible for enrollment. Colposcopy will be performed and cervical biopsies collected during weeks 20-24 (4-8 weeks after completion of Imiquimod therapy) will be centrally reviewed in the Pathology Department at Yale University to assess for the primary outcome. Pathologists will be blinded to study randomization.

5.2 HPV DNA Testing & HPV 16/18 Genotyping: Confirmatory high-risk HPV DNA testing will be performed under supervision of cytopathologist Dr. Angelique Levi in the Pathology Department at Yale University from cervical cytobrush sample obtained on day of enrollment. HPV 16/18 genotyping will be performed via PCR from cervical cytobrush sample by Santin Lab to establish baseline HPV status. This testing will be repeated between weeks 16-18 from cervical cytobrush samples and at final study visit during weeks 20-24 or following at least 3 weeks after completion of imiquimod therapy.

5.3 Exploratory Objectives

5.3.1 In the translational research component of this study we are planning to systematically evaluate and compare T cell infiltration into the cervical dysplasia and correlate with clinical response. The ultimate goal being to determine if the prime and pull strategy of Imiquimod + HPV vaccine leads to the establishment of T_{RM} cells and if this correlates to clinical regression of high-grade cervical dysplasia. The pre- and post-treatment cervical biopsies will be examined to determine the frequencies and anatomical distributions of CD4 and CD8 T cells within the CIN. We will stain paraffin embedded tissue sections with antibodies to CD4 and CD8. These sections will be costained with well-established markers of human T_{RM} (CD69, CD103).[31] The comparison of T_{RM} frequencies and distributions will be conducted both within the individual (pre and post prime and pull) as well as amongst the groups at the relevant time points. We will assess possible correlation between T_{RM} and viral clearance and clinical outcomes. Because the biopsy sample is quite limited in size, we will also collect cells from the endocervical cytobrush to analyze the local T cell populations. The protocol for isolating and analyzing T cells from cytobrush is well established.[32] We will stain the collected CD4 and CD8 T cells with respect to their T_{RM} (CD69, CD103) phenotype, as well as *ex vivo* stimulation with HPV peptide pool (the same one used in the CFSE experiments briefly described below) and measuring TNF-α and IFN-γ secretion by intracellular staining.

5.3.2 In addition, we will explore the changes of pre- and post-immune therapy responses to HPV16/18 E7 antigens in subject's peripheral blood lymphocytes (PBL) and serum. We will utilize CFSE flow cytometry-based proliferative assays after in vitro stimulation with HPV16 or 18 E7 peptides (i.e., a set of 15-mer peptides overlapping by the central ten amino acids and a set of 9-mer peptides overlapping by the central eight amino acids for the HPV 16 or 18 E7 protein) and a streptavidin-biotin capture ELISA method to investigate anti-HPV E7 antibody prevalence in serum as described by our group (references below).[33-37] These explorative assays have been previously validated in studies using reagents obtained from both healthy women as well as cervical cancer subjects vaccinated within Phase I clinical studies with HPV type 16 and 18 E7 antigen-loaded autologous dendritic cells (DC) as a therapeutic cellular vaccine.[33-37]

6.0 TREATMENT PLAN AND ENTRY PROCEDURE/REGISTRATION PROCEDURE

6.1 Study Entry: Subjects will be screened and consented by the P.I. or a member of the study staff. Consent may be obtained remotely. The consent form can be mailed or emailed to the research participant who will sign a printed version of the consent form and fax/scan/email/send a picture of the signed consent form or mail the signed consent form back to the researcher.

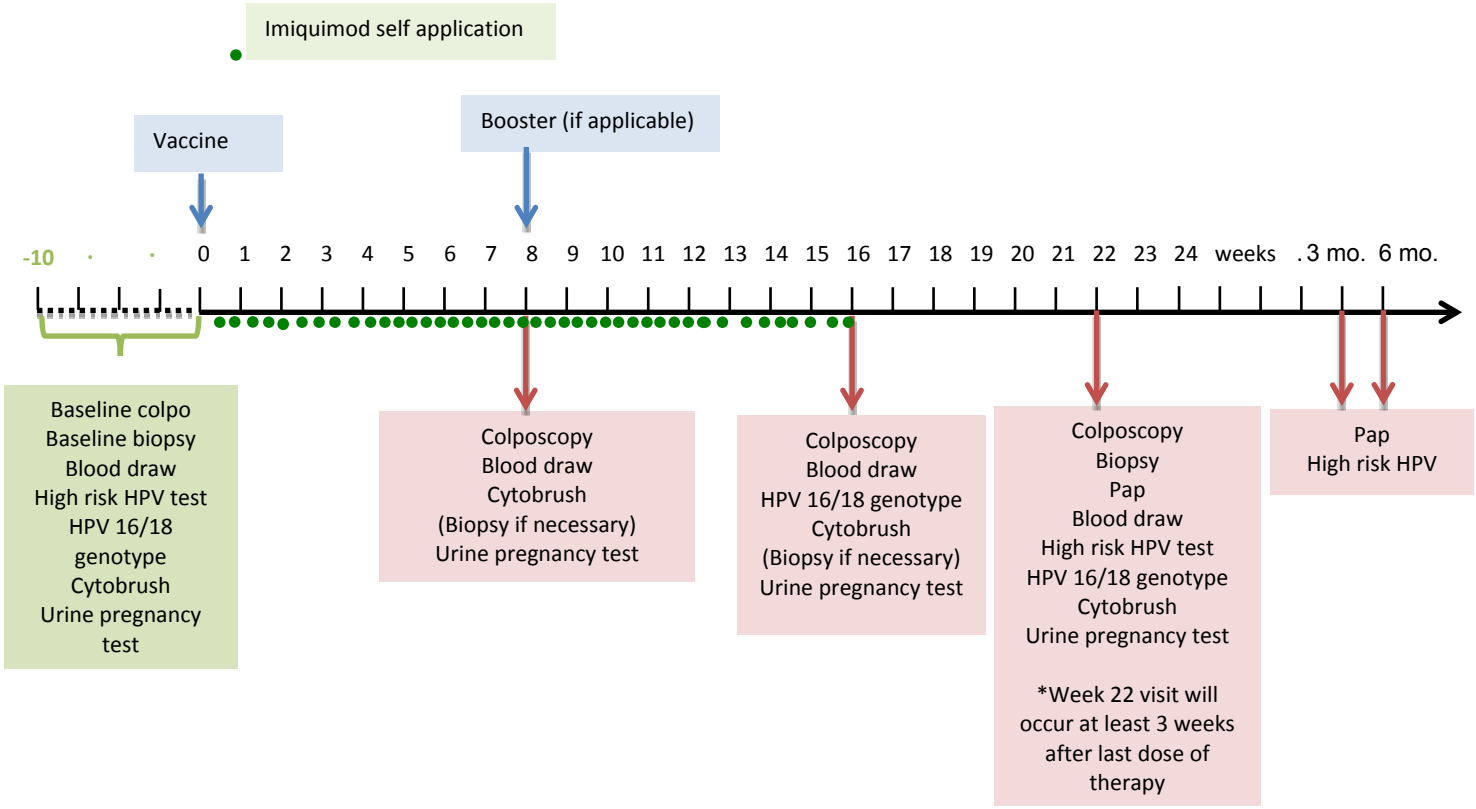

FIGURE 1: Study Timeline

6.2 Treatment Plan This is a randomized Phase II, three arm control trial in subjects with CIN 2/3 high grade cervical dysplasia. Subjects with CIN 2/3 meeting eligibility criteria will have cervical biopsy specimens centrally reviewed by study pathologist to confirm diagnosis. HPV DNA test and HPV 16/18 genotyping will be performed from endocervical cytobrush samples to determine HPV status associated with the dysplasia.

Subjects who have CIN 2/3 with HPV+ disease will be enrolled in this study. Subjects will be randomized to one of three arms: observation only (control), imiquimod only, imiquimod + 9-valent HPV vaccine. All cytology and cervical biopsy specimens related to the study will be centrally reviewed by study pathologists who will be blinded to treatment assignment.

6.2.1 Pre-Treatment Visit/Study Enrollment: After the index cervical biopsy demonstrating CIN 2-3, all subjects will be brought back to clinic to discuss results and management. After obtaining informed written consent the following pre-treatment tests will be performed: medical history, physical exam with pelvic exam, vital signs, urine pregnancy test (must be performed within 7 days of treatment), translational blood

samples (may be drawn after randomization but must be drawn prior to treatment), endocervical specimen to be sent to Yale Pathology for high-risk HPV DNA testing (if high-risk HPV DNA results are already available, this testing may not have to be repeated) and a second endocervical cytobrush specimen for translational research purposes. Blood samples will be collected in sodium heparin tubes with about 28 cc collected each time as follows: 7 cc in each of 3 green top tubes and 7 cc in 1 EDTA/purple top tube (see follow up visits below). Endocervical specimen for high-risk HPV DNA test to be performed by Yale Pathology will be submitted in usual fashion as a SurePath specimen. Endocervical cytobrush sample for study purposes will be collected into a 50ml conical tubes containing PBS using sterile techniques and will be stored on ice until picked up by a laboratory personnel. Once all eligibility has been met the subject will be randomized to one of three arms.
Additional components of visit outline by study group:

(1) Control group: Observation at specified study timepoints.

(2) Imiquimod only: At the baseline visit, this group will receive instruction on imiquimod self application (16 week course) using a 6.25 mg vaginal suppository, storage of medication, possible side effects. Subjects will be provided a diary and case report form to record vaginal suppository application and side effects will be recorded per NCI Common Terminology Criteria for Adverse Events (CTCAE 4.0). Imiquimod self-application regimen will be similar to one previously described[24] and is as follows:

- Treatment weeks 1 and 2: Subjects apply one vaginal suppository per week
- Treatment weeks 3 and 4: Subjects apply two vaginal suppositories per week
- Treatment weeks 5 to 16: Subjects apply three vaginal suppositories per week

Vaginal suppositories will be self-applied by the subjects in the evening right after going to bed. Subjects will be advised not to have sexual intercourse during the nights in which they applied vaginal suppositories and to perform a vaginal douche in the morning. Subjects will be instructed to suspend the application of vaginal suppositories during the first 3 days of their menses. Subjects will be advised to keep the study medication refrigerated. In case of persistent side effects in subjects of the imiquimod group, the dose of the study medication will be modified and participants will receive vaginal suppositories containing only 50% of the original imiquimod dose (vaginal suppositories containing 3.125 mg imiquimod).

Missed doses of imiquimod can be made up the following day. Otherwise, the dose must be skipped and subjects should take the next scheduled dose at the usual time.

Imiquimod will be dispensed in boxes containing 18 vaginal suppositories (treatment weeks 1-8) at the beginning of the treatment course. During follow up visit at week 8 (see below), subjects will be provided a new box of 24 vaginal suppositories to complete treatment course for weeks 9-16. Study drug will be prescribed by the investigator and may be dispensed either by the investigator or study staff.

(3) Imiquimod + HPV vaccine: At the baseline visit, this group will be instructed about the correct method of self-application of imiquimod 6.25mg as a vaginal suppository as described above, storage of medication, possible side effects. Subjects will be provided a diary and case report form to record vaginal suppository application and side effects will be recorded per NCI Common Terminology Criteria for Adverse Events (CTCAE 4.0). In addition, all women (regardless of age) will be administered a dose of the HPV vaccine on day of enrollment (regardless of previous HPV vaccination history). Women previously unvaccinated will receive an additional booster dose at 8 weeks. Local pain will be assessed with a visual analog scale immediately after vaccine administration and 5 minutes later. Subjects will be provided a separate case report form to record vaccine side effects per CTCAE 4.0.

Missed doses of imiquimod can be made up the following day. Subjects are encouraged to complete taking all prescribed doses. If additional time is required toward this goal of treatment, the scheduled dosing period may be extended.

Imiquimod will be dispensed in boxes containing 18 vaginal suppositories (treatment weeks 1-8) at the beginning of the treatment course. During follow up visit at week 8 (see below), subjects will be provided a new box of 24 vaginal suppositories to complete treatment course for weeks 9-16. Study drug will be prescribed by the investigator and may be dispensed either by the investigator or study staff.

6.2.2 Follow-up Visits: Follow-up visits will occur at week 8 (between weeks 7-9), at week 16 (between weeks 16-18) and once at week 22 (between weeks 20-24). For subjects randomized to treatment arms (2) Imiquimod only and (3) Imiquimod + HPV vaccine, the week 22 visit will occur at least 3 weeks after last dose of therapy. Urine pregnancy test will be performed at all follow-up visits. Subjects with exam findings concerning for worsening disease at any point in the study will be taken out of the study and treated with surgical excision procedure.

Week 8 (7-9 weeks) Visit: This visit will be timed to occur part way through the 16 week imiquimod treatment course. For women using imiquimod (Imiquimod only and Imiquimod + HPV vaccine arms), a review of medication diary, monitoring of correct use of study medication, and report of local and systemic side effects using a standardized case report form will occur at all follow up visits. For women who received HPV vaccine as part of study protocol (Imiquimod + HPV vaccine arm), a review of vaccination diary and report of local and systemic side effects using a standardized case report form will occur at all follow up visits. A pelvic exam with colposcopy will be performed to objectively assess for gross cervical lesions in all subjects and also for local side effects from imiquimod use in the Imiquimod only and Imiquimod + HPV vaccine arms. Endocervical cytobrush samples will be obtained at this time for study purposes. Cervical biopsies will be performed as clinically indicated. Blood sample collections will also occur. In addition, women in the Imiquimod + HPV vaccine groups who were previously unvaccinated will receive their booster dose of the HPV vaccine.

Week 16 (16-18 weeks) Visit: This visit will be timed to occur at completion of imiquimod treatment course. For women using imiquimod (Imiquimod only and Imiquimod + HPV vaccine

arms), a review of medication diary, monitoring of correct use of study medication, and report of local and systemic side effects using a standardized case report form will occur at all follow up visits. For women who received HPV vaccine as part of study protocol (Imiquimod + HPV vaccine arm), a review of vaccination diary and report of local and systemic side effects using a standardized case report form will occur at all follow up visits. A pelvic exam will be performed to objectively assess for gross cervical lesions in all subjects and also for local side effects from imiquimod use in the Imiquimod only and Imiquimod + HPV vaccine arms. Endocervical cytobrush samples will be obtained at this time. HPV 16/18 genotyping will be performed using endocervical cytobrush samples at this time point. Colposcopy and cervical biopsies will be performed as clinically indicated. Blood sample collections will also occur.

Week 22 (20-24 weeks): This visit will be timed to occur at least 3 weeks after completion of imiquimod treatment course. For women using imiquimod (Imiquimod only and Imiquimod + HPV vaccine arms), a review of medication diary, monitoring of correct use of study medication, and report of local and systemic side effects using a standardized case report form will occur at all follow up visits. For women who received HPV vaccine as part of study protocol (Imiquimod + HPV vaccine arm), a review of vaccination diary and report of local and systemic side effects using a standardized case report form will occur at all follow up visits. A pelvic exam will be performed to objectively assess for gross cervical lesions in all subjects and also for local side effects from imiquimod use in the Imiquimod only and Imiquimod + HPV vaccine arms. Endocervical cytobrush samples will be obtained at this time. High risk HPV test and HPV 16/18 genotyping will be performed using endocervical cytobrush samples at this time point. Colposcopy and cervical biopsies will be performed on all subjects at this final study visit to assess primary endpoints. Blood sample collections will also occur.

Persistence of CIN 2-3 or worsening cervical dysplasia diagnosed at the 20-24 weeks visit will be recommended for treatment with surgical conization.

At approximately 3 months and 6 months after the last study visit, as per standard of care, it is recommended that subjects will have a pap smear with high-risk HPV testing. The results of this testing will be recorded in the subject's study record.

7.0 TREATMENT MODIFICATIONS: IMIQUIMOD

7.1 Dose reduction levels

7.1.1 Dose reduction for imiquimod

Treatment related toxicities will be managed by treatment interruptions and possible dose reductions of imiquimod according to the schedule described in the below Tables. Dose reductions will apply to individual subjects only. Once the dose has been reduced, it cannot be increased later.

To prevent the development of more severe adverse events, treatment related rash/skin irritation should be managed early and proactive as described in the below Tables. Table 1: Dose reduction scheme for imiquimod

AE type and CTCAE Grade	Action	Dose reduction scheme
Events related to study drug: Grade 2 • Dermatologic or local inflammatory reactions which responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDS, narcotics); prophylactic medications indicated for ≤ 24 hrs • Vulvar edema with readily apparent obscuration of anatomic architecture, obliteration of skin folds, deviation from normal anatomic contour • Any drug related AE Grade ≥3	Pause treatment until subject has recovered to Grade ≤1 or baseline[1]. Resume treatment. May reduce dose according to schedule opposite. If subject has not recovered to Grade ≤1 or baseline[1] within 21 days study treatment must be permanently discontinued[2].	If subject was receiving 6.25 mg, resume treatment at a dose of 3.125. If subject was receiving 3.125 mg, discontinue imiquimod.

1 Baseline is defined as the CTCAE (version 4) Grade at the start of treatment
2 In the event that the subject is deriving obvious clinical benefit according to the investigator's judgement, further treatment with imiquimod will be decided by the investigator.

In the event of any unrelated adverse events, the investigator may choose to interrupt the medication for up to 21 days, but no dose reduction should occur. If the medication is interrupted for more than 21 days, the decision to continue with imiquimod will be made by the investigator.

7.2 Management of adverse events, concomitant therapy, restrictions and rescue treatment

7.2.1 Rescue medication, emergency procedures, and additional treatment(s)

7.2.1.1 Rescue medication

Rescue medications to reverse the actions of imiquimod are not available. There is no specific antidote for overdosage. Potential adverse events should be treated symptomatically. Common adverse events of treatment with imiquimod with specified management recommendations and/or requirements include local inflammatory reactions with site itching or burning or localized edema and occasionally accompanied by dermatologic, respiratory, or infectious symptoms. To improve tolerability and the probability of clinical benefit, subjects should receive prompt and appropriate supportive care at the first signs of symptoms. Suggested treatments for AEs are described below.

7.2.1.2 Concomitant treatment(s)

Concomitant medications or therapy to provide adequate supportive care may be given as clinically necessary.

All concomitant therapy, including anaesthetic agents, vitamins, homeopathic/herbal remedies, nutritional supplements, must be recorded in the (e)CRF during the screening and treatment period, starting from the date of signature of informed consent, and ending at the EOT visit. After the EOT visit, only concomitant therapy indicated for treatment of an AE has to be reported.

In case of major surgery (as judged by the investigator), it is recommended to stop treatment with imiquimod around one week prior to the surgery, and to restart treatment after surgery (unless surgery involves genitourinary or anogenital tracts in which case treatment should be suspended until complete wound healing achieved). If imiquimod is interrupted for more than 21 days, the decision to continue will be made by the sponsor in agreement with the investigator.

7.2.2 Management of expected adverse events

Dermatologic and local inflammatory adverse events are the most common side-effects associated with treatment with imiquimod. Respiratory symptoms are less commonly reported but can include sinusitis, upper respiratory tract infection or flu-like symptoms. Treatment of these side-effects should be proactive and should be started as early as possible after onset of symptoms.

7.2.2.1 Management of dermatologic and local inflammatory adverse events following treatment with imiquimod

Dermatologic adverse events including localized erythema, xeroderma, crusted skin, erosion or ulceration, edema, vulvar pain or pruritus can occur after a few applications of imiquimod. Although usually mild to moderate, dermatologic reactions can cause significant discomfort (Table 7.2.2.1: 2).

Table 2: Grade specific treatment recommendations for imiquimod-related dermatologic/ local inflammatory reactions:

Severity (CTCAE Grading)	**Description**	**Intervention concerning imiquimod treatment**	**Specific intervention**
Pain of skin			
Mild (Grade 1)	Mild pain	Continue same dose	Consider oral acetaminophen or ibuprofen
Moderate (Grade 2)	Moderate pain; limiting instrumental ADL	Continue same dose unless Grade 2 skin pain continues for ≥ 2 days (48 hours) in which case treatment must be interrupted until recovered to ≤ Grade 1 followed by dose reduction	See Grade 1; Continue acetaminophen or ibuprofen; assess for signs of local infection and additional dermatologic findings
Severe (Grade 3)	Severe pain; limiting self care ADL	Dose interruption until recovered to ≤Grade 1 followed by dose reduction*	See Grade 2; plus: an infectious process should be ruled out with pelvic exam and cultures.
Pruritis			
Mild (Grade 1)	Mild or localized; topical intervention indicated	Continue same dose	Topical polidocanol cream. Consider oral antihistamines, e.g. diphenhydramine, dimethindene, cetirizine,

			levocetirizine, desloratidine, fexofenadine or clemastine)
Moderate (Grade 2)	Intense or widespread; intermittent; skin changes from scratching (e.g., edema, papulation, excoriations, lichenification, oozing/crusts); oral intervention indicated; limiting instrumental ADL	Continue same dose unless Grade 2 skin pain continues for ≥ 2 days (48 hours) in which case treatment must be interrupted until recovered to ≤ Grade 1 followed by dose reduction	See Grade 1 plus oral histamines; Consider topical steroids (e.g., topical hydrocortisone)
Severe (Grade 3)	Intense or widespread; constant; limiting self care ADL or sleep; oral corticosteroid or immunosuppressive therapy indicated	Dose interruption until recovered to ≤Grade 1 followed by dose reduction*	See Grade 2; plus: an infectious process should be ruled out with pelvic exam and cultures
Skin Ulceration			
Mild (Grade 1)	Combined area of ulcers < 1cm; nonblanchable erythema of intact skin with associated warmth or edema	Continue same dose	Consider oral acetaminophen or ibuprofen; consider topical polidocanol cream
Moderate (Grade 2)	Combined area of ulcers 1 - 2 cm; partial thickness skin loss involving skin or subcutaneous fat	Continue same dose unless Grade 2 skin ulcers continue to worsen after ≥ 2 days (48 hours) in which case treatment must be interrupted until recovered to ≤ Grade 1 followed by dose reduction	See Grade 1; consider topical antibiotics and additional topical analgesic treatments
Severe (Grade 3)	Combined area of ulcers >2 cm; full-thickness skin loss involving damage to or necrosis of subcutaneous tissue that may extend down to fascia	Dose interruption until recovered to ≤Grade 1 followed by dose reduction*	See Grade 2; Consider oral antibiotics and institute additional symptomatic therapy (topical or systemic) as clinically indicated. Consider dermatology consultation
Life threatening (Grade 4)	Any size ulcer with extensive destruction, tissue necrosis, or damage to muscle, bone, or supporting structures with or without full thickness skin loss	Dose interruption until recovered to ≤Grade 1 followed by dose reduction*	See Grade 3. Consider oral antibiotics
Localized (Vulvar) Edema			
Mild (Grade 1)	Localized to dependent areas, no disability or functional impairment	Continue same dose	Avoid prolonged dependent positions

Moderate (Grade 2)	Moderate localized edema and intervention indicated; limiting instrumental ADL	Continue same dose unless Grade 2edema continue to worsen after ≥ 2 days (48 hours) in which case treatment must be interrupted until recovered to ≤ Grade 1 followed by dose reduction	See Grade 1
Severe (Grade 3)	Severe localized edema and intervention indicated; limiting self care ADL	Dose interruption until recovered to ≤Grade 1 followed by dose reduction*	See Grade 1; Consider Foley catheter placement if urethral obstructed

* If despite optimal supportive care and a treatment interruption, dermatologic reactions does not resolve to CTC AE Grade ≤ 1 within 21 days, treatment with imiquimod must be permanently discontinued. In the event that the subject is deriving obvious clinical benefit according to the investigator's judgement, further treatment with imiquimod will be decided by the investigator.

7.2.2.2 Management recommendations for respiratory AEs following treatment with Imiquimod

Respiratory adverse events including sinusitis, flu-like symptoms, upper respiratory tract infection can occur, although usually mild to moderate (Table 7.2.2.2: 3).

Table 3: Grade specific treatment recommendations for imiquimod-related respiratory reactions:

Severity (CTCAE Grading)	**Description**	**Specific intervention**
Upper respiratory infection		
Mild (Grade 1)	-	-
Moderate (Grade 2)	Moderate symptoms; oral intervention indicated (e.g., antibiotic, antifungal, antiviral)	Consider oral antibiotic, antifungal or antiviral; Consider anti-pyretic
Severe (Grade 3)	IV antibiotic, antifungal, or antiviral intervention indicated; radiologic, endoscopic, or operative intervention indicated	See Grade 2; if not improving within 48 hours of initiation of oral medication, consider IV formulation and also radiologic imaging
Life-threatening (Grade 4)	Life-threatening consequences; urgent intervention indicated	IV antibiotic therapy, radiologic imaging, evaluate for other systemic infections, hospitalization to monitor progress and if urgent respiratory interventions required
Sinusitis		
Mild (Grade 1)	-	
Moderate (Grade 2)	Localized; local intervention indicated (e.g., topical antibiotic, antifungal, or antiviral)	Consider oral antibiotic, antifungal or antiviral; Consider anti-pyretic
Severe (Grade 3)	IV antibiotic, antifungal, or antiviral intervention indicated;	See Grade 2; if not improving within 48 hours of initiation of oral medication, consider IV formulation and also radiologic imaging

	radiologic, endoscopic, or operative intervention indicated	
Life threatening (Grade 4)	Life-threatening consequences; urgent intervention indicated	See Grade 3
Death (Grade 5)	Death	
Flu like symptoms		
Mild (Grade 1)	Mild flu-like symptoms present	Consider anti-pyretic
Moderate (Grade 2)	Moderate symptoms; limiting instrumental ADL	See Grade 1
Severe (Grade 3)	Severe symptoms; limiting self care ADL	See Grade 1

* If despite optimal supportive care and a treatment interruption, respiratory reactions does not resolve to CTC AE Grade ≤ 1 within 21 days, treatment with imiquimod must be permanently discontinued. In the event that the subject is deriving obvious clinical benefit according to the investigator's judgement, further treatment with imiquimod will be decided by the investigator.

7.2.3 Restrictions

7.2.3.1 Restrictions regarding concomitant treatment

Concomitant medications, or therapy to provide adequate supportive care, may be given as clinically necessary.

Additional experimental immunosuppressant treatment and/or standard immunotherapy is not allowed concomitantly with the administration of study treatment.

7.2.3.2 Restrictions on diet and life style

Subjects should be advised there are no dietary restrictions.

To prevent skin related adverse events it is recommended to avoid intense irradiation with UV light and harsh detergents. In addition, subjects will be advised not to have sexual intercourse during the nights in which they applied vaginal suppositories and to perform a vaginal douche in the morning. Subjects will be instructed to suspend the application of vaginal suppositories during the first 3 days of their menses

7.2.3.3 Women of Child-Bearing Potential and Pregnancy Prevention

Subjects who are not of childbearing potential due to being postmenopausal (1 year without menstruation) or surgical sterilisation (bilateral oophorectomy and/or tubal ligation) do not need to use contraception to be eligible for the trial.

All other subjects are considered to have childbearing potential and must use adequate contraception throughout the trial (from screening until end of trial participation).

Acceptable methods of contraception include surgical sterilisation and double barrier method, and must be in accordance with local regulations where applicable. Double barrier method of contraception is defined as two barrier methods used simultaneously each time the subject has intercourse. Accepted barrier methods include diaphragm, female condom, cervical cap, male condom and intrauterine device (IUD) (the diaphragm and cervical cap must be used in conjunction with spermicidal jelly/cream). Those using hormonal contraceptives, or with partners using hormonal contraceptives, must also be using an additional approved method of contraception (as described above). Partner vasectomy, natural "rhythm" and spermicidal jelly/cream are not acceptable methods of contraception.

Women who become pregnant while participating in the study must discontinue study medication immediately. The pregnancy must be reported following procedures detailed in section 12.10.2.

7.3 Treatment compliance

The study medication will be given in accordance with the protocol and the instructions of the investigator.

The appropriate number of imiquimod suppositories for the first *8 weeks* of imiquimod treatment course will be provided to subjects to be self-administered at home. At week 8 follow up visit, the appropriate number of imiquimod suppositories for weeks 9-16 imiquimod treatment course will be provided to subjects. Subjects will be asked to bring the remaining study medication at each follow-up visit (weeks 8, 16) to the investigator site for a compliance check. The remaining suppositories will be counted by the investigator/site staff and recorded at the investigator site. Discrepancies between the number of suppositories remaining and the calculated number the subjects should have used must be documented and explained. At the end of the 16-week period, any remaining medication will be collected.

The investigator can withdraw a subject from the study in the event of serious and persistent non-compliance which jeopardizes the subject's safety or render study results for this subject uninterpretable.

8.0 TREATMENT MODIFICATIONS: 9-valent HPV Vaccine

8.1 Dose reduction levels

8.1.1 Dose reduction for 9-valent HPV Vaccine

As the 9-valent HPV vaccine is not a continuous use medication, dose reduction will not be applied in the setting of treatment related toxicities. Subjects experiencing allergic reactions as described in table below will not receive additional doses of the HPV vaccine per FDA and CDC guidelines. Subjects experiencing local, self-limited site reactions will be eligible to receive additional doses of the vaccine.

Table 4: Discontinuation of HPV vaccine

AE type and CTCAE Grade	**Action**	**Dose reduction scheme**
Events related to study drug: Grade 3: • Anaphylaxis: Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension • Any drug related AE Grade ≥3	Treat adverse event or provide symptomatic relief if treatment not available. Discontinue or resume HPV vaccination protocol as noted in next column.	For anaphylactoid reaction, discontinue additional HPV vaccine administration. For all other Grade 3 AE, if subject has not recovered to Grade ≤2 or baseline[1] within 21 days, discontinue additional HPV vaccine administration. The decision to continue with additional HPV vaccine doses following an AE Grade ≤2 or a Grade 3 AE that resolves within

		21 days will be made by the investigator.

3 Baseline is defined as the CTCAE (version 4) Grade at the start of treatment

In the event of any unrelated adverse events, the investigator may choose to delay HPV vaccine administration for up to 21 days, but no dose reduction should occur. If vaccine administration is delayed for more than 21 days, the decision to continue with vaccination and possibly with study will be made by the investigator.

8.2 Management of adverse events, concomitant therapy, restrictions and rescue treatment

8.2.1 Rescue medication, emergency procedures, and additional treatment(s)

8.2.1.1 Rescue medication

Rescue medications to reverse the actions of 9-valent HPV vaccine are not available. Potential adverse events should be treated symptomatically. Common adverse events of vaccination with 9-valent HPV vaccine with specified management recommendations and/or requirements include local reactions with pain, swelling or erythema at injection site and occasionally headaches or syncope. Rare events include anaphylactoid/hypersensitivity reactions which should serve as contraindication to administration of additional doses of 9-valent HPV vaccine as described above. To improve tolerability and the probability of clinical benefit, subjects should receive prompt and appropriate supportive care at the first signs of symptoms. Suggested treatments for AEs are described below.

8.2.1.2 Concomitant treatment(s)

Concomitant medications or therapy to provide adequate supportive care may be given as clinically necessary.

All concomitant therapy, including anaesthetic agents, vitamins, homeopathic/herbal remedies, nutritional supplements, must be recorded in the (e)CRF during the screening and treatment period, starting from the date of signature of informed consent, and ending at the EOT visit. After the EOT visit, only concomitant therapy indicated for treatment of an AE has to be reported.

If vaccination is delayed by more than 21 days, the decision to continue will be made by the investigator.

8.2.1.3 Emergency procedures

Careful management and assessment of all subjects with syncope should be performed to prevent serious secondary injuries (skull fracture, cerebral haemorrhage). Procedures will be in place to avoid injuries from falling and to restore cerebral perfusion if syncope occurs.

Subjects who develop anaphylactoid/hypersensitivity symptoms (symptomatic bronchospasm with or without urticarial, angioedema, hypotension) should receive immediate treatment including epinephrine 1:1000. If anaphylactoid/hypersensitivity reaction to 9-valent HPV is suspected, additional doses of the vaccine should be discontinued.

8.2.2 Management of expected adverse events

Local injection site and headache adverse events are the most common side-effects associated with administration of 9-valent HPV vaccine. Syncope and anaphylactoid/hypersensitivity reactions are less commonly reported. Treatment of these side-effects should be proactive and should be started as early as possible after onset of symptoms.

8.2.2.1 Management of local injection site adverse events following administration of 9-valent HPV vaccine

Local injection site adverse events including pain, erythema, or swelling can occur shortly after injection. Swelling and erythema have been noted to be increased in some subjects with successive doses. Although usually mild to moderate, local site reactions can cause significant discomfort (Table 8.2.2.1: 5).

Table 5: Grade specific treatment recommendations for local injection site reactions:

Severity (CTCAE Grading)	**Description**	**Specific intervention**
Injection site reaction		
Mild (Grade 1)	Tenderness with or without associated symptoms (e.g., warmth, erythema, itching)	Advise symptomatic treatment with acetaminophen or ibuprofen and local ice application
Moderate (Grade 2)	Pain; lipodystrophy; edema; phlebitis	Continue acetaminophen or ibuprofen; assess for signs of local infection and additional dermatologic findings. Addition of topical antibiotic or corticosteroid if clinically indicated
Severe (Grade 3)	Ulceration or necrosis; severe tissue damage; operative intervention indicated	See Grade 2; Consider oral or IV antibiotic as clinically indicated and institute wound care, including surgical consultation if needed
Life threatening (Grade 4)	Life-threatening consequences; urgent intervention indicated	See Grade 3
Localized edema		
Mild (Grade 1)	Localized to dependent areas, no disability or functional impairment	Avoid prolonged dependent positions
Moderate (Grade 2)	Moderate localized edema and intervention indicated; limiting instrumental ADL	See Grade 1
Severe (Grade 3)	Severe localized edema and intervention indicated; limiting self care ADL	See Grade 1

***** If despite optimal supportive care, reactions do not resolve to CTC AE Grade ≤ 2 within 21 days, administration of additional HPV vaccine doses must be permanently discontinued. The decision to continue with additional HPV vaccine doses following an AE Grade ≤2 or a Grade 3 AE that resolves within 14 days will be made by the investigator.

8.2.2.2 Management recommendations for headache AEs following administration of 9-valent HPV vaccine: Headaches can occasionally develop, although usually mild to moderate (Table 8.2.2.2: 6).

Table 6: Grade specific treatment recommendations for 9-valent HPV vaccine-related headache symptoms:

Severity (CTCAE Grading)	**Description**	**Specific intervention**
Headaches		
Mild (Grade 1)	Mild pain	Consider symptomatic treatment with acetaminophen or ibuprofen
Moderate (Grade 2)	Moderate pain; limiting instrumental ADL	See Grade 1
Severe (Grade 3)	Severe pain; limiting self care ADL	See Grade 1

***** If despite optimal supportive care, reactions do not resolve to CTC AE Grade ≤ 2 within 21 days, administration of additional HPV vaccine doses must be permanently discontinued. The decision to continue with additional HPV vaccine doses following an AE Grade ≤2 or a Grade 3 AE that resolves within 21 days will be made by the investigator.

8.2.2.3 Management recommendations for syncopal AEs following administration of 9-valent HPV vaccine: Syncopal events occur rarely with injectable vaccines and are typically reported in adolescents and young adults, occurring within 15 minutes of vaccination. Avoiding falls is imperative.

Table 7: Grade specific treatment recommendations for 9-valent HPV vaccine related syncopal events:

Severity (CTCAE Grading)	**Description**	**Specific intervention**
Presyncope / Syncope		
Mild (Grade 1)	-	
Moderate (Grade 2)	Presyncope present (e.g., near fainting)	Advise subject to sit down, check vitals, consider oral hydration
Severe (Grade 3)	Fainting; orthostatic collapse	Help subject to lie down and avoid fall, check vitals and EKG, consider IV hydration

***** If despite optimal supportive care, reactions does not resolve to CTC AE Grade ≤ 2 within 21 days, administration of additional HPV vaccine doses must be permanently discontinued. The decision to continue with additional HPV vaccine doses following an AE Grade ≤2 or a Grade 3 AE that resolves within 21 days will be made by the investigator.

8.2.2.4 Management recommendations for anaphylactoid/hypersensitivity reactions following administration of 9-valent HPV vaccine: A rare AE seen with 9-valent HPV vaccine that clinically may present with breathing difficulty, dizziness, hypotension, cyanosis and loss of consciousness.

Table 8: Grade specific treatment recommendations for 9-valent HPV vaccine-related anaphylactoid/hypersensitivity reactions

Severity (CTCAE Grading)	**Description**	**Specific intervention**
Anaphylaxis		
Mild (Grade 1)	-	-
Moderate (Grade 2)	-	-
Severe (Grade 3)	Symptomatic bronchospasm, with or without urticaria; parenteral intervention indicated; allergy-related edema/angioedema; hypotension	Administer epinephrine (1:1000) intramuscularly immediately; additional supportive therapy as indicated including IV fluids, hospitalization for observation and airway management
Life threatening (Grade 4)	Life-threatening consequences; urgent intervention indicated	See Grade 3

8.2.3 Restrictions

8.2.3.1 Restrictions regarding concomitant treatment

Concomitant medications, or therapy to provide adequate supportive care, may be given as clinically necessary.

Additional experimental immunosuppressant treatment and/or standard immunotherapy is not allowed concomitantly with the administration of study treatment.

8.2.3.2 Restrictions on diet and life style

Subjects should be advised there are no dietary or lifestyle restrictions.

8.2.3.3 Women of Child-Bearing Potential and Pregnancy Prevention

Subjects who are not of childbearing potential due to being postmenopausal (1 year without menstruation) or surgical sterilisation (oophorectomy, hysterectomy and/or tubal ligation) do not need to use contraception to be eligible for the trial.

All other subjects are considered to have childbearing potential and must use adequate contraception throughout the trial (from screening until end of trial participation).

Acceptable methods of contraception include surgical sterilisation and double barrier method, and must be in accordance with local regulations where applicable. Double barrier method of contraception is defined as two barrier methods used simultaneously each time the subject has intercourse. Accepted barrier methods include diaphragm, female condom, cervical cap, male condom and intrauterine device (IUD) (the diaphragm and cervical cap must be used in conjunction with spermicidal jelly/cream). Those using hormonal contraceptives, or with partners using hormonal contraceptives, must also be using an

additional approved method of contraception (as described above). Partner vasectomy, natural "rhythm" and spermicidal jelly/cream are not acceptable methods of contraception.

Women who become pregnant while participating in the study may not receive any additional doses of the HPV vaccine. The pregnancy must be reported following procedures detailed in section 12.10.2.

8.3 Vaccine compliance

The vaccine will be administered in accordance with the protocol and the instructions of a site investigator. The 9-valent HPV vaccine will be administered in the clinic by a clinic or study nurse based on study protocol.

The investigator can withdraw a subject from the study in the event of serious and persistent non-compliance with receiving the vaccine dose(s) which jeopardizes the subject's safety or render study results for this subject unacceptable.

9.0 STUDY PARAMETERS

9.1 Observations and Tests

The following observations and tests are to be performed and recorded before, during, and after treatment:

	Pre-Treatment	**Treatment Period**	**Post-Treatment Period**	**End of Treatment ******	**3 and 6 Month Post End of Treatment**
Study week		8	16	22	
Study Procedures Window (weeks)	-10	± 1	+ 2	± 2	± 2
History, PE w/ pelvic exam	X	X	X	X	
Vital signs	X	X	X	X	
Urine pregnancy test	X**	X	X	X	
Pap				X	
High risk HPV test	X***			X	X
HPV 16/18 genotype	X		X	X	
Cytobrush collection*	X	X	X	X	
Colposcopy, cervical biopsy	X	X (biopsy if clinically indicated)	X (biopsy if clinically indicated)	X	
Peripheral blood sample collection*^	X****	X	X	X	
Toxicity assessment	X	X	X	X	
Randomization	X*****				

* For translational studies. Translational blood may be drawn after randomization but must be drawn prior to treatment.
^ See Section 6.2.1 for collection information.
** Urine pregnancy test must be performed within 7 days of treatment
*** If high-risk HPV DNA results are already available, this testing may not have to be repeated
**** Week 22 visit will occur at least 3 weeks after last dose of therapy
***** Randomization to occur following confirmation of eligibility

10.0 EVALUATION CRITERIA

10.1 Objective Response

The major parameters of response to be assessed include treatment efficacy defined as histologic regression of cervical dysplasia to CIN 1 or less 12 to 16 weeks after the end of imiquimod treatment, HPV clearance and treatment tolerance.

10.11 Histologic regression: Only subjects with histologically confirmed CIN 2,3 as reviewed by a central study pathologist will be enrolled in the study. All index biopsy sites noted to have CIN 2,3 at baseline must be assessed via colposcopy. Cervical biopsy at the final colposcopy will be collected from the worst areas of residual disease. Included in the evaluations are the following criteria:

- Histologic regression (HR): Histologic regression of all index lesions to CIN 1 or less at 12 to 16 weeks after end of imiquimod treatment period.
- Histologic remission (HM): Complete regression of cervical dysplasia at all index biopsy sites at 12 to 16 weeks after end of imiquimod treatment period.
- Persistent Disease (PR): One or more index lesions persists with CIN 2,3 high grade dysplasia or new lesions are identified colposcopically and histologically confirmed to be CIN 2,3.
- Progressive Disease (PD): Worsening histology of an index lesion.

10.12 HPV Clearance: HPV clearance will be measured by both the Roche cobas HPV Test utilized by pathology concomitant with the pap test at final study visit which assesses for presence of 14 high risk HPV types as well as HPV 16/18 genotyping performed by Santin Lab.

10.13 Treatment Tolerance: The grade level of the various toxicities will be classified using the Common Toxicity Criteria, version 4 (CTC, v.4) guidelines. Acute toxicities will be scored if occurring </= 30 days from treatment completion, and chronic if > 30 days. Frequency and duration of treatment interruptions due to the treatment toxicity will be assessed.

11.0 DURATION OF STUDY

11.1 This study will continue as long as treatment protocols remain activated.

11.2 The subject will be followed with routine clinical care with a 6 month colposcopy and cytology if histologic remission or regression noted at final study visit and in 12 months if cytology remains negative with normal colposcopy. Any abnormal cytology or colposcopy at 6 months should prompt cervical biopsy and management per guidelines of American Society for Colpsocpy and Cervical Pathology (ASCCP).

Those subjects with persistent CIN 2,3 or progression of disease at end of study period will be offered surgical excisional procedure per routine clinical care and should be followed by ASCCP guidelines.

12.0 STUDY MONITORING AND REPORTING PROCEDURES

12.1 Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency which must be conducted at a minimum of every 6 months (including when re-approval of the protocol is sought). During the review process, the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator, the IRB or the Yale Cancer Center Data and Safety Monitoring Committee (DSMC) have the authority to stop or suspend the study or require modifications.

12.2 The risks associated with the current study are deemed moderate for the following reasons:

1. The risk of progression of CIN 2,3 to invasive cervical cancer over 6-8 months from time of initial diagnosis is low and observation of CIN 2 diagnosed in young women for up to 12 to 24 months is already recommended by the ASCCP.
2. The risks associated with the FDA-approved drugs imiquimod and 9-valent HPV vaccine are minimal.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

12.3 Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator Dr. Alessandro Santin, according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

12.4 Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

12.5 Plan for Determining Seriousness of Adverse Events:

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

Refer to Section 13.0 of the protocol for guidelines

12.6 Plan for reporting Reportable Adverse Events and other unanticipated problems involving risks to subjects or others to the IRB

The principal investigator will report the following types of events to the IRB: a) adverse events that are serious or life-threatening AND unanticipated (or anticipated but occurring with a greater frequency than expected) AND possibly, probably or definitely related to the drug/device/intervention; and b) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. **This information is discussed in Section 13.0 of the protocol.**

12.7 Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., Yale Cancer Center Data and Safety Monitoring Committee (DSMC), Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

X All Co-Investigators listed on the protocol.

X Yale Cancer Center Data and Safety Monitoring Committee (DSMC)

The principal investigator Alessandro Santin will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

12.8 ADVERSE EVENT REPORTING

This study will utilize the CTC version 4.0 for toxicity and Adverse Event Reporting. A copy of the CTC version 4.0 can be downloaded from the CTEP home page.

Toxicity Grade	Type[a]	Local IRB	Study Coordinators Via email/fax/phone
4,5	Unknown	Yes	Yes
5	Known	No	Yes*
2,3	Unknown	No	No
4	Known	No	No

* If clearly related to the commercial agent(s)

[a] Type (Known or unknown) is based on toxicities included in the package insert or literature of known toxicities associated with the study drug(s).

12.9 ASSESSMENT OF SAFETY

12.9.1 SPECIFICATION OF SAFETY VARIABLES

Safety assessments will consist of monitoring all adverse events and reporting adverse events (AEs) and serious adverse events (SAEs) that are considered related to **Imiquimod or 9-valent HPV vaccine**, all events of death, and any study specific issue of concern.

12.9.1.1 Adverse Events

An AE is any unfavorable and unintended sign, symptom, or disease temporally associated with the use of an investigational (medicinal) product or other protocol-imposed intervention, regardless of attribution.

This includes the following:

- AEs not previously observed in the subject that emerge during the protocol-specified AE reporting period, including signs or symptoms associated with high-grade cervical dysplasia CIN 2,3 that were not present prior to the AE reporting period. See Section **12.10.**

- Complications that occur as a result of protocol-mandated interventions (e.g., cervical biopsy).
- If applicable, AEs that occur prior to assignment of study treatment associated with medication washout, no treatment run-in, or other protocol-mandated intervention.
- Preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character during the protocol-specified AE reporting period.

12.9.2 Serious Adverse Events

Refer to Section 13.0 of the study protocol.

12.9.3 Methods and Timing for Assessing and Recording Safety Variables

The investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, as outlined in Section 12.10 and 13.0 respectively, are collected and reported to the appropriate IRB(s).

12.9.4 Adverse Event Reporting Period

The study period during which all AEs and SAEs must be reported begins after informed consent is obtained and initiation of study treatment and ends 30 days following the last administration of study treatment or study discontinuation/termination, whichever is earlier. After this period, investigators should only report SAEs that are attributed to prior study treatment.

12.9.5 Assessment of Adverse Events

All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported appropriately.

Each reported AE or SAE will be described by its duration (i.e., start and end dates), regulatory seriousness criteria if applicable, suspected relationship to imiquimod or 9-valent HPV vaccine (see following guidance), and actions taken.

To ensure consistency of AE and SAE causality assessments, investigators should apply the following general guideline:

Yes

There is a plausible temporal relationship between the onset of the AE and administration of imiquimod or 9-valent HPV vaccine and the AE cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to imiquimod or 9-valent HPV vaccine and/or the AE abates or resolves upon discontinuation of imiquimod or dose reduction and, if applicable such as in case of 9-valent HPV vaccine, reappears upon re-challenge.

No

Evidence exists that the AE has an etiology other than imiquimod or 9-vvalent HPV vaccine (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the AE has no plausible temporal relationship to imiquimod or 9-valent HPV vaccine administration (e.g., cancer diagnosed 2 days after first dose of study drug).

Expected adverse events are those adverse events that are **listed** or characterized in the Package Insert or current Investigator Brochure.

Unexpected adverse events are those **not listed** in the Package Insert (P.I.) or current Investigator Brochure (I.B.) or not identified. This includes adverse events for which the specificity or severity is not consistent with the description in the P.I. or I.B. For example, under this definition, hepatic necrosis would be unexpected if the P.I. or I.B. only referred to elevated hepatic enzymes or hepatitis. **Refer to Section 13.0 for further guidelines**.

12.10 PROCEDURES FOR ELICITING AND RECORDING ADVERSE EVENTS

12.10.1 Eliciting Adverse Events

A consistent methodology for eliciting AEs at all subject evaluation time points should be adopted. Examples of non-directive questions include:

"How have you felt since your last clinical visit?

"Have you had any new or changed health problems since you were last here?"

12.10.2 Specific Instructions for Recording Adverse Events

Investigators should use correct medical terminology/concepts when reporting AEs or SAEs. Avoid colloquialisms and abbreviations. All information will be recording using Oncore Clinical Trials Management System.

a. Diagnosis vs. Signs and Symptoms

If known at the time of reporting, a diagnosis should be reported rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, it is ok to report the information that is currently available. If a diagnosis is subsequently established, it should be reported as follow-up information.

b. Deaths

All deaths that occur during the conduct of the research must be reported to the Yale Principal Investigator and the Remote Monitoring Coordinator immediately (if possible) to ensure that all over site committees are properly informed. All information will be entered into Oncore Clinical Trials Management System and updated appropriately. Further determination regarding the need to submit to the subjects local IRB, Yale University HHRP following IRB Policy 710 for reporting Unanticipated Problems Involving Risk to Subjects will be reviewed. Specific information is outlined in section 13.0 of the protocol.

When recording a death, the event or condition that caused or contributed to the fatal outcome should be reported as the single medical concept. If the cause of death is unknown and cannot be ascertained at the time of reporting, report "Unexplained Death".

c. Preexisting Medical Conditions

A preexisting medical condition is one that is present at the start of the study. Such conditions should be reported as medical and surgical history.

A preexisting medical condition should be re-assessed throughout the trial and reported as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When reporting such events, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

d. Hospitalizations for Medical or Surgical Procedures

Any AE that results in hospitalization or prolonged hospitalization should be documented and reported as an SAE.

If a subject is hospitalized to undergo a medical or surgical procedure as a result of an AE, the event responsible for the procedure, not the procedure itself, should be reported as the SAE. For example, if a subject is hospitalized to undergo coronary bypass surgery, record the heart condition that necessitated the bypass as the SAE.

Hospitalizations for the following reasons do not require reporting:

Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for preexisting conditions

Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study or

Hospitalization or prolonged hospitalization for scheduled therapy of the target disease of the study.

e. Pregnancy

If a female subject becomes pregnant while receiving imiquimod or 9-valent HPV vaccine series or within 30 days of awareness after the last dose of study drug, a report should be completed and expeditiously submitted to Yale HIC. Follow-up to obtain the outcome of the pregnancy should also occur.

Abortion of a pregnancy in a study subject receiving Imiquimod, whether accidental, therapeutic, or spontaneous, should always be classified as serious, and expeditiously reported as an SAE. Similarly, any congenital anomaly/birth defect in a child born to a female subject exposed to imiquimod or 9-valent HPV vaccine should be reported as an SAE.

f. Post-Study Adverse Events

The investigator should expeditiously report any SAE occurring after a subject has completed or discontinued study participation if attributed to prior **Imiquimod or 9-valent HPV vaccine** exposure.

If the investigator should become aware of the development of cancer or a congenital anomaly in a subsequently conceived offspring of a female subject who participated in the study, this should be reported as an SAE.

13.0 PROCEDURES FOR REPORTING UNANTICIPATED PROBLEMS INVOLVING RISKS TO SUBJECTS OR OTHERS, INCLUDING ADVERSE EVENTS (UPIRSOs)

13.1 Expedited Reporting of UPISROs Occurring at Yale

AEs classified as "serious" and "unexpected" that are possibly, probably, or definitely attributed to drug administration, or SAEs whose frequency exceeds expectations, require expeditious handling and reporting.

Serious Adverse Event (SAE)
Any adverse event that results in any of the following outcomes:

- death,
- a life-threatening experience,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- a congenital anomaly/birth defect, or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

The PI will promptly investigate all safety information related to an adverse experience. If the results of the PI's investigation show an adverse drug experience not initially determined to be reportable (based on whether the event is serious, unexpected, and associated with drug administration) is so reportable, the PI will report such experience. Follow-up information to a safety report shall be submitted as soon as the relevant information is available.

Reporting to the Yale Human Investigation Committee

Timeframe for Reporting

1. Events that may require a temporary or permanent interruption of study activities by the Principal Investigator or sponsor to avoid potential harm to subjects should be reported to the IRB **immediately** (if possible), followed by a written report to the IRB using the UPIRSO Reporting Form (710 FR 4) **no more than 5 calendar days** after the Yale Principal Investigator becomes aware of the event**.**

2. Internal Events (defined above) should be reported to the IRB using the UPIRSO Reporting Form (710 FR 4) **within 5 calendar days** of the Principal Investigator becoming aware of the event.

3. External Events (defined above) should be reported to the IRB using the UPIRSO Reporting Form (710 FR 4) **within 15 calendar days** of the Yale University Principal Investigator (PI) becoming aware of the event ONLY IF either of the following are true:

   (a) The Yale PI has concluded that an immediate change to the protocol is necessary to address the risks raised by the event, and BI agreed to the changes OR

   (b) A monitoring entity (e.g., an external IRB at the site where the problem or event occurred, the sponsor, or the Data Safety Monitoring Board) has required modifications/amendments to the research protocol or consent documents as a result of the event.

For all reports of external events, the UPIRSO Reporting Form (710 FR 4) must include the following information:

(a) a clear explanation of why the event or series of events has been determined to meet criteria for reporting;

(b) a description of the proposed protocol changes and any corrective actions to be taken by the PI in response to the external event; and

(c) any aggregated data and an analysis or summary from the sponsor or DSMB, when applicable and available, sufficient to explain the significance of the event or series of events in order to ensure the information is interpretable and relevant to the IRB's task of protecting the rights and welfare of human participants.

MedWatch 3500A Reporting Guidelines:

In addition to completing appropriate subject demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome if known
- Supportive laboratory results and diagnostics

Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Follow-up information:

Additional information may be added to a previously submitted report by any of the following methods:

- Adding to the original MedWatch 3500A report and submitting it as follow-up
- Adding supplemental summary information and submitting it as follow-up with the original MedWatch 3500A form

Summarizing new information and faxing it with a cover letter including subject identifiers (i.e. D.O.B. initial, subject number), protocol description and number, if assigned, brief adverse event description, and notation that additional or follow-up information is being submitted (The subject identifiers are important so that the new information is added to the correct initial report)

MedWatch 3500A (Mandatory Reporting) form is available at http://www.fda.gov/medwatch/getforms.html

At all times, the Remote Monitoring Coordinator is available to facilitate submissions and answer any questions regarding the process for all sub site staff.

14.0 STUDY MANAGEMENT AND COORDINATION

14.1 Overview

This is a single site trial where **Dr. Alessandro D. Santin** is the Principal Investigator. The research team at Yale University consisting of a Site Research Coordinator, Data Manager, Clinical Research Nursing Coordinator, Specimen Procurement Coordinator and an Administrative Assistant will provide research support. The YCCI clinical trials management support staff will provide clinical database systems support and training through the use of Oncore Clinical Trials Management System.

14.2. Data Collection

Study will use Oncore Clinical Trials Management System for all data associated with the protocol. Data manager will receive Oncore access, complete an Oncore access request and submit HIPAA training certification. Individuals will receive Oncore training by a member of the YCCI clinical trials management support staff. Subjects entered into the system will be identified by a subject number assigned after randomization. The confidentiality of records that could identify subjects will be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

14.3 Study Enrollment Procedures

Any modification of the original consent document must be approved in advance by the IRB. Rationale will be provided with any request for a change. A copy of the IRB and approved informed consent document must be on file at the Regulatory office before subjects may be enrolled.

To register eligible subjects on this study, contact the Site Coordinator; Lisa Baker, RN (203-785-6398) or Martha Luther, RN (203-737-2781) and provide the signed and dated eligibility checklist, completed signature page of the consent form and additional source documents if requested by the Principal Investigator or Site Coordinator. Once the Principal Investigator or Site Coordinator verifies eligibility, a unique subject study number will be issued. The subject will not be identified by name.

14.4 Responsibilities of the Principal Investigator Dr. Alessandro D. Santin:

The principal investigator is responsible for the overall conduct of the study and for monitoring the safety and quality of the data as well as compliance to the protocol and with applicable federal regulations and Good Clinical Practice (GCP).

The principal investigator will monitor accrual rates for adequate progress.
The principal investigator will ensure appropriate coordination, submission and approval of the protocol as well as the consent documents and any subsequent amendments. There will be only one version of these documents and the principal investigator is responsible for assuring that the correct versions are used.

In addition, this study is subject to YCC Discovery Fund Grant Oversight. The principal investigator will provide periodic reports to YCC leadership and attend meetings in accordance with the YCC Discovery Fund Grant Monitoring Plan to ensure study progress toward milestones and timelines are being achieved.

14.5 IRB Approvals

No subjects are to be entered on study without full IRB approval. IRB re-approval must be appropriately maintained. Documentation of reapproval must be obtained in a timely manner or registration will be halted.

14.6 Amendments and Consents

The Study Coordinator will maintain a copy of all amendments, consent forms, and approvals. Consent forms will be reviewed and approved by the Yale IRB. Should changes to the protocol or consent become necessary, protocol amendments will be issued by the Yale IRB.

15.0 DATA ANALYSIS / STATISTICAL METHODS

The analyses and methods described in this section, for this randomized, Phase II, single-blinded, 3-arm clinical trial, apply to the primary, secondary, and exploratory/correlative objectives provided in Sections 1.1 to 1.3.

15.1 General Statistical Considerations

- Type I error for hypothesis testing will be $\alpha = 0.05$, and 95% confidence intervals will be used for parameter estimation, unless otherwise specified. Type II error will be based on $\beta = 0.10$, unless otherwise specified. Only the primary analysis will use an adjustment for multiple comparisons.
- Summary statistics for continuous variables will include (but may not be limited to) the number of observations, mean, standard deviation, minimum, median and maximum. For categorical variables, summary statistics will include (but may not be limited to) counts, percentages, and cross-tabulation tables.
- Standard diagnostics will be performed to assess whether the assumptions for specific parametric statistical methods are adequately supported by the data. Should parametric methods be inappropriate, the analogous non-parametric methods will be used and only these results will be reported.
- Imputation of missing values will not be applied.
- No interim analyses for efficacy or futility are planned, although the trial may be stopped early based on observations from continual safety monitoring.
- Data analysis will be conducted using SAS® v9.4 (or later).

15.2 Analysis Set Definitions

- **full analysis set**—all randomized subjects
- **safety analysis set**—all subjects who have received at least one dose of any study drug, and/or have had any assessments at baseline or later that could be associated with an AE; analyzed by treatment received
- **ITT analysis set**—all randomized subjects with valid baseline measurements; analyzed by treatment assigned
- **modified ITT analysis set**—all randomized subjects with valid baseline measurements, receiving at least one dose of any study drug, and have at least one valid post-baseline measurement of outcome; analyzed by treatment assigned

- **modified ITT completers analysis set**—all randomized subjects who receive at least one dose of any study drug and have complete and valid post-baseline assessments of outcome; analyzed by treatment assigned
- **per protocol analysis set**—all randomized subjects who receive all doses of their assigned study drug, have complete and valid post-baseline assessments of outcome, and have no protocol deviations (e.g. dose modification, assessment outside of time window, non-compliance)

15.3 Subject Disposition

Using a flowchart with counts and percentages, a breakdown of the study dispositions for **all screened subjects** will be provided. 'Disposition' refers to eligibility, consent, enrollment, randomization assignment, dosing, drop-out prior to completion of full protocol, completion of protocol, follow-up. Reasons for drop-out will also be summarized.

15.4 Demographics, clinical measures, and safety

Summary statistics will be used to describe demographics and clinical measures collected at **baseline** using **ITT analysis set**, and will be presented by assigned treatment group and overall. Baseline characteristics will be tested for homogeneity across treatment arms using a 2-sided Fisher's Exact Test for categorical measures and ANOVA or Wicoxon Rank-Sum Test for continuous variables. Standard tables and listings will be provided for ongoing assessments, including (but not limited to) study drug dose received, treatment modifications, concomitant medications, labs, vital signs, and clinical tests, and will be reported by subject, by time point, and by treatment group, as appropriate. AEs/SAEs will be reported by subject, by time point, and by treatment group, using the **safety analysis set**.

15.5 Analysis for the Primary Objective

The co-primary objectives of this study are to test superior treatment efficacy of Imiquimod + HPV vaccine (Trt1) compared to control (Trt0), and likewise Imiquimod only (Trt2) compared to control (Trt0). The **primary outcome** is ***histologic regression* (*yes/no*)** after completion of the 12 to 16 weeks imiquimod course. The full definition of *histologic regression* and how it is determined is described in Section 5.1.

The **co-primary hypothesis tests** are:
$\{H_{01}: p_1 - p_0 = 0$ versus $H_{11}: p_1 - p_0 > 0\}$
and
$\{H_{02}: p_2 - p_0 = 0$ versus $H_{12}: p_2 - p_0 > 0\}$
p_0, p_1, and p_2 are the proportion of subjects on Trt0, Trt1, and Trt2, respectively, who are determined to have *histologically regressed*. Fisher's Exact Test will be used to assess if the data support rejecting the null hypothesis H_{01}, and likewise for H_{02}. To control Type I error, we will use a conservative Bonferroni adjustment for multiple comparisons with one-sided $\alpha = (0.05)/2 = 0.025$ as the significance level for each test. Rejection of a particular null hypothesis suggests superior efficacy of that specific treatment compared to control. Exact 95% confidence intervals will be calculated for $p_1 - p_0$ and for $p_2 - p_0$. Confidence intervals for 'risk ratios' (p_1/p_0, p_2/p_0) in addition to 'risk differences' ($p_1 - p_0$, $p_2 - p_0$) may also be estimated. The main analysis will use the **modified ITT analysis set**. Sensitivity analyses for the same statistical method using the **modified ITT completers** and/or **per protocol analysis sets** may also be provided.

15.6 Analysis for the Secondary Objectives

The **secondary objectives** are:

- to estimate treatment efficacy of Imiquimod + HPV vaccine (Trt1) compared to control (Trt0), and likewise Imiquimod only (Trt2) compared to control (Trt0), for the **secondary outcome *histologic remission* (*yes/no*)** after completion of the 12 to 16 weeks imiquimod course. Exact 95% confidence intervals for $p_1 - p_0$ and for $p_2 - p_0$ will be calculated. The main analysis will use the **modified ITT analysis set**. Sensitivity analyses for the same statistical method using the **modified ITT completers** and/or **per protocol analysis sets** may also be provided.

- to estimate treatment efficacy of Imiquimod + HPV vaccine (Trt1) compared to control (Trt0), and likewise Imiquimod only (Trt2) compared to control (Trt0), for the **secondary outcome HPV clearance (*yes/no*)** after completion of the 12 to 16 weeks imiquimod course. Exact 95% confidence intervals for $p_1 - p_0$ and for $p_2 - p_0$ will be calculated. The main analysis will use the **modified ITT analysis set**. Sensitivity analyses for the same statistical method using the **modified ITT completers** and/or **per protocol analysis sets** may also be provided.

- to characterize **treatment safety and tolerability** using descriptive statistics (**safety analysis set**)

15.7 Exploratory/Correlative Objectives

To characterize the association of **T cell infiltration** into the cervical dysplasia (i.e. frequencies and anatomical distributions of CD4 and CD8 T cells within the CIN) and pre- and post-immune therapy responses to HPV16/18 E7 antigens in subject peripheral blood lymphocytes (PBL) and serum with **clinical outcomes** (histologic regression/remission) and **viral clearance**. T_{RM} frequencies ,distributions and responses will be measured within subjects (pre and post 'prime and pull'), and changes from baseline will be compared between the treatment groups at relevant time points. Descriptive summary statistics will be used to evaluate associations and ANOVA will be the used to test the statistical significance of associations. The main analysis will use the **modified ITT analysis set**. Sensitivity analyses for the same statistical method using the **modified ITT completers** and/or **per protocol analysis sets** may also be provided.

15.8 Sample Size Justification

Anticipated annual accrual is approximately 40-45 subjects.

Our general philosophy regarding Phase II trials of new drug combinations is to facilitate the prompt discovery of inactive agents, yet allow for a reasonable estimate of the response rates for those regimens demonstrating some degree of activity. However, excessive accrual to inactive study regimens to document precise response rates is to be avoided. The purpose of this study is to evaluate the activity and potential toxicity of the combination therapy of Imiquimod + HPV vaccine in subjects with untreated high-grade CIN 2,3 cervical dysplasia. This study will implement a 3 arm randomized trial design that will allow us to estimate the treatment effect of Imiquimod + HPV vaccine compared to observation as well as Imiquimod alone compared to observation.

Based on published literature for the primary outcome,[38] we assume a 28% spontaneous histological regression rate in CIN 2-3 lesions with observation alone (control group). From Grimm et al.'s efficacy

trial of imiquimod for treatment of CIN 2-3, we would like to have sufficient power to detect a 35% absolute increase in rate of histologic regression in the imiquimod only arm compared to control. Based on this data, we consider a conservative effect size estimate also of an absolute 35% higher rate of histologic regression in the imiquimod + HPV vaccine intervention arm compared with the control arm. We therefore aim to recruit a total sample size of 114 subjects (38 in each arm). Calculations based on Fisher's Exact Test indicate we will have ≥ 90% power to detect a treatment effect of ≥ 29% in both treatment arms independently compared to the observation arm ($\alpha = 0.025$ for each comparison to control). In addition, with n = 38 subjects per arm, we would have 80% power to detect a treatment effect size of ≥ 25% in one treatment arm compared to placebo (while maintaining 90% power to detect the treatment effect size of ≥ 29% in the other arm). Assuming a subject dropout rate of 20%, a total sample size of 138 (n = 46 per arm) subjects is calculated which will be recruited over 5 years.

Some operating characteristics of the study design are as follows:

Exact Operating Characteristics for the Treatment Effect Size Δ
given control group rate=0.28, n=38 subjects per arm, one-sided α

Exact binomial probabilities for α given minimum required power = (1-β)								
Δ =	**0.00**	**0.10**	**0.20**	**0.30**	**0.32**	**0.35**	**0.37**	**0.40**
(1-β) ≥ 0.80	0.8080	0.8008	0.8101	0.8126	0.8011	0.8168	0.8119	0.8113
α =	0.5321	0.7745	0.1537	0.0366	0.0220	0.0118	0.0058	0.0027
(1-β) ≥ 0.90	0.9033	0.9009	0.9049	0.9060	0.9068	0.9062	0.9012	0.9009
α =	0.4835	0.6574	0.2876	0.0863	0.0616	0.0363	0.0203	0.0103
Exact binomial probabilities for power = (1-β) given maximum required α								
Δ =	**0.00**	**0.10**	**0.20**	**0.30**	**0.32**	**0.35**	**0.37**	**0.40**
α ≤ 0.025	0.0147	0.0147	0.0143	0.0140	0.0140	0.0143	0.0146	0.0149
1-β =	0.0147	0.1050	0.3472	0.6860	0.7472	0.8269	0.8706	0.9215
α ≤ 0.05	0.0305	0.0327	0.0272	0.0304	0.0317	0.0330	0.0331	0.0323
1-β =	0.0305	0.1795	0.4847	0.7870	0.8328	0.8898	0.9197	0.9535

Software: G*Power version 3.1.9.2, ©1992-2014, Franz Faul, Universität Kiel, Germany

At 7-8 months from time of initial CIN 2,3 diagnosis, persistent or progressing dysplasia will be surgically excised per current standard of care. Risk of progression of high-grade cervical dysplasia during the study period in the observation group is very low and observation periods of 6-9 months for CIN 2,3 are commonly described in the literature.[24,39] In this regard, studies estimating the risk of clinical progression from CIN2/3 lesions to cervical cancer found that the median time was 23.5 years with only 1.6% of these lesions becoming invasive within 10 years[40]. In addition, any clinical concern for disease progression based on history, physical exam or colposcopy at an interval follow up visit will trigger cervical biopsies to objectively evaluate histopathology and, should disease progression be noted, the subject will be removed from study and offered surgical excision. We will also employ early stopping rules as follows:

Evaluability for Efficacy and Toxicity:
Only those subjects who are deemed "ineligible" will be eliminated from the analysis. All subjects who receive any therapy will be evaluated for both treatment efficacy and toxicity. While on occasion, circumstances may prevent the determination of treatment efficacy, such subjects will be included in the

analysis and labeled as “unknown”. This category will be listed and be reflected in the calculation of the response rate.

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