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DCP Protocol #: UAZ2016-08-02
Local Protocol #: 1712083061

A feasibility trial of alternating intravaginal application of 5-fluorouracil and imiquimod for treatment of high-grade cervical squamous intraepithelial lesions

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IND Sponsor:	DCP
IND#	[redacted]
Agent(s)/Supplier:	5-fluorouracil and imiquimod/commercial sources
NCI Contract #	HHSN2612012000311
Protocol Version Date:	04/9/2020
Protocol Revision or Amendment #	Version 3, Amendment 11

SCHEMA

A feasibility trial of alternating intravaginal application of 5-fluorouracil and imiquimod for treatment of high-grade cervical squamous intraepithelial lesions

Women with biopsy-confirmed high grade cervical squamous intraepithelial lesions (i.e., CIN3 lesions, and p16-immunohistochemistry-positive CIN2 lesions)

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Screening/Consenting Visit

Informed consent, medical history, conmeds, baseline signs and symptoms, Karnofsky performance status, height, weight, vital signs, blood and urine collection for clinical laboratory tests, urine pregnancy test

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Baseline Visit

Colposcopy with digital imaging of the cervix
Cervical specimen, blood, and urine collection for research endpoints
Weight, vital signs, urine pregnancy test, conmeds, adverse event (AE) evaluation, tobacco and alcohol assessment
dispense 5-fluorouracil for 4 applications

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Agent Intervention*

- Applications of intravaginal 5-fluorouracil (5%) self-administered once every other week (Initiation phase: weeks 1, 3, 5, 7; If optional extension phase: weeks 9, 11, 13, 15**)
- Applications of intravaginal imiquimod cream 5% provider-administered once every other week (Initiation phase: weeks 2, 4, 6, 8; If optional extension phase: weeks 10, 12, 14, 16**)

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Regular contact

Alternating weekly reminder of 5-flurouracil application and
for AE and compliance evaluation

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Clinic visits for imiquimod application
(Initiation phase: weeks 2, 4, 6, 8; Optional extension phase: weeks 10, 12, 14, 16)**
Colposcopy with digital imaging of the cervix (and further management if evidence of disease progression)
Application of imiquimod by provider, collect unused agents
Weight, vital signs, urine pregnancy test, concomitant medications, AE evaluation

Additional procedures at week 8 visit only if continuing into optional extension phase**:
Blood for clinical labs, cervical specimen, blood, and urine collection for research endpoints

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End of Study Visit

(4-6 weeks after last agent application)
Weight, vital signs, urine pregnancy test, conmeds and AE evaluation, collect unused agents
Blood for clinical labs, urine for urinalysis, cervical specimen, blood, and urine collection for research endpoints
Tobacco and alcohol assessment
Colposcopy with digital imaging of the cervix
LEEP or cone biopsy or colposcopy directed biopsy, HPV testing, cytology, optional participant survey

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Follow-up

Collect results from the follow-up cervical exams/procedures/tests (LEEP, biopsies, cytology, HPV) performed within 14 months after the End of Study Visit from the medical records.

Endpoints

- Primary: Feasibility
- Secondary: histological regression, type-specific high risk HPV clearance, biomarkers of local immune activation

*Agent application may be delayed until the end of menstrual cycle if application date occurs during menses. No dose will be applied any sooner than 7 days apart even in the setting of dose delay. **Participants will be offered option to participate in an optional extension phase for an additional 8 weeks for a total intervention period of 16 weeks.

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1. OBJECTIVES

1.1 Primary Objectives

Assess feasibility, evaluated based on safety and tolerability, of a combination agent intervention (once-weekly self-administered intravaginal application of 5-fluorouracil alternating with once-weekly provider-applied imiquimod) for treatment of high-grade cervical squamous intraepithelial lesions

1.2 Secondary Objectives

1.2.1 Assess efficacy of the combination agent intervention on cervical disease regression (endpoint based on histologic regression from high-grade lesions to low-grade or no lesions and clearance of high risk-HPV detection) between baseline and study exit visits.

1.2.2 Assess efficacy of the combination agent intervention on genotype-specific HPV clearance between baseline and study exit visits.

1.2.3 Assess efficacy of the combination agent intervention on biomarkers of local immune activation (measurement of changes in expression of Toll-like receptors (TLR) and T-regulatory cells and the levels of innate, immune mediating and proinflammatory cytokines with intravaginal 5-FU and imiquimod) between baseline and study exit visits.

The study will also collect specimens for exploratory studies of vaginal microbiome, proteomics, and metabolomics to identify factors affecting histologic regression, HPV clearance, and immune activation.

2. BACKGROUND

2.1 Cervical Cancer

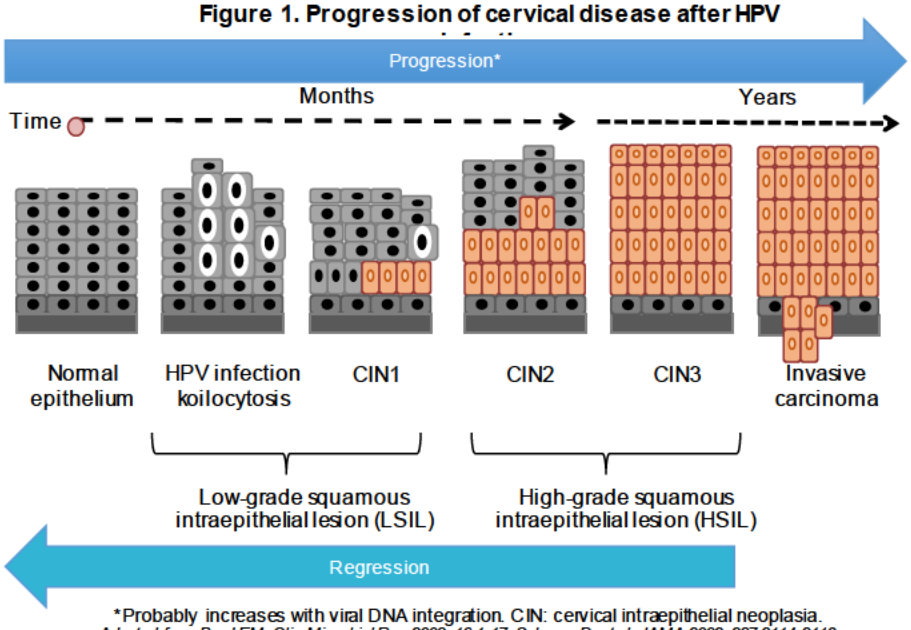
High-risk human papillomavirus (HR-HPV) infection is a precursor to cervical cancer, the leading cause of gynecologic cancer worldwide.[1] HR-HPV genotypes 16 and 18 are associated with approximately 70% of cervical cancers.[2] Roughly 25 million women in the U.S. are actively infected with HPV, and an estimated 80% of sexually active women will contract genital HPV by age 50.[3] While most HR-HPV infections are transient, persistent HR-HPV infections are considered the most significant risk factor for the development of high-grade cervical intraepithelial lesions (CIN 2-3) and cancer.[4] Long standing clinical practice has been to treat all high grade CIN similarly by recommending immediate treatment for all women regardless of age or risk factors. Current clinical guidelines recognize that the prevalence of HPV is high in young women (particularly in the 3rd decade of life) and that disease can be transient. [5] Therefore, intervals of follow up have been lengthened and options for observation of high grade CIN 2 lesions have been encouraged. [5] This approach has been implemented to decrease the over-treatment of women of childbearing age, which can put them at a higher risk for preterm delivery, pain, and bleeding.[6-8]

Current screening for and management of cervical precancer is based on surveillance and destructive surgical therapy. Pap smear surveillance has significantly reduced the incidence of and mortality from cervical cancer by early identification of high-grade cervical intraepithelial lesions and preventative treatment through excisional or ablative surgical procedures.[9] A large majority of women with low-grade CIN (also known as CIN 1) regress to normal within 12-36 months (Figure 1).[10] Approximately 40-58% of CIN 2 lesions regress spontaneously, 30-35% persist, and 10-22% progress to CIN 3.[10-13] More than 12% of women with CIN 3 will advance to invasive cancer over 5-10 years.[14] A key variable in risk is age; young women are more likely to experience regression of cervical dysplasia. Therefore, current clinical guidelines suggest observation with Pap smears and colposcopies at 6 month intervals for "young women" with high-grade CIN.[9] The term "young woman" is loosely defined as women planning future pregnancies.[15]

Surgical therapy for high-grade cervical intraepithelial lesions includes cryotherapy, laser therapy, and excisional procedures within the cervical transformation zone.[9] Excisional options consist of Loop Electrosurgical Excision Procedures (LEEP) or cold-knife-cone (CKC), which remove large biopsies (1-3 cm) from the cervical transformation zone. Although surgical therapy is highly successful in 90-95% of women, 5-16% of women with high-grade CIN will have a recurrence of disease within 5 years of an excisional procedure, with most recurring within 2 years of treatment.[15,16] The exact reasoning for recurrence is unclear, although it may be because of residual disease,[17] re-infection

with another HR-HPV subtype, or reactivation of underlying HR-HPV infection in the genital tract.[18]

Figure 1. Progression of cervical disease after HPV



*Probably increases with viral DNA integration. CIN: cervical intraepithelial neoplasia.
Adapted from Burd EM. Clin Microbiol Rev 2003; 16:1-17; Solomon D, et al. JAMA 2002; 287:2114-2119.

Development of effective, female controlled biologically-rational medical therapies for cervical precancer is a significant need. Despite the overall success and relatively low short-term risk of excisional treatments, there are long-term side effects of excisional treatment to consider in women of childbearing age. Women who undergo excisional procedures potentially carry a 2 to 3-fold increased risk of preterm delivery compared with women without excision history.[8,19] Women undergo psychological distress associated with the need for invasive procedures.[7,20] There is significant economic burden associated with HR-HPV related disease.[21,22] An estimated 412,000 women are diagnosed with CIN annually, with a total cost of approximately $570 million per year. The cost per single episode of care for high-grade cervical intraepithelial lesions is roughly $1634.[23] In fact, most of the cost and burden of disease in HR-HPV-related cervical disease is related to cervical dysplasia as opposed to cancer. Only 10% of expenditures related to cervical cancer are dedicated to the treatment of invasive cervical cancer, whereas 17% is dedicated to the management of CIN.[24] The development of a noninvasive patient-controlled mode of treatment has the potential to lower cost and long-term morbidity for women as well as fulfill the NIH's Strategic Plan for Women's Health Research: to actualize personalized prevention, diagnostics, and therapeutics for girls and women. Although HPV vaccination fit well in this strategic plan, uptake is low in the U.S.[32] In addition, currently available prophylactic HPV vaccines are not therapeutic to women already infected with HR-HPV.[25,26] Successful medical therapy may also serve as (i) adjunct to excisional methods, or (ii) allow women to delay excisional therapy.

2.2 5-Fluorouracil and Imiquimod

5-Fluoruracil (5-FU)

5-FU is an anti-metabolite which has been widely used to treat a variety of malignancies, particularly colon cancer.[27] The primary mechanism of 5-FU is to block synthesis of thymidine and prevent DNA replication. Topical 5-FU is approved for the treatment of dermatologic conditions, but is also used off-label for treatment of vulvar and vaginal dysplasia.[28,29] Previously, 5-FU treatment was limited by side effects, including burning, erythema, erosion, pain, and chronic ulceration, because standard treatment regimens required multiple daily applications.[30] However, limiting the application of 5-FU to biweekly dosing (as is proposed in our protocol) or decreasing the concentration of 5-FU has shown improved participant tolerance.[31-34]

Topical 5-FU induces regression of CIN 2 and has the potential to be an effective medical therapy. Our group hypothesized that low-frequency dosing of self-administered intravaginal 5-FU could serve as primary treatment of CIN 2 in healthy women and avoid the side effects of prior dosing regimens. Our randomized controlled trial in 60 healthy women, aged 18-29 years, with CIN 2 demonstrated that women treated with a 16-week course of biweekly 5-FU (2 g 5% 5-FU per application once every 2 weeks) were more likely to show disease regression (to CIN 1 or normal) than observation alone (84% 5-FU versus 52% control under the intent-to-treat analysis).[34] A relative risk for disease regression of 1.62 [95% confidence interval (CI) 1.10, 2.56, ($p \leq 0.01$)] was found between the 5-FU and observation arms. When histology, Pap smear, and HR-HPV results were combined, women in the 5-FU group were more likely to have all categories normal [RR = 2.25 (95% CI = 1.05, 5.09, $p < 0.05$)] compared with the observation group post-treatment. Using a validated scale,[35] 48% of women in the intervention group reported a side effect (e.g. pain, bleeding, discharge, burning/itching/irritation, urinary concerns, other). No participants reported that symptoms caused interference with usual activities, and there were no moderate or severe side effects in the intervention group. Most (86%) participants reported feeling satisfied with the use of the 5-FU cream. This preliminary work provides valuable data for acceptability, feasibility, and efficacy regarding the use of 5-FU in young women with CIN 2. However, to test efficacy on cervical precancers overall, we propose we need to test this treatment in women with CIN 3 as well since they have no other alternatives to surgical therapy.

Imiquimod

Imiquimod functions at the TLR7 receptor to stimulate the innate immune system and lead to cytokine production, particularly interferon α, IL-6, and TNF-α. It is a treatment for genital warts and vulvar/vaginal dysplasia. It has also been studied as a medical therapy for high-grade squamous intraepithelial lesions.[36-39] Imiquimod is typically used as a topical cream applied 3-7 times per week for 8 to 16 weeks. Local reactions can include erythema, pruritus, excoriation, erosion, edema, scabbing or crusting. Though used topically with minimal systemic absorption, imiquimod therapy can produce systemic reactions such as myalgia, headache, fatigue and nausea. A Cochrane Review of its use for treatment of external genital warts showed a higher rate of local reactions compared to placebo (RR 1.71, 95% CI 1.18 to 2.53; 5 trials, 1225 participants) but no significant difference on systemic reactions compared to placebo (RR 0.91, 95% CI 0.63 to 1.32, 2 trials, 313 participants).[40] A review of its use in cervical, vulvar and vaginal dysplasia also described mild to moderate local and systemic side effects but overall a well-tolerated treatment by patients.[41]

We wish to improve overall treatment success by adding imiquimod to the therapeutic regimen. Though there was a high rate of histologic regression in the 5-FU treated patients in our prior study, only 56% of women were HPV-negative after treatment.[34] A randomized controlled trial conducted by Grimm et al. [36] showed that a 16-week course of dose escalating intravaginal imiquimod treatment in women with high-grade squamous intraepithelial lesions led to histologic regression and HPV clearance (73% versus 39%, $p < 0.05$ for histologic regression and 60% versus 14%, $p < 0.05$ for HPV clearance) similar to that observed in our 5-FU trial. The treatment regimen was more intensive than our 5-FU trial as it required increased dosing over time (one dose (6.25 mg imiquimod) per week for 2 weeks followed by 2 doses per week for 2 weeks and 3 doses per week for 12 weeks). More than 90% of participants in the imiquimod arm reported both local and systemic (flu-like symptoms and fatigue). [36]

Successful medical therapy for high-grade cervical intraepithelial lesions must suppress cellular proliferation within the cervical epithelium and promote a sufficient local immune response to suppress HR-HPV infection. Both 5-FU and imiquimod have similar efficacy as mono-therapies; however, ideal therapy is needed to achieve higher HR-HPV clearance. We propose that dual therapy with 5-FU and imiquimod is biologically rational: 5-FU's mechanism of action is based on local cellular death; imiquimod's mechanism of action is based on modulation of the host immune response. We propose that dual therapy with 5-FU and imiquimod allows for lower dose, less intensive treatment that will result in superior efficacy but fewer side effects for treatment of high-grade cervical intraepithelial lesions.

2.3 Summary of Justification/Rationale

- Many young women need treatment for cervical disease and no conservative medical options are available. Based on the burden of disease, high likelihood of exposure to HPV, costs, and long-term consequences described above, alternatives to surgery are necessary.

- Successful medical therapy for high-grade cervical intraepithelial lesions must suppress cellular proliferation within the cervical epithelium and promote a sufficient local immune response to clear HR-HPV infection. Both 5-FU and imiquimod have similar efficacy as mono-therapies. We propose that dual therapy with 5-FU and imiquimod would lead to improved efficacy because 5-FU's mechanism of action is based on local cellular death whereas imiquimod's mechanism of action is based on modulation of the host immune response. Successful medical therapy may also serve as (i) adjunct to excisional methods, or (ii) allow women to delay excisional therapy.
- Due to lack of safety data of combining therapeutic agents on the cervix, we propose to conduct a Phase 1 study to evaluate the safety and tolerability of dual intravaginal therapy of 5-FU and imiquimod prior to conducting an efficacy trial. Both 5-FU and imiquimod are approved by the Federal Drug Administration (FDA) for other topical indications. [42,43] We hypothesize that dual therapy will not lead to significant increase side effects of either treatment individually. The anticipated improved side effect profile is primarily due to the lower dosing of both medications compared to usual treatment dosing for vaginal/vulvar dysplasia and genital warts.
- We are limiting the study population to women younger than 45 years of age since less women are interested in childbearing after 45 years old and risks of cervical cancer increase in older age groups. [44] Additionally, as women become post-menopausal, vaginal dryness and atrophy may be associated with less tolerance of intravaginal therapies.[45]

Increasing evidence suggests that tobacco and alcohol use are risk factors in the development of intraepithelial neoplasia and cancer. In addition, tobacco and alcohol use may adversely affect agent intervention, for example by altering the safety profile or metabolism of a drug. Standardized assessments of tobacco and alcohol use during clinical trials will aid in understanding the potential relationship between the use of these products and clinical endpoints or cancer prevention biomarkers. Therefore, NCI, DCP is including assessment of tobacco and alcohol use at baseline and follow-up, to determine the potential impact of tobacco and alcohol use on 1) treatment toxicity and symptom burden, and 2) the efficacy of treatment intervention.

3. SUMMARY OF STUDY PLAN

This is a pilot study in women aged 18-45 years with biopsy confirmed high-grade cervical intraepithelial lesions to assess the feasibility of once-weekly intravaginal application of 5-FU and imiquimod used on alternating weeks for 8 to 16 weeks.

The study will have two phases:

1. Initiation phase, which will include 12 to 14 participants and applications of 5-FU and imiquimod on alternating weeks for 8 applications. Participants will self-administer intravaginal 5-FU (2 g of 5% 5-fluorouracil, Effudex) once every other week for a total of 4 applications (wks 1, 3, 5, 7)*. Participants will receive provider-applied commercially available imiquimod cream 5% (12.5 mg imiquimod in 250 mg cream) directly to the cervix once every other week for a total of 4 applications (wks 2, 4, 6, 8)*.
2. Extension phase, which will include no more than the original 12 to 14 participants and continue their participation for applications of 5-FU and imiquimod on alternating weeks for 8 additional applications as described in the initiation phase. Participants will self-administer intravaginal 5-FU (2 g of 5% 5-fluorouracil, Effudex) once every other week for a total of 4 applications (wks 9, 11, 13, 15)*. Participants will receive provider-applied commercially available imiquimod cream 5% (12.5 mg imiquimod in 250 mg cream) directly to the cervix once every other week for a total of 4 applications (wks 10, 12, 14, 16)*.

*Agent application may be delayed until the end of menstrual cycle if application date occurs during menses. No dose will be applied any sooner than 7 days apart even in the setting of dose delay.
The projected accrual rate is 4/month.
At the Screening/consenting Visit, participants will sign informed consent, sign medical records and tissue release form, undergo evaluation of medical history and concomitant medications, have a blood sample collected for complete blood count with differential (CBC with diff), comprehensive metabolic panel (CMP) and HIV test, and have a urine sample collected for urinalysis as well as tests on pregnancy, *Chlamydia trachomatis*, and *Neiserria Gonorrhea.* Participants will

undergo a brief physical exam including weight, height, vital signs including temperature, blood pressure, and heart rate. Performance status will be collected. Participants will be given a symptom diary to record adverse events (AEs) during study participation.

Participants who meet the eligibility assessment will return to the clinic for a Baseline Visit. Vital signs will be collected including weight, temperature, blood pressure and heart rate. Urine will be collected for pregnancy test. Participants will complete tobacco and alcohol assessment forms. Adverse events and concomitant medications will be reviewed. Participants will undergo a colposcopic examination with digital imaging of the cervix and cervical specimen collection (cericovaginal lavage, swab of the cervix, swab of the vagina, and cervical brushing for research endpoints and banking). Blood and urine will be collected and banked for future research. Participants will receive education regarding intravaginal 5-FU self-application, condoms and contraception use, and scheduling information about study visits for imiquimod application. They will receive an initial supply of the following: intravaginal 5-FU preparation, condoms, and pregnancy test kits and instructions on agent application. The 5-FU will be supplied to participants in a commercially available tube with vaginal applicators for 4 applications. Participants will be taught how to remove the 5-FU cream from the tube to fill the applicator with 2 grams of the cream prior to each self-application. This method of application has been chosen in order to preserve the shelf-life of the commercially available 5-FU cream. Once the study agent has been applied, if needed, participants may apply lidocaine jelly or a topical protective barrier cream to the vulva or take oral ibuprofen to minimize vulvar irritation or pain. If a participant experiences an allergic reaction (swelling, rash, hives), she may use diphenhydramine and should notify the study physician. Use of these products will be recorded on the study calendar.

Participants will self-apply 5-FU intravaginally on weeks 1, 3, 5, and 7. Study staff will contact participants via phone call, text, or email to remind them to use the 5-FU, to answer questions participants may have and to review AEs and concomitant medications. Participants will also be reminded to perform pregnancy testing prior to using the study medication with each dose, to avoid sexual activity for at least 48 hours after each application of study medication, and to use two forms of birth control including condoms and one other form of contraception when participating in sexual activity. Agent application will be delayed until the end of menstrual cycle if application date occurs during menses. No dose will be applied any sooner than 7 days apart even in the setting of dose delay. Participants will record study agent applications on a study calendar. If a safety concern is identified during the regular contact, participants will be asked to come in for an interim clinic exam.

Participants will return to the clinic every other week (weeks 2, 4, 6, and 8) to assess safety and compliance and for provider application of imiquimod. Prior to study visits, participants will be reminded to bring unused applicators, tube of 5-FU, symptom diary and study calendar to the study visits. Visits may be delayed due to menstrual cycle. The following tests/assessments will be performed at each of these clinic visits: weight, vital signs, concomitant medications, review of symptom diary and study calendar, and a urine pregnancy test. The tube of 5-FU cream will be weighed at each visit to check compliance. At these visits, participants will also undergo a colposcopic examination with digital imaging of the cervix and examination of genital tissues for any adverse outcomes. Imiquimod will be applied by the provider directly on the cervix. If there is any evidence of disease progression, they will undergo biopsy confirmation. Participants will be taken off study for biopsy confirmed invasive cervical cancer or cervical adenocarcinoma. Similarly, participants will be reminded to avoid sexual activity for at least 48 hours after each application of study medication, and to use two forms of birth control including condoms and one other form of contraception when participating in sexual activity.

At the Week 8 Visit, participants will also undergo blood collection for CBC with diff and CMP. Participants will return unused agents. At this point, they will be offered the option to continue study participation for applications of 5-FU and imiquimod on alternating weeks for 8 additional applications (extension phase).
Participants who opt into the extension phase will be provided with 5-FU for 4 additional applications. The timeline and visits for the extension phase will be the same as for the 8 applications of the initiation phase. Cervical and vaginal specimens, blood, and urine collection for research endpoints and for future research will be collected at this visit only for participants who choose to continue participation for an additional 8 weeks. Participants will self-administer intravaginal 5-FU once every other week for a total of 4 applications (wks 9, 11, 13, 15), and will receive provider-applied imiquimod cream 5% directly to the cervix once every other week for a total of 4 applications (wks 10, 12, 14, 16).

Four to six weeks after 8 (or 16) applications of agent (or 4-6 weeks after the last agent application, if off agent before completing the 8 or 16 week cycle), participants will return for an End of Study Visit. They will be assessed for weight, vital signs, concomitant medications, review symptom diary and study calendar with study staff. Paricipants will complete tobacco and alcohol assessment forms and will be given the option to complete a survey about their experiences while participating in the study. Participants who completed participation in the study and were not given the opportunity to complete the survey at that time may do so by telephone, mail or email once verbal consent has been obtained. They will undergo blood and urine collection for future research. In addition, a blood sample will be collected for CBC with diff and CMP and urine will be collected for a urinalysis and urine pregnancy test. They will undergo a colposcopic examination with digital imaging of the cervix. Cervical specimens will be collected for research endpoints and future research. Additional cervical cells will be collected and sent to the institutional pathology lab for Pap smear and HR-HPV testing. Participants will undergo a LEEP or Cold Knife Cone procedure at this time. If participants decline LEEP or are young women with CIN 2 whose standard of care recommendation had been to observe disease for 6 months, then participants will be given the option to first undergo colposcopy-directed biopsy to assess for residual disease. If subjects opt to have colposcopy-directed biopsy first, then subjects will undergo a minimum of 2 cervical biopsies (one will be at the site of previously noted disease, second will be an endocervical curettage) for histopathological confirmation to verify no microscopic disease. If there is histologic confirmation of residual high-grade squamous intraepithelial lesions or cytologic evidence of ASC-H/HSIL, participants will undergo a LEEP or cone biopsy at future clinical visit according to current clinical care recommendations. If they have evidence of regression (i.e., < CIN2 on histology or cytologic evidence of LSIL/ASC-US/NILM), participants will be recommended to return 6 months later to repeat colposcopic examination with Pap smear and HR-HPV testing (to be performed as the standard of care).

Digital photos will be evaluated for repeat assessment and lesion measurement by a board-certified Gynecologist or Gynecologic Oncologist.

Study staff will contact the participant within 2 weeks of receiving pathology reports of the colposcopically-directed biopsies Women with CIN 1-3 disease or adenocarcinoma-in-situ will be offered current clinical management. Any participant with cancer will be referred for management by gynecologic oncology.

The study team will collect results from the follow-up cervical exams/procedures/tests performed within 14 months after the End of Study Visit from the medical records of all participants.

4. PARTICIPANT SELECTION

4.1 Inclusion Criteria

4.1.1 Women with biopsy confirmed high grade cervical squamous intraepithelial lesions (i.e., CIN3 lesions, and CIN2 lesions with diagnosis confirmed by positive p16 immunohistochemistry staining) within 12 weeks of Baseline Visit

4.1.2 Age 18-45 years

4.1.3 Karnofsky ≥70%

4.1.4 Participants must have normal organ and marrow function as defined below:

Leukocytes	≥3,000/microliter
Absolute neutrophil count	≥1,500/microliter
Platelets	≥100,000/microliter
Serum creatinine	≤ the upper institutional limits

4.1.5 Participants must have a negative HIV antibody/antigen test and negative C. trachomatis/N. gonorrhea NAAT.

4.1.6 Agree to use an effective form of contraception. The effects of intravaginal 5-fluorocuracil and imiquimod on the developing human fetus at the recommended therapeutic dose are unknown. For this reason and because 5-fluorouracil is known to be teratogenic, women of child-bearing potential must agree to use adequate dual methods of contraception (hormonal method of birth control, intrauterine device, or tubal ligation - plus condoms) or abstinence prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her study physician immediately.

4.1.7 Ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Women treated previously with 5-fluorouracil or imiquimod or other medications for high-grade squamous intraepithelial lesions will be excluded from the study.

4.2.2 Concurrent vaginal, vulvar, anal lesions or symptomatic infections.

4.2.3 Pregnant or planning pregnancy within the next 6 months, or breastfeeding. Pregnant women are excluded from this study because 5-fluorouracil is an antimetabolite with the potential for teratogenic effects. Because there is an unknown but potential risk for AEs in nursing infants secondary to treatment of the mother with 5-fluorouracil, breastfeeding should be discontinued if the mother is treated with 5-fluorouracil.

4.2.4 Inability to speak or read English or Spanish

4.2.5 Prior hysterectomy

4.2.6 Use of anticoagulant medications

4.2.7 Subjects who have a known immunocompromised condition (HIV+, use of immunosuppressive medications or systemic steroids, organ transplant recipients) or autoimmune conditions (e.g. psoriasis, rheumatoid arthritis or other known autoimmune condition).

4.2.8 Evidence of invasive anal, vulva, vaginal, or cervical carcinoma; prior LEEP or ablative treatment within 6 months prior to study entry; other invasive malignancies, with the exception of non-melanoma skin cancer, within the last 5 years;

4.2.9 Pathologic findings consistent with
- atypical endometrial cells or serious glandular-cell atypia (atypical glandular cells, favor neoplasia cytology diagnosis)
- evidence of cervical carcinoma on Pap smear or biopsy
- more than two cervical quadrants of CIN 3 as visualized by colposcopy
- nonvisual squamous columnar junction on colposcopy with no concurrent endocervical sampling performed

4.2.10 Use of other investigational agents within 6 months prior to enrollment.

4.2.11 History of allergic reactions attributed to compounds of similar chemical or biologic composition to 5-fluorouracil or imiquimod.

4.2.12 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (other than HPV), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

4.2.13 Subjects with known partial or complete dihydropyrimidine dehydrogenase (DPD) enzyme deficiency.

4.3 Inclusion of Women and Minorities

Women and members of all races and ethnic groups are eligible for this trial. Men are not included because they do not have a cervix or vagina.

4.4 Recruitment and Retention Plan

Study participants will be recruited from patients seen at the Protocol PI's UNC OBGYN outpatient clinics, and from outside referring physicians. The Protocol PI is the Medical Director of the UNC Cervical Dysplasia Clinic and Senior Medical Director of all the UNC Obstetrics & Gynecology clinics and will have access to all patients seen in them. These include UNC OBGYN at Hillsborough, UNC OBGYN Weaver Crossing, and UNC GYN Oncology at Rex Hospital in Raleigh. Potentially eligible patients will be identified and contacted about the study. If they express interest in participation, they will present for an in person consent and screening visit. Patients contacted for the study will be listed on the AQUIP enrollment log as required by DCP. Reasons for refusal of study participation will be recorded by the study coordinator and evaluated regularly by the Protocol PI.

Retention will be centered upon follow-up telephone or other contacts (e.g., email or in person). The study team will provide a friendly and comfortable study setting for participants from initial contact through the completion of their study activities. Demands upon the subjects will be minimized to foster comfort while preserving the research goals. Wherever possible, flexibility will be built into the study schedule to promote compliance.

5. AGENT ADMINISTRATION

Intervention will be administered on an out-patient basis. Reported AEs and potential risks are described in Section 6.2.

5.1 Dose Regimen and Dose Groups

The study will have two phases:

1. Initiation phase, which will include a total of 12 to 14 participants and applications of 5-FU and imiquimod on alternating weeks for 8 applications. Participants will self-administer intravaginal 5-FU (2 g of 5% 5-fluorouracil, Effudex) once every other week for a total of 4 applications (wks 1, 3, 5, 7). Participants will receive provider-applied commercially available imiquimod cream 5% (12.5 mg imiquimod in 250 mg cream) directly to the cervix once every other week for a total of 4 applications (wks 2, 4, 6, 8).
2. Extension phase, which will include no more than the original 12 to 14 participants and continue their participation for applications of 5-FU and imiquimod on alternating weeks for 8 additional applications as described in the initiation phase.

Weekly treatment regimen*

WEEKS (Initiation phase)	1	2	3	4	5	6	7	8	WEEKS (if continuing to Extension phase)	9	10	11	12	13	14	15	16
5-FU (self-applied)	X		X		X		X		5-FU (self-applied)	X		X		X		X	
Imiquimod (provider-applied)		X		X		X		X	Imiquimod (provider-applied)		X		X		X		X

*Agent application may be delayed until the end of menstrual cycle if application date occurs during menses. No dose will be applied any sooner than 7 days apart even in the setting of dose delay.

5.2 5-Fluorouracil and Imiquimod Administration

5-FU Administration

- The 5-FU will be supplied to participants in a commercially available multi-dose tube with vaginal applicators for individual use. Participants will be taught how to remove the 5-FU cream from the tube to fill the applicator with 2 grams of the cream prior to each self-application. This method of application has been chosen in order to preserve the shelf-life of the commercially available 5-FU cream.
- Participants will receive phone call, text or email reminders to use the 5-FU cream every other week.
- Participants will self-administer 5-FU on alternating weeks via vaginal applicators. They will perform home pregnancy testing prior to each application.
- 5-FU will be self-applied prior to bedtime. After agent application, the participant will place a tampon in the vagina overnight and remove it in the morning. She will wash the agent preparations off externally and wear panty liners that will be replaced every 2-3 hours or as needed throughout the next day.
- Agent application will be delayed until the end of menstrual cycle if application date occurs during menses.
- Participants will be advised to avoid agent application in circumstances where pregnancy is suspected for any reason by the participant. If this is the case, she will notify study staff.
- Participants will be instructed to dispose the used applicators in trash, away from the reach of other household individuals.
- The participant will enter date and time of drug administration into the study calendar to document adherence to regimen.

Imiquimod Administration

- Commercially available imiquimod cream 5% will be applied by the provider directly to the cervix in the clinic on alternating weeks. Urine pregnancy test will be performed prior to each application.
- Visits may be delayed until the end of menstrual cycle if application date occurs during menses. No dose will be applied any sooner than 7 days apart even in the setting of dose delay.
- Date and time of imiquimod application will be documented in the study calendar by the provider.
- After the application, the participant will lay in the supine position for at least 30 min. The participant will then place a tampon in the vagina. Before going to bed, she will remove the tampon and shower to wash any agent preparations off externally and wear a pad during the night. The pad will be replaced every 2-3 hours or as needed throughout the next day.

5.3 Run-in Procedures

Not Applicable.

5.4 Contraindications

Participants will be informed to avoid intercourse for 48 hours after each agent application. Dual contraception with condoms and another hormonal or long-acting method will be required. Condoms will be recommended for additional contraception, for the prevention of sexually transmitted infections, and to potentially protect male partners from any residual medication.

5.5 Concomitant Medications

Women will be excluded if they are using anticoagulants or immunosuppressive medications.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (*e.g.*, biopsy) and those used to minimize vulvular irritation or pain should also be included.

5.6 Dose Modification

Participants will be asked to contact the study team when experiencing a possibly related adverse event. Section 11.1.3.1 describes the assessment of the severity of an adverse event. Briefly, adverse events (except female genital AEs) will be assessed according to the grade associated with the CTCAE term. Female genital AEs will be assessed according to the NIAID DAIDS Female Genital Grading Table (FGGT) (Appendix B), rather than using CTCAE.

For a Grade 1 toxicity of any genital lesion(s) (blisters, ulcerations, or pustules), that is possibly, probably, or definitely related to the study medication, the participant will not receive applications of the study drug until the toxicity has resolved. No dose modification will be made for other Grade 1 adverse events.
For Grade 2 adverse events definitely, probably or possibly related to study agent persisting until the next scheduled agent application, the next application(s) will be omitted until toxicity is resolved to Grade 1 or less.
Participants with Grade 3 or 4 events that are definitely, probably or possibly related to the study agent will receive no further doses and be followed for resolution of adverse events. If appropriate, they should remain on-study and undergo the post-intervention biopsy.

For adverse events not considered related to study agent persisting until the next agent application, the scheduled agent application may be continued or omitted at the discretion of the study physician.

5.7 Adherence/Compliance

5.7.1 Participants will be considered compliant if they have applied/received ≥ 80% of the assigned number of applications.

5.7.2 The compliance will be assessed by the number of applications based on the study calendar and provider applications. The information will be further confirmed by remaining weight of the returned 5-FU cream.

6. PHARMACEUTICAL INFORMATION

6.1 5-Fluoruracil and Imiquimod

5% 5-FU cream will be acquired from Valeant Pharmaceuticals or Spear Dermatology and will be ordered by the UNC Outpatient Pharmacy and distributed by Investigational Drug Services (IDS). Every effort will be made to acquire the agent from the same supplier throughout the study. The 5-FU will be supplied to participants in the commercially available tube with vaginal applicators. Participants will be taught how to remove the 5-FU cream from the tube to fill the applicator to the 2 gram mark with the cream prior to each self-application. This method of application has been chosen in order to preserve the shelf-life of the commercially available 5-FU cream.

Commercially available imiquimod cream 5% (trade name Aldara) will be acquired from a commercial source (e.g., Fougera, Glenmark, Perrigo) and will be ordered by the UNC Outpatient Pharmacy and distributed by Investigational Drug Services (IDS). Every effort will be made to acquire the agent from the same supplier throughout the study.

6.2 Reported Adverse Events and Potential Risks

5-FU: Participants may have side effects from the 5-FU medication. Mild side effects include symptoms, such as burning, redness, or vaginal discharge. Serious side effects include pain, bleeding or vaginal ulcers. This treatment (5% 5-FU once every 2 weeks) has been previously studied in other women and 2% of women (1 out 50) reported mild side effects related to the cream.[31] Our research team assessed women undergoing the same treatment regimen and 48% reported some genital side effect (e.g. burning, irritation, spotting). However, none reported that these side effects interfered with usual

activities, and 83% reported that they had a good experience using the cream. No women reported serious side effects.[34]

Because systemic 5-FU is listed as Federal Drug Administration (FDA) Category X, participants should not be pregnant, and should not become pregnant or breast-feed while using the 5-FU cream because the drug in this study could be teratogenic.[42] To minimize this risk, women will be required to use 2 forms of birth control while consuming the study drug if they are using a short acting form of birth control, such as hormonal oral, transdermal, intravaginal or injectable contraception. Condoms will still be recommended for women who are using long acting forms of contraception or have had a tubal ligation to prevent acquisition of a sexually transmitted infection. Use of condoms will also minimize risk of irritation to a sexual partner. We completed a randomized-controlled trial in women ages 18-29 years and reported no pregnancies during use of the 5-FU study drug using these parameters. Participants will be advised to wash hand thoroughly after use of the cream and bring any unused medications back to study team at the subsequent study visit.

Imiquimod: Imiquimod is FDA approved for treatment of external genitalia and perineal warts, condyloma acuminate as well as actinic keratosis and primary superficial basal cell carcinoma in immunocompetent adults. [43] Imiquimod may induce both local and systemic side effects as has been noted in prior studies. A previous trial of women with high-grade squamous intraepithelial lesions reported women having both local and systemic side effects using self-administered imiquimod therapy intravaginally once a week for 2 weeks, twice a week for 2 weeks and then 3 times a week for 12 weeks (total 16 week therapy, total 42 doses). Mild local side effects (vulvar pain or pruritus) were reported in 92% of participants. Flu-like symptoms and fatigue were reported in 97% of imiquimod participants and 34% of placebo participants. Investigators reported findings, such as erythema, edema, erosion, and ulceration during treatment. Severe findings were improved by holding one dose of therapy.[36] Another trial of self-administered imiquimod (twice a week or every other day dosing for up to 12 doses) for women with CIN 1-3 reported the most common side effect as vaginal discharge. Other reported events included vulvar pain, pruritus, erythema, erosion, myalgia and flu-like symptoms; however, no participant discontinued the study due to these side effects.[38]

Our dosing in this trial (one dose every 2 weeks, up to a total of 8 doses) will be much lower than the above noted studies. Therefore, we expect an improved safety profile. Participants will be advised to use nonsteroidal anti-inflammatory drugs (NSAIDs) (or acetaminophen, if not tolerable to NSAIDs) in the event of systemic symptoms which has been shown to be beneficial and not interfere with efficacy of imiquimod.[46]

6.3 Availability

5% 5-FU (trade name Efudex,Valeant Pharmaceuticals) and vaginal applicators will be ordered by the UNC Outpatient Pharmacy and distributed by Investigational Drug Services (IDS).
Commercially available imiquimod cream 5% (trade name Aldara) will be acquired from a commercial source (e.g., Fougera, Glenmark, Perrigo) by the UNC IDS. Every effort will be made to acquire the agent from the same supplier throughout the study.

6.4 Agent Distribution

The 5-FU cream and imiquimod cream will be sent directly to the UNC IDS from UNC outpatient pharmacy.

UNC IDS will distribute the study agents to the study staff. 5-FU cream will be distributed to the study participants. Imiquimod cream will be applied by the study physician.

6.5 Agent Accountability

The Investigator, or a responsible party designated by the Investigator, must maintain a careful record of the inventory and disposition of all agents received from DCP using the NCI Drug Accountability Record Form (DARF). The Investigator is required to maintain adequate records of receipt, dispensing and final disposition of study agent. This responsibility has been delegated to Lisa Rahangdale, MD, MPH and the UNC Investigational Drug Service. Include on receipt record from

whom the agent was received and to whom study agent was shipped, date, quantity and batch or lot number. On dispensing record, note quantities and dates study agent was dispensed to and returned by each participant.

6.6 Packaging and Labeling

5-FU will be packaged by Valeant Pharmaceuticals or Spear Dermatology. It will be ordered by the UNC Outpatient Pharmacy and stored at the UNC Investigational Drug Service.

Commercially available imiquimod cream 5% will be packaged by the supplier (e.g., Fougera, Glenmark, Perrigo), ordered by the UNC Outpatient Pharmacy and stored at the UNC Investigational Drug Service. Every effort will be made to acquire the agent from the same supplier throughout the study.

6.7 Storage

Medications will be stored at room temperature (between 59°F and 86°F) at the UNC IDS. Participants will be instructed to store the agent at room temperature away from the reach of other individuals.

6.8 Registration/Randomization

Participants will be considered registered on the date they sign the approved informed consent document with a member of the study staff. A participant identification number (PID) will be assigned by the coordinator from a predetermined list once the subject has been consented. This study does not involve randomization.

6.9 Blinding and Unblinding Methods

Not applicable

6.10 Agent Destruction/Disposal

The UNC IDS will receive all unused study agents for disposal according to the institutional standards.

7. CLINICAL EVALUATIONS AND PROCEDURES

7.1 Schedule of Events

Evaluation/ Procedure	Screening/ Consenting Visit	Baseline Visit	Self-Application of 5-FU Wks 1, 3, 5, 7[5]	Imiquimod Application Visits Wks 2, 4, 6[5]	Wk 8 Imiquimod Application Visit[5]	Self-Application of 5-FU Wks 9, 11, 13, 15[5]	Imiquimod Application Visits - Wks 10, 12, 14[5]	Wk 16 Imiquimod Application Visit[5]	End of Study Visit [7,13]
						OPTIONAL EXTENSION PHASE			
Informed Consent	X								
Assess Eligibility; Pathology Review[3]	X								
Medical History	X								
Vitals (height[1], weight, temp, BP, heart rate)	X[1]	X		X	X		X	X	X
Karnofsky performance status	X								
Blood for CBC-diff, CMP[2]	X				X			X	X
Urinalysis	X								X
Tobacco Assessment		X							X
Alcohol Assessment		X							X
Blood for HIV test	X								
Urine for Chlamydia, Gonorrhea NAAT	X								
Urine pregnancy test[4]	X	X	X	X	X	X	X	X	X
Colposcopy with digital image		X		X	X		X	X	X
Cericovaginal lavage, swab of the cervix, swab of the vagina, and cervical brushing for research endpoints		X			X[12]				X
Cervical cells for Pap smear and HR-HPV									X
Blood and urine for exploratory endpoints		X			X[12]				X
LEEP or Cone biopsy or Colposcopy-directed biopsy[10]									X
Concomitant Medications	X	X	X	X	X	X	X	X	X
Dispense Study Agent (5-FU)		X			X[6]				
Self administered 5-FU			X			X			
Provider applied imiquimod				X	X		X	X	
Collect Study Agent					X			X	
Review Study Calendar				X	X			X	
Participant Survey[14]									X

Adverse Events		X	X	X	X	X	X	X	X
Telephone/email Contact[8]			X			X			
Collection of results from additional tests/procedures[11]									

[1] Height required at Visit 1 only.

[2] CMP includes serum glucose, urea nitrogen, creatinine, sodium, potassium, chloride, bicarbonate, total protein, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin.

[3] Biopsy confirmed high grade cervical squamous intraepithelial lesions (i.e., CIN3 lesions and CIN2 lesions diagnosis confirmed by positive p16 immunohistochemistry staining) within 12 weeks prior to Baseline Visit.

[4] Urine pregnancy test will be done at each visit. Participants will be provided with home pregnancy tests and will be instructed to use them prior to each self-application.

[5] Self-administered 5-FU and provider-administered imiquimod visits will be scheduled on alternating weeks. Agent applications will be delayed during menstrual cycles or omitted for occurrence of study related ≥ grade 2 adverse events. No dose will be applied any sooner than 7 days apart even in the setting of dose delay.

[6] Following the first 8 applications of study agent, participant will be offered option to continue agent intervention for extension phase for an additional 8 weeks.

[7] End of Study Visit to occur 4-6 weeks after the last agent application unless encountering scheduling difficulty[9].

[8] Contact via phone call, text or email will be used to remind participants to use the study medication, to answer questions participants may have and to review AEs and concomitant medications. Participants will also be reminded to perform pregnancy testing prior to using the study medication, to avoid sexual activity for at least 48 hours after each application of study medication, and to use two forms of birth control including condoms and one other form of contraception when participating in sexual activity. Prior to study visits, participants will be reminded to bring unused applicators/agents, symptom diary and study calendar to the study visits.

[9] When encountering scheduling difficulties, all efforts will be made to ensure that the visit date does not deviate for more than 5 business days of the specified timeframe.

[10] If not done at the End of Study Visit, the procedure will be scheduled within 2 weeks of the End of Study Visit, when feasible.

[11] The study team will collect results from the follow-up cervical exams/procedures/tests performed within 14 months after the End of Study Visit from the medical records for all participants.

[12] Cericovaginal lavage for collection of specimens for research endpoints and blood and urine for exploratory endpoints will only be collected at Visit 8 if participant opts to extend treatment for an additional 8 weeks.

[13] Participants who initiate study agent but terminate prior to study completion should return to the clinic for End of Study procedures.

[14] Participant Survey is optional. Participants who completed participation in the study and were not given the opportunity to complete the survey at that time may do so by telephone, mail or email once verbal consent has been obtained.

7.2 Baseline Testing/Prestudy Evaluation

Participants will undergo a Screening Evaluation to be assessed for study eligibility. The informed consent form, medical records release and tissue release form will be signed. Participants must have biopsy confirmed high grade cervical squamous intraepithelial lesions (i.e., CIN3 lesions and CIN2 lesions diagnosis confirmed by positive p16 immunohistochemistry staining) within 12 weeks prior to the Baseline Visit.

Participants will be evaluated for concomitant medications, medical history and baseline symptoms and signs.

Participants will undergo a brief physical exam including weight, height, vital signs including temperature, blood pressure, and heart rate. Performance status will be collected.

Blood will be collected for complete blood count with differential (CBC-diff) and comprehensive metabolic panel (CMP) and HIV test. Urine will be collected for urinalysis, tests on pregnancy, Chlamydia, and Gonorrhea.

A symptom diary for recording adverse events will be provided to participants at this visit.

7.3 Evaluation During Study Intervention

Participants who meet the eligibility assessment will return to the clinic for a Baseline Visit. Vital signs will be collected including weight, temperature, blood pressure and heart rate. Participants will complete tobacco and alcohol assessment forms. Urine will be collected for pregnancy test. Adverse events and concomitant medications will be reviewed.

Participants will undergo colposcopic examination with digital imaging of the cervix and cervical specimen collection (cericovaginal lavage, swab of the cervix, swab of the vagina, and cervical brushing for research endpoints and banking; see section 10.2.2 for detailed information). Blood and urine will be collected and banked for future research. Participants will receive education regarding 5-FU self-application, condoms and contraception use, and scheduling information about study visits for imiquimod application. They will receive an initial supply of the following: intravaginal 5-FU, condoms, pregnancy test kits and instructions on agent application. The 5-FU will be supplied to participants in a commercially available tube with vaginal applicators for 4 applications. Participants will be shown how to remove the 5-FU cream from the tube to fill the applicator with 2 grams of the cream prior to each self-application. If needed, participants may use lidocaine jelly or a topical protective barrier cream to the vulva and/or take oral ibuprofen to minimize vulvar irritation or pain from the application of study agent. If the participant experiences an allergic reaction (swelling, rash, hives), she may use diphenhydramine and should notify the study physician. Use of these products will be recorded on the study calendar.

Participants will self-apply 5-FU intravaginally on weeks 1, 3, 5 and 7. They will be contacted via phone call, text or email to remind them to use the 5-FU, to answer questions participants may have and to review AEs and concomitant medications. Participants will also be reminded to perform pregnancy testing prior to each time they use the study medication, to avoid sexual activity for at least 48 hours after each application of study medication, and to use two forms of birth control including condoms and one other form of contraception when participating in sexual activity. Agent application will be delayed until the end of menstrual cycle if application date occurs during menses. No dose will be applied any sooner than 7 days apart even in the setting of dose delay. Participants will record study agent applications on study calendar. If a safety concern is identified during the regular contact, participants will be asked to come in for an interim clinic exam.

Participants will return to the clinic every other week (weeks 2, 4, 6 and 8) to assess safety and compliance and for provider applications of imiquimod. Prior to study visits, participants will be reminded to bring unused applicators, tube of 5-FU, symptom diary and study calendar to the study visits. Visits may be delayed due to menstrual cycle. No dose will be applied any sooner than 7 days apart even in the setting of dose delay. At each of these interim visits, participants will be assessed for weight, vital signs, concomitant medications, review of symptom diary and study calendar with study staff, and undergo a urine pregnancy test. The tube of 5-FU cream will be weighed at each visit to check compliance. Participants will undergo a colposcopic examination with digital imaging of the cervix and examination of genital tissues

for any adverse outcomes. They will undergo a urine pregnancy test prior to receiving application of study agent. Imiquimod will be applied by the provider directly onto the cervix. If there is any evidence of disease progression, they will undergo biopsy confirmation. Participants will be taken off study for biopsy confirmed invasive squamous cervical cancer or cervical adenocarcinoma or adenocarcinoma-in-situ. Similarly, participants will be reminded to avoid sexual activity for at least 48 hours after each application of study medication, and to use two forms of birth control including condoms and one other form of contraception when participating in sexual activity.

At the Week 8 Visit, participants will also undergo blood collection for CBC with diff and CMP. Participants will return unused agents. At this point, they will be offered the option to continue with study participation for applications of 5-FU and imiquimod on alternating weeks for an additional 8 applications (extension phase). Participants who opt into the extension phase will be provided with 5-FU for 4 additional applications. The timeline and visits for the extension phase will be the same as for the 8 applications of the initiation phase, however, cervical and vaginal specimens, blood, and urine collection for research endpoints and for future research will only be collected at this visit for participants who choose to continue participation for an additional 8 weeks.

7.4 Evaluation at Completion of Study Intervention

The use of the study agents will be completed after 8 applications of agents or after 16 applications if the participant opts to continue participation for an additional 8 applications of treatment.
Four to six weeks after 8 (or 16) applications of agent (or 4-6 weeks after the last agent application, if off agent before completing the 8 or 16 application cycle), participants will return for an End of Study Visit. Participants will be assessed for weight, vital signs, and concomitant medications; review symptom diary and study calendar with study staff. Participants will complete tobacco and alcohol assessment forms and will be given the option to complete a survey about their experiences while participating in the study. Participants who completed participation in the study and were not given the opportunity to complete the survey at that time may do so by telephone, mail or email once verbal consent has been obtained. They will undergo blood and urine collection for future research. In addition, a blood sample will be collected for CBC with diff and CMP and urine will be collected for a urinalysis and urine pregnancy test. Participants will undergo a colposcopic examination with digital imaging of the cervix. Cervical and vaginal specimens will be collected for research endpoints and future research. Additional cervical cells will be collected and sent to the institutional pathology lab for Pap smear and HR-HPV testing. Participants will be scheduled for LEEP or Cold Knife Cone procedure at this time. If participants decline LEEP or are young women with CIN 2 whose standard of care recommendation had been to observe disease for 6 months, then participants will be given the option to first undergo colposcopy-directed biopsy to assess for residual disease. If subjects opt to have colposcopy-directed biopsy first, then subjects will undergo a minimum of 2 cervical biopsies (one will be at the site of previously noted disease, second will be an endocervical curettage) for histopathological confirmation to verify no microscopic disease. If there is histologic confirmation of residual high-grade squamous intraepithelial lesions or ASCH/HSIL pap, participants will undergo a LEEP or cone biopsy at future clinical visit according to current clinical care recommendations. If they have evidence of regression (LSIL or normal histology or LSIL, ASCUC, normal pap), participants will be recommended to return 6 months later to repeat colposcopic examination with Pap smear and HR-HPV (to be performed as the standard of care).

Digital photos will be evaluated for repeat assessment and lesion measurement by a board-certified Gynecologist or Gynecologic Oncologist.

7.5 Post-intervention Follow-up Period

Study staff will contact the participant within 2 weeks of receiving pathology reports of the colposcopically-directed biopsies. Women with CIN 1-3 disease will be offered current clinical management. Any participant with more severe lesions, such as adenocarcinoma-in-situ or cancer, will be referred for management by gynecologic oncology.

The study team will collect results from the follow-up cervical exams/procedures/tests performed within 14 months after the End of Study Visit from the medical records for all participants.

7.6 Methods for Clinical Procedures

Colposcopy and biopsy will be carried out according to standard clinical procedures. Colposcopic images will be obtained at low magnification (10X/20X) with acetic acid according to standard clinical practice. Cervical biopsies will be taken after application of acetic acid.

8. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITION

8.1 Primary Endpoint

The primary endpoint is feasibility of a combination agent intervention (once-weekly self-administered intravaginal 5-FU alternating with once-weekly provider-administered imiquimod) in women with biopsy confirmed high grade cervical squamous intraepithelial lesions (i.e., CIN3 lesions and CIN2 lesions diagnosis confirmed by positive p16 immunohistochemistry staining).

Feasiblity is evaluated based on safety and tolerability of the study intervention. For safety, we will assess the number of participants experiencing the dose limiting toxicity (DLT). DLT is defined as Grade 2 or greater toxicity (or Grade 1 toxicity of any genital lesion (blisters, ulcerations, or pustules)) that is possibly, probably, or definitely related and lasts for more than 5 days. For tolerability, we will assess the number of participants who are not able to apply at least 50% of the treatment due to DLT.

8.2 Secondary Endpoints

8.2.1 Response to the combination of agent intervention on cervical disease regression (endpoint based on histologic regression from high-grade lesions to low-grade- or no lesions and clearance of high risk-HPV detection) between baseline and study exit visits.

8.2.2 Effect of the combination agent intervention on genotype-specific HPV clearance between baseline and study exit visits.

8.2.3 Effect of the combination agent intervention on biomarkers of local immune activation (measurement of changes in expression of Toll-like receptors (TLR2, TLR 3, TLR7, TLR8 and TLR9) and T-regulatory cells (Foxp3) and the levels of mRNA expression and the innate (IFN-α2), immune mediating (IFN-Y, IL-10, IL-12), and pro-inflammatory (IL-1α, -1β, -6, -8, MIP-1α, TNF) cytokine with intravaginal 5-FU and imiquimod between baseline and study exit visits.

The study will also collect specimens for exploratory studies of vaginal microbiome, proteomics and metabolomics to identify factors affecting histologic regression, HPV clearance and immune activation.

8.3 Off-Agent Criteria

Participants may stop taking study agent for the following reasons: completed the protocol-prescribed intervention, AE or serious adverse event (SAE), inadequate agent supply, noncompliance, concomitant medications, medical contraindication. Participants will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.4 Off-Study Criteria

Participants may go ‘off-study’ for the following reasons: the protocol intervention and any protocol-required follow-up period is completed, AE/SAE, lost to follow-up, non-compliance, concomitant medication, medical contraindication, withdraw consent, death, determination of ineligibility (including screen failure), pregnancy.

8.5 Study Termination

NCI, DCP as the study sponsor has the right to discontinue the study at any time.

9. CORRELATIVE/SPECIAL STUDIES

9.1 Rationale for Methodology Selection

HR-HPV DNA testing and genotyping: The genomic DNA will be isolated using a QIAampMinElute Media Kit (QiagenInc, Valencia, CA). The purified genomic DNA will be amplified (PCR Cycler, AB 2720, Applied Biosystems, Grand Island, NY) and genotyped for HPV 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS39, and CP6108 using a Roche Linear Array (RLA) assay (Roche, Pleasanton, CA). This is a quantitative test that detects 37 high-risk and low-risk HPV genotypes.

Cytokine testing by Luminex. Cytokines (IFN-alpha2, IFN-gamma, IL-1alpha, IL-1beta, IL-6, IL-8, IL-10, IL-12 [p70 heterodimer], MIP-1alpha, and TNF) in cervicovaginal lavage will be measured by Luminex technology using Milliplex Human Cytokine Magnetic-bead Kits (Millipore Corporation, Billerica MA).[47,48] Luminex assays will allow us to simultaneously detect and quantify multiple cytokines of interest. They require small sample volumes, are cost-effective, and allow researchers to collect more data in less time than other assays.

TLR and T-regulatory expression: RNA will be extracted using TRI Reagent (Molecular Research Center, Cincinnati, OH)[49]. DNase treatment (TURBO DNA-free, Ambion), reverse transcription (OmniScript RT, Qiagen, Germantown, Maryland), and quantitative PCR on TLR and Foxp3 (T-regulatory cells) mRNA expression will be performed as previously described.[50,51] Using real time RT-PCR is a feasible option since the samples can be stored and processed in batches. Other options such as flow cytometry are not feasible because of the small amount of sample from the cervix and the feasibility of collecting samples.

9.2 Comparable Methods

Each of the proposed methods for the correlative studies has been used in previous research. We will be able to compare data generated from this study with prior research.

10. SPECIMEN MANAGEMENT

10.1 Laboratories

Clinical laboratory tests and histopathology will be performed at the UNC Clinical and Pathology Labs.

Oncogenic HPV testing and genotyping, TLR, T-regulatory, and cytokine assays will be performed in the laboratory of Dr. Barbara Moscicki at UCLA.

10.2 Collection and Handling Procedures

10.2.1 Clinical laboratory tests

CBC with diff: One 3 ml EDTA vacutainer tube to be collected at Screening, Week 8 and End of Study Visits. Blood tubes will be prepped, labeled, and packaged according to the recommendations from the diagnostic laboratory.

CMP: One 7 ml SST or tiger top Vacutainer tubes at Screening, Week 8 and End of Study Visits. Blood tubes will be prepped, labeled, and packaged according to the recommendations from the diagnostic laboratory.

HIV: A minimum of 5 mL of blood will be collected at Screening in a blood/separator tube/gold top. Blood tubes will be prepped, labeled, and packaged according to the recommendations from the diagnostic laboratory.

Urinalysis: A minimum of 4 mL of urine will be collected for point of care testing by the research team.

Urine Chlamydia, and Gonorrhea: No more than 15 mL of urine will be obtained at Screening from the initial stream of urine. Sample will be prepped, labeled, and packaged according to the recommendations from the diagnostic laboratory.

Urine pregnancy: A minimum of 4 mL of urine will be collected for point of care testing by the research team.

10.2.2 Specimens for research endpoints

The following cervical specimens will be collected in the sequence listed below.

Cervicovaginal lavage (CVL) will be collected at Baseline, Week 8 (only for participants who choose to continue participation for an additional 8 weeks), and End of Study Visits. A continuous stream of 10 ml of normal saline will be aimed directly at and into the cervical opening to bathe the endocervix and ectocervix. The fluid will pool in the posterior fornix and then be aspirated. Repeat this procedure three times with the same fluid. The aspirate will be aliquoted into 5 x 2 ml sterile cryovials (~1.5 ml volume each) and labeled with study ID, subject ID, and visit type/date. The samples will be stored at -70 ºC or below for cytokines and HPV testing.

Swab of the cervix will be collected at Baseline, Week 8 (for participants who choose to continue participation for an additional 8 weeks) and End of Study Visits. A Dacron swab will be inserted to swab the endo and exocervix. The swab will then be placed into sterile tube spiked with 2 ml normal saline. Rotate the swab against the side of the tube to remove as much fluid as possible. Vortex the tube, if possible. The fluid will be placed in a 2 ml cryovial and labeled with study ID, subject ID, and visit type/date. The samples will be stored at -70ºC or below for future microbiome and metabolome analysis.

Swab of the vagina will be collected at Baseline, Week 8 (for participants who choose to continue participation for an additional 8 weeks) and End of Study Visits. A Dacron swab will be inserted to swab the walls of vagina. The swab will then be placed into sterile tube spiked with 2 ml normal saline. Rotate the swab against the side of the tube to remove as much fluid as possible. Vortex the tube, if possible. The fluid will be placed in a 2 ml cryovial and labeled with study ID, subject ID, and visit type/date. The samples will be stored at -70ºC or below for future microbiome analysis.

Cervical brushing will be collected at Baseline, Week 8 (for participants who choose to continue participation for an additional 8 weeks) and End of Study Visits. A cervical cytobrush will be placed into the endocervical os and rotated gently 2 times. The cytobrush will be placed into a 5 ml cryotube prespiked with RNA later. The end of the cytobrush will be snapped off so that the brush portion can fit into the cryotube. The tube will be labelled with study ID, subject ID, and visit type/date. The samples will be stored at -70ºC or below for mRNA analysis.

Cervical cells for Pap smear and HR-HPV will be obtained at End of Study Visit using ThinPrep collection devices and cytology medium. The specimen will be prepped, labeled, and packaged according to the recommendations from the UNC pathology laboratory. The sample will be submitted to UNC pathology for cytology analysis and HR-HPV testing. The remaining sample will be stored for any potential future study.

Cervical biopsies will be collected at the End of Study Visit or at a subsequent clinical visit. The specimen will be prepped, labeled, and packaged according to the recommendations from the UNC pathology laboratory. The sample will be submitted to UNC pathology for histopathology and p16 staining.

One 7 ml SST or tiger top Vacutainer tubes at Baseline, Week 8 (for participants who choose to continue participation for an additional 8 weeks) and End of study Visits. The SST tube will be held at room temperature for 30 min and then centrifuged. Serum will be aliquoted evenly into 5 x 2 ml cryovials and stored at -70°C or below. The cryovials will be labeled with study ID, subject ID, and visit date.

Urine will be collected at Baseline, Week 8 (for participants who choose to continue participation for an additional 8 weeks) and End of study Visits and aliquoted 5 x 2 ml cryovials (~1.5 ml volume each) and stored at -70°C or below. The cryovials will be labeled with study ID, subject ID, and visit type/date.

10.3 Shipping Instructions

All samples will be shipped in compliance with the International Air Transport Association (IATA) Dangerous Goods Regulations. Current shipper and institutional procedures must be followed. Biologic specimens (Category B, UN3373) will be in leak-proof primary and secondary receptacles with puncture resistant packaging and absorbent material.

Shipments are to be preceded with phone contact to the receiving lab to assure the shipment will be met and stored promptly.

Cervical specimens for cytokines, HPV testing, and mRNA analysis will be shipped overnight in batches on dry ice to the laboratory of Dr. Barbara Moscicki at UCLA.

Dr. Anna-Barbara Moscicki
10833 Le Conte Ave # MDCC 22-452
Los Angeles CA 90095
Telephone: (310) 206-6345
Fax: (310) 206-4855
amoscicki@mednet.ucla.edu

10.4 Tissue Banking

Biologic specimens collected during the conduct of each clinical trial that are not used during the course of the study will be considered deliverables under the contract and thus the property of the NCI. At study completion, NCI reserves the option to either retain or relinquish ownership of the unused biologic specimens. If NCI retains ownership of specimens, the Contractor shall collect, verify and transfer the requested biologic specimens from the site to a NCI-specified repository or laboratory at NCI's expense.

In concordance with the NCI Genomic Data Sharing Policy, genomic data generated in this study will be deposited in dbGaP and will be available in a controlled-access manner.

11. REPORTING ADVERSE EVENTS

DEFINITION: AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes all deaths that occur while a participant is on a study.

Please note that all abnormal clinical laboratory values that are determined to be of clinical significance based on a physician's assessment are to be reported as AEs. Those labs determined to be of no clinical significance or of unknown clinical significance (per the physician's assessment) should not be reported as AEs. Any lab value of unknown clinical significance should continue to be investigated/followed-up further for a final determination, if possible.

A list of AEs that have occurred or might occur can be found in §6.2 Reported Adverse Events and Potential Risks, as well as the Investigator Brochure or package insert.

11.1 Adverse Events

11.1.1 Reportable AEs

All AEs that occur after the informed consent is signed and baseline assessments are completed (including run-in) must be recorded on the AE CRF (paper and/or electronic) whether or not related to study agent.

11.1.2 AE Data Elements:

The following data elements are required for AE reporting.

- AE verbatim term
- NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAE v4.0) AE term (MedDRA lowest level term)
- CTCAE (MedDRA) System Organ Class (SOC)
- Event onset date and event ended date
- Treatment assignment code (TAC) at time of AE onset
- Severity grade
- Attribution to study agent (relatedness)
- Whether or not the event was reported as a SAE
- Whether or not the subject dropped due to the event
- Outcome of the event

11.1.3 Severity of AEs

11.1.3.1 Identify the AE using the CTCAE version 4.0. The CTCAE provides descriptive terminology (MedDRA lowest level term) and a grading scale for each AE listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

AEs (except female genital AEs) will be assessed according to the grade associated with the CTCAE term. Female genital AEs will be assessed according to the NIAID DAIDS Female Genital Grading Table (FGGT) (weblinks below; Appendix B), rather than using CTCAE:
http://www.mtnstopshiv.org/sites/default/files/attachments/Addendum_1_Female_Genital_Grading_Table_v1_Nov_2007.pdf or https://rsc.tech-res.com/docs/default-source/safety/addendum_1_female_genital_grading_table_v1_nov_2007.pdf

If an AE can be found in both CTCAE and FGGT, FGGT will be used for the assessment.

Other AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

CTCAE v4.0 general severity guidelines:

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Life-threatening	Life-threatening consequences; urgent intervention indicated.

Grade	Severity	Description
5	Fatal	Death related to AE.

ADL

*Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc*.

**Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

11.1.4 Assessment of relationship of AE to treatment

The possibility that the AE is related to study agent will be classified as one of the following: not related, unlikely, possible, probable, definite.

11.1.5 Follow-up of AEs

All AEs, including lab abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious Adverse Events

11.2.1 DEFINITION: Regulations at 21 CFR §312.32 (revised April 1, 2014) defines an SAE as any untoward medical occurrence that at any dose has one or more of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to perform normal life functions
- A congenital anomaly or birth defect
- Important medical events that may not be immediately life-threatening or result in death or hospitalization should also be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require intervention to prevent one of the other outcomes.

11.2.2 Reporting SAEs to DCP

11.2.2.1 The Lead Organization and all Participating Organizations will report SAEs on the DCP SAE Report Form found at http://prevention.cancer.gov/clinical-trials/clinical-trials-management/protocol-information-office/pio-instructions-and-tools/2012-consortia.

11.2.2.2 Contact the DCP Medical Monitor by phone within 24 hours of knowledge of the event.

DCP Medical Monitor:
Edward Sauter, MD, PhD
Breast and Gynecologic Cancer Research Group
Division of Cancer Prevention
9609 Medical Center Dr., Rm 5E326
Bethesda, MD 20892-9783
Phone: (240)276-7657
Email: edward.sauter@nih.gov

Include the following information when calling the Medical Monitor:

- Date and time of the SAE
- Date and time of the SAE report
- Name of reporter
- Call back phone number
- Affiliation/Institution conducting the study
- DCP protocol number
- Title of protocol
- Description of the SAE, including attribution to drug

11.2.2.3 The Lead Organization and all Participating Organizations will email written SAE reports to DCP's Regulatory Contractor CCS Associates, Inc. (CCSA; phone: 650-691-4400) at safety@ccsainc.com within 48 hours of learning of the event using the fillable PDF SAE Report Form.

11.2.2.4 The DCP Medical Monitor and CCSA regulatory and safety staff will determine which SAEs require FDA submission as IND safety reports.

11.2.2.5 The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB/IEC.

11.2.3 Follow-up of SAE

Site staff should send follow-up reports as requested when additional information is available. Additional information should be entered on the DCP SAE Report Form in the appropriate format. Follow-up information should be sent to DCP as soon as available. SAE related to the study agent will be followed until resolved, or deemed unlikely to further resolved by the Protocol Chair, or until the subject withdraws consent for further follow-up. SAE unrelated or unlikely to be related to study agent will be followed for at least 30 days after the last dose of study agent.

12. STUDY MONITORING

12.1 Data Management

This study will report clinical data using the OnCore application from Forte Research Systems, Inc., as stated in the Master Data Management Plan. All users of the database will have appropriate education, training and experience to perform assigned tasks. The data collection and management will be done according to the Consortia 2012 DMP.

12.2 Case Report Forms

Participant data will be collected using protocol-specific case report forms (CRF) developed from the standard set of DCP Chemoprevention CRF Templates and utilizing NCI-approved Common Data Elements (CDE). The approved CRFs will be used to create the electronic CRF (e-CRF) screens in the OnCore application. Consortia site staff will enter data into the e-CRF for transmission to DCP according to pre-established DCP standards and procedures. Amended CRF will be submitted to the DCP Protocol Information Office for review and approval. Approved changes will be programmed into the OnCore database by the Consortium Data Management staff.

CRF Submission Information:
University of Arizona Early Phase Chemoprevention Consortium Office
Attn: Bonita Weible
1430 E. Fort Lowell, Suite 304
Tucson, AZ 85719
Phone: (520) 318-7178
Fax: (520) 514-6015

Email: UACC-CPRE@UACC.arizona.edu

12.3 Source Documents

Source documentation for this trial will consist of protocol-specific source documents as well as clinical and research laboratory reports. In the event of a Serious Adverse Event, medical records related to the event will be sought for source documentation of the event and its treatment, if any.

12.4 Data and Safety Monitoring Plan

The University of Arizona Cancer Center (UACC) Data and Safety Monitoring Board (DSMB) will provide oversight for subject safety for all UA Consortium clinical trials consistent with the National Institutes of Health Policy for Data and Safety Monitoring dated June 10, 1998; further guidance statement issued by the NIH on June 5, 2000, and the policy for Data and Safety Monitoring by Data and Safety Monitoring Boards. The UACC DSMB meets quarterly.

Regular monthly meetings of the UA Consortium, are used as a forum to review accrual rates, problematic issues relating to accrual and protocol implementation, adverse events occurrence, follow-up, and reporting; submission of all required study reports; and progress and outcomes of laboratory analyses.

12.5 Sponsor or FDA Monitoring

The NCI, DCP (or their designee), pharmaceutical collaborator (or their designee), or FDA may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.6 Record Retention

Clinical records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, *etc.*), as well as IRB records and other regulatory documentation will be retained by the Investigator in a secure storage facility in compliance with Health Insurance Portability and Accountability Act (HIPAA), Office of Human Research Protections (OHRP), Food and Drug Administration (FDA) regulations and guidances, and NCI/DCP requirements, unless the standard at the site is more stringent. The records for all studies performed under an IND will be maintained, at a minimum, for two years after the approval of a New Drug Application (NDA). For NCI/DCP, records will be retained for at least three years after the completion of the research. NCI will be notified prior to the planned destruction of any materials. The records should be accessible for inspection and copying by authorized persons of the Food and Drug Administration. If the study is done outside of the United States, applicable regulatory requirements for the specific country participating in the study also apply.

12.7 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA)

Not applicable.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Description

This is a pilot study aiming to assess the feasibility of a combination regimen of 5-FU and imiquimod. As mentioned in Section 3, the study will have two phases:

1. Initiation phase, which will include a total of 12 to 14 participants

2. Extension phase, which will include no more than the original 12 to 14 participants.
   Study participants will be given the option to continue treatment during the extension phase. Data collected during this phase will be used to further assess the safety, tolerability, and efficacy of the combination therapy.

13.2 Randomization/Stratification

No randomization/stratification will be performed.

13.3 Accrual and Feasibility

We plan to screen/consent 14 women, assuming a screen fail rate of 10%, to have at least 12 initiating agent intervention. The projected accrual rate is 4/month and it is anticipated that the accrual will take 3-4 months. In addition, it is expected that additional delays and deferrals would occur due to menstrual periods and/or other medically-indicated reasons.

13.4 Primary Objective, Endpoint(s), Analysis Plan

The primary objective of the study is to assess the feasibility of intravaginal use 5-FU and imiquimod on alternating weeks in women with biopsy confirmed high grade cervical squamous intraepithelial lesions (i.e., CIN3 lesions and CIN2 lesions diagnosis confirmed by positive p16 immunohistochemistry staining).

Feasiblity is evaluated based on safety and tolerability of the study intervention. For safety, we will assess the number of participants experiencing the dose limiting toxicity (DLT). DLT is defined as Grade 2 or greater toxicity (or Grade 1 toxicity of any genital lesion (blisters, ulcerations, or pustules)) that is possibly, probably, or definitely related and lasts for more than 5 days. For tolerability, we will assess the number of participants who are not able to apply at least 50% of the treatment due to DLT.

The proportion of participants experiencing DLT and the proportion of tolerable participants will be reported along with their 95% confidence intervals. We plan to accrue at least 12 participants to evaluate the feasibility of the intervention to determine whether this intervention is worth exploring further in a subsequent trial. If $\geq$ 33% experience DLT, we will not proceed with further exploration using the combined agent intervention.

In the prior trial conducted by Dr Rahangdale, self-administration of 5FU once every two weeks for a total of 16 weeks did not result in adverse events that were Grade 2 or higher[34]. Addition of imiquimod in alternating weeks to the 5FU alone treatment may increase the type or severity of the adverse events than those observed with 5FU alone. Nevertheless, our proposed intervention is expected to have an improved safety profile than prior trials of intravaginal imiquimod[36,38] because of the less frequent dosing of imiquimod employed in our trial. Therefore, we do not expect to observe a toxicity rate of greater than 30% with our study intervention.

The probabilities of observing DLT in 33% or more participants for toxicity rates no greater than 30% are listed below:

Projected toxicity rate	Probability of observing DLT in ≥ 33% participants
≤ 5%	< 1%
10%	2.6%
20%	20.5%
30%	50.7%

The probabilities of having less than 66% tolerable participants with projected tolerability rates of 50-80% are listed below:

Projected tolerability rate	Probability of < 66% tolerable participants
80%	7.3%

70%	27.6%
60%	56.2%
50%	80.6%

13.5 Secondary Objectives, Endpoints, Analysis Plans

The secondary objectives include describing the response (composite endpoint based on histologic regression and clearance of HR-HPV) to intravaginal 5-FU and imiquimod and type specific HR-HPV clearance and measuring changes in the expression of biomarkers of local immune activation (including IFN-α2, IFN-Y, IL-10, IL-12, IL-1α, -1β, -6, -8, MIP-1α, TNF) after treatment with self-administered intravaginal use of 5-FU and imiquimod. The response and type-specific HR-HPV clearance will be reported along with their 95% confidence intervals. For each biomarker, the mean change and the associated standard deviation will be reported. Analyses will be performed on all participants who provide post-intervention specimens for endpoint evaluation. Assume an attrition rate of 15% (for completing intervention and providing post-intervention specimens). There will be at least10 participants available for the secondary analyses. Based on a sample size of 10, it allows us to construct a 95% confidence interval for the overall response and clearance rate, respectively, with a width ≤0.72. It also allows us to construct a 95% confidence interval for the overall change in expression of each biomarker with a distance from the mean change ≤ 0.72 standard deviations. With an anticipated attrition rate of 15%, we will try to reduce the fraction of participants with missing outcomes as much as possible. Also, multiple imputation techniques will be used to handle missing data. Similar to the primary endpoints, each of the secondary endpoints will be also reported by whether or not the participants continue to the expansion phase.

13.6 Reporting and Exclusions

The study will report the primary and secondary study endpoints based on the intent-to-treat population. The study may also report the response rate in those who have applied ≥80% of the assigned dose.

13.7 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of self-administered intravaginal use 5-FU and imiquimod. Descriptive statistics of the type and frequency of all adverse events will be generated, including 95% confidence intervals.

13.8 Evaluation of Response

As described in Section 13.5, the analysis the response rate will be based on the intent-to-treat population. All subjects with endpoint data will be assessed for response to intervention.

Sub-analyses may be performed on the subsets of participants, excluding those who have applied < 80% of the assigned application or for whom major protocol deviations have been identified (e.g., early discontinuation of intervention, major protocol violations, etc.). However, sub-analyses may not serve as the basis for drawing conclusions concerning efficacy, and the reasons for excluding participants from the analysis should be clearly reported.

13.9 Interim Analysis

No formal interim statistical analyses are planned for this pilot/feasibility trial. Accrual, data collection, and any adverse events will be monitored on a regular basis

13.10 Ancillary Studies

None.

14. ETHICAL AND REGULATORY CONSIDERATIONS

14.1 Form FDA 1572

Prior to initiating this study, the Protocol Lead Investigator at the Lead or Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators, at each site that will participate in the protocol. All personnel directly involved in the performance of procedures required by the protocol and the collection of data should be listed on Form FDA 1572.

14.2 Other Required Documents

14.2.1 Current (within two years) CV or biosketch for all study personnel listed on the Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.2 Current medical licenses (where applicable) for all study personnel listed on Form FDA 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.3 Lab certification (*e.g.*, CLIA, CAP) and lab normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.

14.2.4 Documentation of training in "Protection of Human Research Subjects" for all study personnel listed on the FDA Form 1572 and Delegation of Tasks form for the Lead Organization and all Participating Organizations.

14.2.5 Documentation of Federalwide Assurance (FWA) number for the Lead Organization and all Participating Organizations.

14.2.6 Signed Investigator's Brochure/Package Insert acknowledgement form

14.2.7 Delegation of Tasks form for the Lead Organization and all Participating Organizations signed by the Principal Investigator for each site and initialed by all study personnel listed on the form

14.2.8 Signed and dated NCI, DCP Financial Disclosure Form for all study personnel listed on Form FDA 1572 for the Lead Organization and all Participating Organizations

14.3 Institutional Review Board Approval

Prior to initiating the study and receiving agent, the Investigators at the Lead Organization and the Participating Organization(s) must obtain written approval to conduct the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the DCP PIO according to DCP Amendment Guidelines. The DCP-approved amended protocol must be approved by the IRB prior to implementation

14.4 Informed Consent

All potential study participants will be given a copy of the IRB-approved Informed Consent to review. The investigator will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, he/she will be asked to sign and date the Informed Consent document. The study agent(s) will not be released to a participant who has not signed the Informed Consent document. Subjects who refuse to participate or who withdraw from the study will be treated without prejudice.

Participants must be provided the option to allow the use of blood samples, other body fluids, and tissues obtained during testing, operative procedures, or other standard medical practices for further research purposes. If applicable, statement of this option may be included within the informed consent document or may be provided as an addendum to the consent. A Model Consent Form for Use of Tissue for Research is available through a link in the DCP website.

Prior to study initiation, the informed consent document must be reviewed and approved by NCI, DCP, the Consortium Lead Organization, and the Central IRB. Any subsequent changes to the informed consent must be approved by NCI, DCP, and the Central IRB.

14.5 Submission of Regulatory Documents

All regulatory documents are collected by the Consortia Lead Organization and reviewed for completeness and accuracy. Once the Consortia Lead Organization has received complete and accurate documents from a participating organization, the Consortium Lead Organization will forward the regulatory documents to DCP's Regulatory Contractor:

Paper Document/CD-ROM Submissions:
Regulatory Affairs Department
CCS Associates, Inc.
2001 Gateway Place
Suite 350 West
San Jose, CA 95110
Phone: 650-691-4400
Fax: 650-691-4410

E-mail Submissions:
regulatory@ccsainc.com

Regulatory documents that do not require an original signature may be sent electronically to the Consortium Lead Organization for review, which will then be electronically forwarded to DCP's Regulatory Contractor.

14.6 Other

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

15. FINANCING, EXPENSES, AND/OR INSURANCE

Study procedures performed during study visits will be covered by the study budget. Research tests will not be billed to the subject. Subjects may incur minimal out-of-pocket expenses for transportation but will not be charged for study agent or any study-related activities. Subjects will receive monetary compensation which they may use at their discretion for out of pocket cost such as transportation. If injury occurs, medical care will be provided and charged to the subject's insurer.

REFERENCES

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CONSENT FORM

Study Title for Study Participants:
Intravaginal use of 5-FU and imiquimod for women with cervical precancer.

Official Study Title for Internet Search on http://www.ClinicalTrials.gov:
A feasibility trial of alternating intravaginal application of 5-fluorouracil and imiquimod for treatment of high-grade cervical squamous intraepithelial lesions.

Introduction

This is a clinical trial, a type of research study. Your study doctor will explain the clinical trial to you. Clinical trials include only people who choose to take part in the research. Please take your time to make your decision about volunteering. You may discuss your decision with your friends and family. You can also discuss this study with your health care team. If you have any questions, you can ask your study doctor for more of an explanation. You should only agree to participate in this study when you are comfortable enough with the information so that you can make an informed decision about joining.

What is the usual approach to my cervical intraepithelial lesions?

You are being asked to take part in this study because you have cervical precancer (moderate/severe abnormality of the cervix tissue) which increases your risk for cervical cancer. People who are at increased risk for cervical cancer and choose not to participate in a study have other options for treatment, including cryotherapy (use of cold substance to freeze and remove lesions), laser therapy (use of light beam to remove lesions), or surgery for treatment or removal of their cervix.

What are my other choices if I do not take part in this study?

If you decide not to take part in this study, you have other choices. For example:

- you may choose to have the usual approach described above,
- you may choose to take part in a different study, if one is available,
- or you may choose to do nothing.

Why is this study being done?

The purpose of this study is to test the safety of alternating weekly applications of two intravaginal products, 5-fluorouracil (5-FU) cream and imiquimod cream in women with cervical precancer. The study will also find out what effects, if any, the two products have on cervical abnormality. The abnormal tissue will be removed from the cervix at the end of the study. 5-FU is a drug approved in the US which has been widely used to treat several types of cancers. Imiquimod has been used to stimulate the body's immune response and is also approved for use in the US to treat genital warts. However, 5-FU and imiquimod have not been approved for treating cervical precancer and the use of this drug combination is not standard of care. There will be about 12-14 women taking part in this study.

What are the study groups?

Two study drugs, 5-FU cream and imiquimod cream, will be used in this study. There will be two phases in the study:

Initiation Phase: In the first phase, participants will self-administer one dose of the 5-FU cream into the vagina every other week (weeks 1, 3, 5, and 7). On alternate weeks (weeks 2, 4, 6, and 8) the study physician will apply one dose of the imiquimod cream into the vagina. This phase will last for 8 applications and approximately 8 weeks.

Initial Phase - Weekly treatment schedule

WEEK	1	2	3	4	5	6	7	8
5-FU (self-applied)	X		X		X		X	
Imiquimod (study physician-applied)		X		X		X		X

Extension Phase: Participants who complete the first 8 weeks of treatment will be offered the option to continue treatment for 8 additional applications. During this phase, participants will self-administer one dose of the 5-FU cream into the vagina every other week (weeks 9, 11, 13, and 15). On alternate weeks (weeks 10, 12, 14, and 16) the study physician will apply one dose of the imiquimod cream into the vagina. This phase will also last for 8 applications (in other words, for 8 additional weeks).

Extension Phase - Weekly treatment schedule

WEEK	9	10	11	12	13	14	15	16
5-FU (self-applied)	X		X		X		X	
Imiquimod (study physician-applied)		X		X		X		X

A final study visit will be conducted 4-6 weeks following the final application of study medication.

How long will I be in this study?

Participation in the study will last 12 weeks or longer for those who receive 8 applications of treatment and 20 weeks or longer for those who receive 16 applications of treatment. You will apply the 5-FU cream once every other week at home and the study physician will apply the imiquimod cream on alternate weeks in the clinic. The periods for study participation and study medication could be longer due to delays caused by your menstrual cycle. If you stop the study medications at any time before the end of the study, you will be asked to return to the clinic for an additional visit for follow up safety procedures including blood and urine tests and other procedures which the doctor may feel are necessary for your safety. The study team will continue to collect results from your medical records on any follow-up cervical exams/procedures/tests performed within 14 months after the End of Study Visit.

What extra tests and procedures will I have if I take part in this study?

You will need to have the following exams, tests or procedures if you take part in the study. These exams, tests or procedures may be part of regular care for someone with cervical precancer. However, they may be done more often because you are in this study. All exams, tests and biopsies will be performed in the clinic.

Before you begin the study:
You will need to have the following extra tests, and/or procedures in a Screening Visit to find out if you can be in the study.

- The study staff will discuss this consent form with you and answer any questions you may have. Once you have signed it, the following procedures will be done.
- A brief physical exam including height, weight, and vital signs (blood pressure, pulse, and temperature).

- A review of your medical history and current medications, including any symptoms you may be currently experiencing.
- Collection of 2 teaspoons of blood for routine blood tests for clinical care (a complete blood count and a group of blood tests to check the condition of your kidney, liver, electrolytes and blood sugar level).
- Collection of 1 teaspoon of blood for HIV testing. If the test is positive, you will be brought in to the clinic and your doctor will inform you the result and refer you to specialty care.
- Urine test for urinalysis, pregnancy, Chlamydia and Gonorrhea. Your doctor will refer you to specialty care if any of the tests is positive.
- You will be given a symptom diary to record any illness or injury (adverse events) during the study.

Once you are determined to be eligible to participate in the study, you will return to the clinic for the following procedures in a Baseline Visit:

- A review of your symptom diary and current medications.
- A brief physical exam including weight, and vital signs (blood pressure, pulse, and temperature).
- You will complete tobacco and alcohol assessment forms.
- Pelvic exam will be performed and will include the following:
    - Colposcopy, a procedure using a special lighted microscope to visualize the cervix and vaginal walls
    - Digital photos of the cervix
    - Collection of cells from your cervix for Human Papillomavirus (HPV) testing using a pap smear brush
    - Cervicovaginal lavage, or a washing of the tissues of the vagina and cervix, to collect research samples
    - Collection of cells from your cervix and vaginal walls using a swab for research testings
- Collection of 1½ teaspoons of blood for research testings.
- Collection of urine for research testings.
- You will be given study medication for home use and instructions for using it.
- You will be given a study calendar to mark each day you use the study medication.
- You will be given urine pregnancy tests to use at home prior to using the study medication and directions for using them.

Weeks 1, 3, 5 and 7

- Each week, before using the study medication, you will use a home pregnancy test. You should only use the study medication if your pregnancy test is negative.
- Study staff will contact you by telephone, text or email on weeks 1, 3, 5, and 7 to remind you to apply the study medication, and to review any changes in your current medications, and any symptoms you may be experiencing. Study staff will also answer questions you may have about the study medication.
- Using a vaginal applicator, you will apply the 5-FU cream into the vagina on weeks 1, 3, 5, and 7.
- If you are menstruating when it is time to use the 5-FU cream, you should not use the cream until your menstrual period has ended. You should contact the study staff for any delay in applying study medication (including delay for your menstrual period) as there must be a minimum of 7 days between study doses. The staff will work with you to adjust the study medication calendar.

Weeks 2, 4, 6, 8

You will return to the clinic during weeks 2, 4, 6, and 8 for the procedures listed below. If you are menstruating when it is time for your appointment, you should call the study staff to reschedule your appointment as the study medication cannot be applied during your menstrual period.

- A review of your study calendar, symptom diary, current medications.
- A brief physical exam including weight, and vital signs (blood pressure, pulse, and temperature).
- Urine test for pregnancy.
- Pelvic exam including colposcopy and digital photos of the cervix. Biopsies may be taken from your cervix if there is any evidence of worsening disease based on viewing your cervix through the colposcope. You will come off study if the test on the biopsies confirms worsening of the disease.
- The study physician will apply imiquimod cream to your cervix. You will be provided with instructions for your care following the application of the medication.
- You will return unused study medication at each visit and be given more study medication to take home.

At Week 8 the following additional procedures will be performed:

- Collection of 2 teaspoons of blood for routine blood tests for clinical care (a complete blood count and a group of blood tests to check the condition of your kidney, liver, electrolytes and blood sugar level).

If you complete all eight applications of study medication, you may be offered the option to continue participation in the Extension Phase of the study for an additional 8 applications (that is, for an additional 8 weeks). The procedures for the additional 8 applications during the Extension Phase will be same as for the first 8 applications you did during the Initial Phase.

If you choose to participate in the Extension Phase, you will also have the following procedures performed at Visit 8:

- Collection of 1½ teaspoons of blood for research testings.
- Collection of urine for research testings.
- Collection of samples from the cervix and vagina for research testings.

End of Study Visit - You will return to the clinic for the following procedures 4-6 weeks after the final application of study medication (either after 8 applications at the end of the Initial Phase, or if you opt to continue participation In the Extension Phase for an additional 8 applications, this visit will occur after the 16th application):

- A review of your study calendar, symptom diary, current medications.
- A brief physical exam including weight, and vital signs (blood pressure, pulse, and temperature).
- Urine test for urinalysis and pregnancy.
- Collection of 2 teaspoons of blood for routine blood tests for clinical care (a complete blood count and a group of blood tests to check the condition of your kidney, liver, electrolytes and blood sugar level).
- You will complete tobacco and alcohol assessment forms.
- Pelvic exam will be performed and will include the following:
    - Colposcopy
    - Digital photos of the cervix
    - Collection of samples from cervix and vagina as described for Baseline Visit
    - Collection of additional samples from cervix using a pap smear brush and spatula for cervical screening test to check for abnormal cells and HPV testing
- Biopsies will be taken of your cervix. This may be done by loop electrosurgical excision procedure (LEEP) using a wire loop heated by electric current to remove cells and tissue in the cervix or vagina, or by cone biopsy where a cone shaped piece of tissue is removed, or by colposcopy-directed biopsy. The study physician will decide which procedure would be best for you and will discuss this with you. You will sign a separate consent from for that procedure.
- Collection of 1½ teaspoons of blood for research testings.

- Collection of urine for research testings.
- Any remaining study medication will be collected.
- You will be asked to complete a survey about your experience while participating in the study. The survey will take about 10 minutes to complete. The survey is optional, you do not have to complete it if you don't want to.
- Once the results of the biopsy and Pap smear are available, you will be contacted by the study staff or the study physician to discuss additional tests/procedures with you.

Follow-up – The study physician may recommend additional exams/procedures/tests for your cervix after you complete the End of Study Visit. These exams/procedures/tests will be part of your standard of care. The study team will review your medical records and collect the results from the additional exams/procedures/tests performed within 14 months after the End of Study Visit.

Instructions for using the study medication

The study medications may be irritating if they come in contact with normal skin. Therefore, please follow the instructions below closely.

Home application of 5-FU cream:

- Store study medication at room temperature away from the reach of other individuals. **Remember to perform a urine pregnancy test prior to using each dose of the study medication**. Record the date of the test and the test result on the study calendar. **You should only use the study medication if your pregnancy test is negative.**
- Using the vaginal applicator, insert the 5-FU study medication into the vagina at night once every other week, or as directed by the study staff.
- After inserting the study medication, place a tampon in the vagina to keep the study medication from spilling out. Applying the study medication at night, just before bed, is best since you will be lying down. Wash your hands thoroughly after using the study medication to avoid spreading the cream to other areas of your body and causing skin irritation.
- Discard the used applicators in trash, away from the reach of other household individuals.
- In the morning remove the tampon and take a shower. Let all of the study medication run out and make sure that you wash any skin that may have come into contact with the medication.
- During the day wear a pad (not a tampon). If there is a lot of discharge, remember to change pads often so that you are not sitting for a long period of time in any excess study medication. You may have vaginal discharge from the study medication for several days following the last application so remember to continue to wear a pad. Also, remember to wash your hands.
- Do not use the study medication if you are having your menstrual period. You will use the study medication once your menstrual period has finished. The dates of your menstrual period, and the date you used the study medication once your period finished should be noted on the study calendar. You should contact the study staff for any delay in applying study medication (including delay for your menstrual period) as there must be a minimum of 7 days between study doses. The staff will work with you to adjust the study medication calendar.
- Return unused study medication at your next study visit.

Following applications of imiquimod cream in the clinic:

- After the application, you will remain lying on your back for at least 30 min.
- You will then be asked to place a tampon in the vagina. Wash your hands thoroughly to avoid spreading the cream to other areas of your body and causing skin irritation.

- Remove the tampon before going to bed and take a shower. Let all of the study medication run out and make sure that you wash any skin that may have come into contact with the medication.
- During the night wear a pad (not a tampon). You may have vaginal discharge from the study medication for several days following the last application so remember to continue to wear a pad. If there is a lot of discharge, remember to change pads often so that you are not sitting for a long period of time in any excess study medication. Also, remember to wash your hands.

General instructions following application of study creams:

- Remember to avoid sexual activity (including oral sex) for at least 48 hours after each application. After the 48 hour waiting period, you may resume sexual activity but remember to use condoms to help prevent skin irritation to your sexual partner.
- Contact the study team if you experience any symptoms or negative effects you think may be associated with the use of the study medication. If you experience symptoms suggestive of infection in the urinary tract and genital region contact the study team for further evaluation.
    - Symptoms of urinary tract infects may include a strong urge to urinate often, burning pain during urination, cloudy urine, blood in urine, strong urine smell, pelvic pain especially above the pubic bone.
    - Symptoms of infections in the genital region may include ulcers, pain, soreness, itching, burning, tingling, redness, swelling of the vaginal area.
    - Symptoms of an allergic reaction may include swelling, rash or hives. You may use Benadryl (diphenhydramine) for the symptoms. This should be recorded on the study calendar.
- Throughout your participation in the study, you must use 2 forms of birth control during sexual activity. For example, use birth control pills *and* condoms; intrauterine device (IUD) *and* condoms; tubal ligation *and* condoms.
- Vaginal douching is **not** permitted during the study participation.
- You may apply the over-the-counter lidocaine jelly or a topical protective barrier cream to the outer vaginal area and/or take oral ibuprofen, as necessary, to reduce irritation or discomfort that you may experience from the study agent.
- Please call the study physician, _____ *(insert name of study doctor)*, at _____ *(insert phone number)* or email at _____ *(insert email)* if you have any questions or problems.

As part of this study you will also be asked to answer questions about your tobacco and alcohol use, both before you begin the study and again at the End of Study visit. Researchers want to see if tobacco and alcohol use affects the side effects people might get while on this study, or if tobacco and alcohol use modifies the effects of the study agents.

What possible risks can I expect from taking part in this study?

If you choose to take part in this study, there is a risk that you may:

- Lose time at work or home and spend more time in the hospital or doctor's office than usual.
- Be asked sensitive or private questions which you normally do not discuss, for example about your tobacco and alcohol use. There is a risk someone could get access to the personal information in your medical records or other information researchers have kept about you. Someone might be able to trace this information back to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

The 5-FU and imiquimod products used in this study have not been approved for intravaginal use and may affect how different parts of your body work, such as your liver, kidneys, heart, and blood. The study doctor will be testing your blood and will let you know if changes occur that may affect your health.

There is also a risk that you could have side effects.

Here are important points about side effects:

- The study doctors do not know who will or will not have side effects.
- Some side effects may go away soon, some may last a long time, or some may never go away.
- Some side effects may be serious and may even result in death.

Here are important points about how you and the study doctor can make side effects less of a problem:

- Tell the study doctor if you notice or feel anything different so they can see if you are having a side effect.
- The study doctor may be able to treat some side effects.
- The study doctor may adjust the schedule of study drugs application to try to reduce side effects.

Intravaginal use of 5-FU cream has been previously studied in women with cervical precancer. Based on the previous studies, possible side effects for intravaginal use of 5-FU cream include:

Common, some may be serious In 100 people receiving intravaginal 5-FU, more than 20 may have:
• itching • burning • irritation in the vagina • redness in the vagina • vaginal discharge • vaginal bleeding

If you notice ulceration in the vagina or on the outside surface of the genital area indicated by pain or unusual bleeding, do not administer the 5-FU cream.

5-FU should not be used in people with partial or complete dihydropyrimidine dehydrogenase (DPD) enzyme deficiency. This is a metabolic disorder in which the body cannot break down certain substances and can cause neurological problems. This condition can also cause toxic reactions to some types of drugs such as 5-FU. It is possible to have this condition and not be aware of it.

Stop using 5-FU and notify your study physician immediately if you experience severe abdominal pain, bloody diarrhea, vomiting, fever, chills. mouth sores, any abnormal bleeding, redness, swelling, numbness, peeling of the skin on the palms and soles (hand-foot syndrome); shortness of breath; or hair loss. If you are unable to contact the study physician, call <*insert phone number*> and ask for the gynecologist on call.

Exposure to ultraviolet light (e.g., tanning beds, excessive unprotected sun exposure) should be avoided during treatment with 5-FU since it can increase the intensity of a drug reaction.

Intravaginal application of imiquimod has also been studied in a previous study of women with cervical precancer. Based on the previous study, the possible side effects for intravaginal application of imiquimod include:

Common, some may be serious In 100 people receiving intravaginal imiquimod, more than 20 may have:
• pain on the outside surface of the genital area • itching on the outside surface of the genital area • redness in the vagina and on the outside surface of the genital area • swelling in the vagina and on the outside surface of the genital area • erosion in the vagina and on the outside surface of the genital area • ulceration in the vagina and on the outside surface of the genital area (symptoms include pain, unusual bleeding) • flu-like symptoms • fatigue • chills • vaginal discharge • headache • muscle aches
Occasional, some may be serious In 100 people receiving intravaginal imiquimod, from 4 to 20 may have
• fever

If you have an allergic reaction (swelling, rash, hives) to the study medication, call the study physician to report it. You may use Benadryl (diphenhydramine) for the symptoms. This should be recorded on the study calendar.

In the previous study, the imiquimod product was applied to the vagina more often (total 42 applications over 16 weeks) than the frequency to be used in this study (total 8 applications over 16 weeks). There is a possibility that a participant in this study may experience fewer side effects.

The side effects from using 5-FU and imiquimod vaginal products in alternating weeks are not known. There is a risk that you may experience vaginal scarring as a result of this experimental treatment although this has not been reported in prior studies of intravaginal application of 5-FU or imiquimod.

Careful hand washing after using the cream is important to avoid skin irritation to other parts of the body.

Reproductive risks: You should not get pregnant or breastfeed while in this study. The drugs used in this study could be very damaging to an unborn baby. You must use two forms of contraception while participating in sexual activity throughout the study. For example, use birth control pills *and* condoms, intrauterine device (IUD) *and* condoms, tubal ligation *and* condoms. If you are pregnant, you will not be enrolled on this study. If you become pregnant or suspect that you are pregnant, you must tell your study doctor right away. Getting pregnant will result in your removal from this study.

Risks from biopsy: You may have cramping during the biopsy. You may have a brownish discharge for one or two days. If LEEP is done, you may experience some mild pain, discomfort, or bleeding. Notify your doctor if you have heavy bleeding (heavier than your usual menstrual period) or significant pain.
Risks related to blood tests: Blood tests can cause mild discomfort, bruising and/or bleeding at the blood draw site. Less likely is the possibility of significant bleeding or infection.

What possible benefits can I expect from taking part in this study?
This study may or may not help you because we do not know how the study drugs will compare to the usual approach for your condition. This study may help us learn things that could help people in the future.

Can I stop taking part in this study?

Yes. You can decide to stop at any time. If you decide to stop for any reason, it is important to let the study doctor know as soon as possible so you can stop safely and get the treatment you need for your cervical disease. If you stop, you can decide whether or not to let the study doctor continue to provide your medical information to the organization running the study.

For the tobacco and alcohol use questions, you can decide not to answer some or all of the questions. Your decision will not affect whether you can participate in the study, and it will not affect your relationship with your doctor or the study staff.

The study doctor will tell you about any new information or changes in the study that could affect your health or your willingness to continue in the study.

The study doctor may take you out of the study:

- If your health changes.
- If you become pregnant.
- If your cervical disease worsens.
- If the study is no longer in your best interest.
- If new information becomes available.
- If you do not follow the study rules.
- If the study is stopped early for any reason by the sponsor, IRB or FDA.

What are my rights in this study?

Taking part in this study is your choice. No matter what decision you make, and even if your decision changes, there will be no penalty to you. You will not lose medical care or any legal rights. For questions about your rights while in this study, call the _____ *(insert name of center)* Institutional Review Board at _____ (*insert telephone number*).

What are the costs of taking part in this study?

The 5-FU and imiquimod products will be supplied at no charge while you take part in this study. Condoms, pregnancy test kits, and instructions on agent application will also be supplied at no cost. The cost of study-specific exams, tests, and any other procedures such as HIV testing and the colposcopies will be paid for by the study.

Some costs associated with your care may be considered standard of care (such as biopsies, pap smear, LEEP), and will be billed to you or your insurance company. You will have to pay for any costs (including deductibles and co-payments) not covered by your health insurer.

Before you decide to be in the study, you should check with your health plan or insurance company to find out exactly what they will pay for.

You will receive $50 per visit for completing each of the following visits: Baseline Visit, Week 2, Week 4, Week 6 and Week 8 visits. You will receive $60 per visit for completing each of the following visits: Week 10, Week 12, Week 14 and Week 16 visits, if you decide to continue into the extension phase of the study. You will receive an additional $100 for the End of Study Visit, regardless of whether the end of study occurs at 8 weeks or 16 weeks. If extra visits to the clinic are required to find out if you can be in the study, you will receive $50 for each extra visit. You will receive an additional $10 for completing the survey at the end of the study. This

money is to reimburse you for your time and help cover any costs you may have in being on the study.

What happens if I am injured or hurt because I took part in this study?

If you feel you have been injured or hurt as a result of taking part in the study, it is important that you tell the study doctor immediately. You will get medical treatment if you are injured or hurt as a result of taking part in this study.

The study sponsors will not offer to pay for medical treatment for injury. Your insurance company may not be willing to pay for study-related injury. If you have no insurance coverage, you would be responsible for any costs. Even though you are in a study, you keep all of your legal rights to receive payment for injury caused by medical errors.

Who will see my medical information?

Your privacy is very important to us and we will make every effort to protect it. Your information may be given out if required by law. For example, certain states require doctors to report to health boards if they find a disease like tuberculosis. However, we will do our best to make sure that any information that is released will not be able to identify you. Some of your health information, and/or information about your specimen, from this study will be kept in a central database for research. Your name or contact information will not be put in the database.

There are organizations that may inspect your records. These organizations are required to make sure your information is kept private. Some of these organizations are:

- The study sponsor, the National Cancer Institute (NCI) and NCI agents and partners.
- The authorized representatives of the study Coordinating Center, the University of Arizona Cancer Center.
- The Institutional Review Board, IRB, is a group of people who review the research with the goal of protecting the people who take part in the study.
- The Food and Drug Administration and the National Cancer Institute in the US.
- The National Cancer Institute will obtain information for this clinical trial under data collection authority Title 42 U.S.C. 285.
- Every health care personnel who provides services to you in connection with this study.
- Any laboratories, other individuals/organizations that analyze your health information in connection with this study as defined by protocol.

Where can I get more information?

The National Cancer Institute will obtain information from this clinical trial under data collection authority Title 42 U.S.C. 285.

You may visit the NCI website at http://cancer.gov/ for more information about studies or general information about cancer. You may also call the NCI Cancer Information Service to get the same information at: 1-800-4-CANCER (1-800-422-6237).

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who can answer my questions about this study?

You can talk to the study doctor about any questions or concerns you have about this study or to report side effects or injuries. Contact the study doctor _____ *(insert name of study doctor[s])* at _____ *(insert telephone number).*

THIS SECTION IS ABOUT OPTIONAL STUDIES YOU CAN CHOOSE TO TAKE PART IN.

There may be some specimens (cervical samples, blood, urine) remaining once the study is complete. The researchers would like to store and use your remaining samples for future medical research. The research that may be done is unknown at this time but your samples could help researchers to find new ways to prevent, detect, treat, or cure health problems, including cervical cancer. Some of these studies may be about genes. Genes carry information about features that are found in you and in people who are related to you. Researchers are interested in the way that genes affect how your body responds to treatment. You can take part in the main research study described above without giving your consent for your samples to be stored.

WHAT IS INVOLVED?

If you agree to take part, here is what will happen next:

1) Your remaining samples will be stored at the study institution until the end of the study.
2) After study completion, your remaining samples and some related information may be transferred to a central facility (Biobank) supported by the National Institutes of Health and stored in the Biobank along with samples and information from other people who take part.
3) Qualified researchers can submit a request to use the materials stored. A research committee will review each request. There will also be an ethics review to ensure that the request is necessary and proper. Researchers will not be given your name or any other information that could directly identify you.
4) Neither you nor your study doctor will be notified if/when research is conducted using your samples.
5) Some of your genetic and health information may be placed in central databases that may be public, along with information from many other people. Information that could directly identify you will not be included.

WHAT ARE THE POSSIBLE RISKS?

1) There is a risk that someone could get access to the personal information in your medical records or other information we have stored about you.
2) There is a risk that someone could trace the information in a central database back to you. Even without your name or other identifiers, your genetic information is unique to you. The researchers believe the chance that someone will identify you is very small, but the risk may change in the future as people come up with new ways of tracing information.

   There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

   A new Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:

   - Health insurance companies and group health plans may not request your genetic information that we get from this research.

- Health insurance companies and group health plans may not use your genetic information when making decisions regarding your eligibility or premiums.

Employers with 15 or more employees may not use your genetic information that we get from this research when making a decision to hire, promote, or fire you or when setting the terms of your employment. All health insurance companies and group health plans must follow this law by May 21, 2010. All employers with 15 or more employees must follow this law as of November 21, 2009.

Be aware that this new Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

3) There are laws against the misuse of genetic information, but they may not give full protection. New health information about inherited traits that might affect you or your blood relatives could be found during a study. The researchers believe the chance these things will happen is very small, but cannot promise that they will not occur.

HOW WILL INFORMATION ABOUT ME BE KEPT PRIVATE?

Your privacy is very important to the researchers and they will make every effort to protect it. Here are just a few of the steps they will take:

1) When your sample(s) is sent to the researchers, no information identifying you (such as your name or social security number) will be sent. Samples will be identified by a unique study code only.
2) The list that links the unique code to your name will be kept separate from your sample and health information. Any Biobank and the study team with access to the list must sign an agreement to keep your identity confidential.
3) Researchers to whom the study team sends your sample and information will not know who you are. They must also sign an agreement that they will not try to find out who you are.
4) Information that identifies you will not be given to anyone, unless required by law.
5) If research results are published, your name and other personal information will not be used.

WHAT ARE THE POSSIBLE BENEFITS?

You will not benefit from taking part in the optional studies. The researchers, using the samples from you and others, might make discoveries that could help people in the future.

ARE THERE ANY COSTS OR PAYMENTS?

There are no costs to you or your insurance. If any of the research leads to new tests, drugs, or other commercial products, you will not share in any profits.

WHAT IF I CHANGE MY MIND?

If you decide you no longer want your samples to be used, you can call the study doctor, _____, *(insert name of study doctor for main trial)* at _____ *(insert telephone number of study doctor for main trial)* who will let the researchers know. Then, any sample that remains in the bank will no longer be used. Samples or related information that have already been given to or used by researchers will not be returned.

WHAT IF I HAVE MORE QUESTIONS?

If you have questions about the use of your samples for research, contact the study doctor, _____, *(insert name of study doctor for main trial)* at _____ *(insert telephone number of study doctor for main trial)*.

Please circle your answer to show whether or not you would like to take part in each option.

SAMPLES AND INFORMATION FOR FUTURE RESEARCH STUDIES:

Indicate your choice of "yes" or "no" for each of the following studies.

1. My remaining samples and related information may be kept in a Biobank for use in future health research.
Yes No

2. The information from my tobacco and alcohol use questionnaires may be used in future health research.
Yes No

3. I agree that my study doctor, or their representative, may contact me or my physician to see if I wish to participate in other research in the future.
Yes No

4. My genetic data and health information can be released, with no direct identifiers, into scientific databases.
Yes No

This is the end of the section about optional studies.

My Signature Agreeing to Take Part in the Main Study

I have read this consent form or had it read to me. I have discussed it with the study doctor and my questions have been answered. I will be given a signed copy of this form. I agree to take part in the main study and any additional studies where I circled 'yes'.

Participant's signature _____

Date of signature _____

Signature of person(s) conducting the informed consent discussion

_____

Date of signature _____

APPENDIX A
Performance Status Criteria

Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B
Female Genital Grading Table for use in Microbicide Studies

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENERAL					
Odor	No complaint	Mild-moderate unpleasant odor	Severe unpleasant odor	NA	NA
PAIN AND TENDERNESS (Specify Area: Vulvar/Perineum, Vagina, Cervix (including cervical motion tenderness), Uterus, Adnexae, Pelvic/Lower Abdominal, or Ovulatory) *Note – if both pain and tenderness are present, only report the one with the most severe grade					
Pain* [1]	None	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social & functional activities or the need for narcotic medication	Disabling pain causing inability to perform basic self-care functions OR hospitalization (other than emergency room visit) indicated
Tenderness* [1]	None	Mild tenderness	Moderate tenderness	Severe tenderness	NA
Dyspareunia (pain with sexual activity)	None	Pain causing no or minimal interference with sexual function	Pain causing greater than minimal interference with sexual function	NA	NA
Dysmenorrhea/cramping with menses	None	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities or the need for non-narcotic medication	Pain causing inability to perform usual social or functional activities or the need for narcotic medication	NA

[1] If pain or tenderness is included in the grading of another category (e.g., PID), it should not be graded again in the pain or tenderness category.

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INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENITOURINARY SIGNS/SYMPTOMS – VULVA					
Vulvar/vaginal itching	None	Itching causing no, mild, or moderate interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities; may require intervention such as antihistamine or bathing to provide relief	NA	NA
Vulvar edema	None	Mild, non-pitting edema	Moderate, 1-2+ pitting edema	3+ pitting edema, severe enough to require urinary drainage, or weeping edema ± skin breakdown	NA
Vulvar erythema	None	Erythema covering < 50% of vulvar surface	Erythema covering ≥ 50% of vulvar surface	NA	NA
Vulvar lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules - no treatment indicated	Blisters, ulcerations or pustules, with treatment indicated	Severe epithelial disruption with hospitalization indicated	NA
Vulvar rash	None	Rash covering < 50% of vulvar surface	Rash covering ≥ 50% of vulvar surface	Severe epithelial disruption with hospitalization indicated	NA
Bartholin's or Skene's gland	No findings	Cyst with no inflammation	Cyst or abscess with outpatient intervention indicated	Cyst or abscess with hospitalization indicated	Necrotizing fasciitis from Bartholin's abscess
GENITOURINARY SIGNS/SYMPTOMS – VAGINA **** Note – if vaginal discharge is present both by history and on examination, only report the one with the most severe grade**					
Vaginal edema	None	Mild-moderate engorgement	Loss of ruggae and friability	NA	NA
Vaginal erythema	None	Erythema covering < 50% of vaginal surface	Erythema covering ≥ 50% of vaginal surface	NA	NA

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INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Vaginal dryness	No complaint	Dryness causing no or minimal interference with usual sexual, social, & functional activities	Dryness causing greater than minimal interference with usual sexual, social, & functional activities	NA	NA
Vaginal discharge by participant report **	Participant's usual amount of discharge, regardless of color or quantity	Mild-moderate increase in amount above participant baseline - no sanitary protection required	Profuse increase in discharge requiring pad use or other hygienic intervention	NA	NA
Vaginal discharge as observed by clinician ** (red or brown discharge should be reported under bleeding, not discharge)	Slight amount of discharge, any color	Mild-moderate increase in amount	Significant increase in amount with pooling in vagina on examination	NA	NA
Vaginal abrasions or lacerations (including probable applicator injuries)	None	Superficial disruptions and disruptions extending through the mucosa with minimal impact on life	Large disruptions extending through the mucosa or large superficial disruptions, hospitalization not indicated	Large disruptions extending through the mucosa or large superficial disruptions, hospitalization indicated	Lacerations extending into the peritoneal cavity, bladder, or rectum
Vaginal lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations, or pustules with treatment indicated	Severe epithelial disruption requiring hospitalization	NA
Vaginal and Cervical masses (polyps, myomas, or possible malignancy)	None or normal variants such as Nabothian cyst or Gartner duct cyst	Polyp or myoma or undiagnosed mass without symptoms	Polyp, myoma, or undiagnosed mass causing mild symptoms, e.g., bleeding/pain not requiring more than mild analgesia	Polyp, myoma, or undiagnosed mass causing severe symptoms, e.g., bleeding/pain affecting bladder and bowel function	Visible cervical cancer
GENITOURINARY SIGNS/SYMPTOMS – CERVIX					
Cervical edema and friability	None	Edema without friability	Friable cervix	NA	NA

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Female Genital Grading Table for Use in Microbicide Studies

INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Cervical erythema	None	Erythema covering < 50% of cervix	Erythema covering ≥ 50% of cervix	NA	NA
Cervical discharge	White or clear discharge	Small amount of purulent discharge at os	Purulent discharge extending onto cervix or vagina	NA	NA
Visible cervical lesions (findings seen only by colposcopy should not be included here)	Normal variants including skin tags, moles, scars, etc.	Blisters, ulcerations, or pustules, no treatment indicated	Blisters, ulcerations, or pustules with treatment indicated	NA	NA
GENITOURINARY SIGNS/SYMPTOMS – UTERUS					
Uterine masses/enlargement based on bimanual examination	Normal to 8 week size, no palpable myomas	Enlarged uterus and mild symptoms, e.g., bleeding/pain requiring mild analgesics	Enlarged uterus/myoma with moderate pain or symptoms, e.g., bleeding	Mass causing severe bleeding/pain or with impact on bowel/bladder function	Uterine mass that requires transfusion or surgery
Polyp, submucosal fibroid, or thickened endometrium detected by transvaginal ultrasound (new or increasing in size from prior exam)	None or unchanged/reduced in size from prior exam	New myomas < 6 cm diameter (single or multiple) or diameter increased < 6 cm since prior exam	New myomas ≥ 6 cm diameter (single or multiple) or diameter increased ≥ 6 cm since prior exam	Hospitalization and/or surgery indicated	NA
GENITOURINARY SIGNS/SYMPTOMS – ADNEXA					
Not pregnancy- or infection-related adnexal masses based on bimanual exam (use if no ultrasound done; if ultrasound done, use ultrasound categories below)	None, ≤ 4 cm, normal size ovary	> 4 cm with minimal or no symptoms	> 4 cm with severe symptoms, e.g., pain, but hospitalization not indicated (see footnote #1)	> 4 cm with severe symptoms, e.g., pain and hospitalization indicated (see footnote #1)	NA
Hydrosalpinx based on ultrasound	None	Asymptomatic, suspected hydrosalpinx	Hydrosalpinx with pain, but without evidence of infection or ectopic pregnancy	Signs/symptoms of infection with hospitalization and/or surgery indicated	NA
Adnexal mass based on ultrasound	None	Simple cyst, asymptomatic	Simple cyst, symptomatic	Mass suspicious for malignancy	Malignant mass

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INDIVIDUAL SIGNS/SYMPTOMS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENITOURINARY SIGNS/SYMPTOMS – ABDOMEN					
Abdominal mass not palpable on pelvic exam of unknown diagnosis	None or known (pre-existing) mass unchanged in size	New mass or increased size of known mass requiring mild analgesia with minimal impact	New mass or increased size of known mass with moderate symptoms	Mass causing severe bleeding/ pain with impact on bladder/bowel function or with hospitalization indicated	Malignancy
GENITOURINARY SIGNS/SYMPTOMS – URINARY TRACT					
Urinary frequency	None	Up to 2 times participant's normal frequency	> 2 times participant's normal frequency	NA	NA
Dysuria	None	Superficial only	Deep ± superficial	Inability to void due to pain	NA
Hematuria	None	Microscopic, no intervention indicated (beyond evaluation for infection)	Gross blood in urine or medical intervention/ evaluation indicated (beyond evaluation for infection)	Persistent bleeding with transfusion, hospitalization or intervention indicated to obtain hemostasis (endoscopy, interventional radiology, or operative)	Profuse hemorrhage with shock or orthostatic dizziness

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COMPOSITE SIGNS/SYMPTOMS (Use instead of individual categories if 2 or more signs/symptoms are present)					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD** (Use if all signs/ symptoms would individually be Grade 0 or 1)	**GRADE 2 MODERATE** (Use if one or more signs/symptoms would individually be Grade 2 and all others Grade 0 or 1)	**GRADE 3 SEVERE** (Use if one or more signs/symptoms would individually be Grade 3)	**GRADE 4 POTENTIALLY LIFE-THREATENING**
NO ORGANISM IDENTIFIED BUT INADEQUATE TESTING PERFORMED					
Vulvovaginitis (combinations of pain, itching, erythema, edema, rash, tenderness, or discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
Cervicitis (combinations of dyspareunia, erythema, edema, tenderness, and discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
PID (if Gonorrhea or Chlamydia identified use that category)	None	NA	Cervicitis with mild uterine tenderness, ± mild cervical motion tenderness, no signs of peritoneal irritation	More diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution
NO ORGANISM IDENTIFIED AFTER APPROPRIATE TESTING PERFORMED					
Vulvovaginitis (combinations of pain, itching, erythema, edema, rash, tenderness, or discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
Cervicitis (combinations of dyspareunia, erythema, edema, tenderness, and discharge)	None	Mild signs/ symptoms	Moderate signs/ symptoms	Severe signs/ symptoms	NA
PID (if Gonorrhea or Chlamydia identified use that category)	None	NA	Cervicitis with mild uterine tenderness, ± mild cervical motion tenderness, no signs of peritoneal irritation	More diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution

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INFECTIONS AND DYSPLASIA					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
GENITOURINARY INFECTIONS					
Genital herpes	No lesions	Characteristic ulcerative or vesicular lesions confirmed by culture, PCR, Tzanck prep or other diagnostic test of lesion or previous type-specific serology, covering < 25% of vulva, vagina, or cervix	Same criteria as mild but covering 25-50% of vulvar, vaginal, or cervical surface	Same criteria as mild but covering > 50% of vulvar, vaginal, or cervical surface	Symptoms of significant systemic involvement, e.g., encephalitis, hepatitis
Candida	Absence of symptoms regardless of candida test results	Positive culture, wet mount, or other laboratory test for yeast, with mild symptoms	Positive culture, wet mount, or other laboratory test for yeast, with moderate to severe symptoms	NA	NA
Trichomonas	Negative	NA	Positive wet mount, culture, PCR or other licensed test, excluding pap smear, showing T. vaginalis, regardless of symptoms	NA	NA
Bacterial Vaginosis (BV)	Negative	Asymptomatic BV diagnosed by Amsel criteria, wet mount, Gram stain, or licensed diagnostic test	Symptomatic confirmed by wet mount, Gram stain, or any licensed diagnostic test	NA	NA

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INFECTIONS AND DYSPLASIA					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Chlamydia	Negative	NA	Positive culture or other diagnostic test for Chlamydia, asymptomatic or with mild uterine or cervical motion tenderness (no signs of peritoneal irritation)	Positive test for Chlamydia with abdominal or uterine or adnexal tenderness on examination, with or without adnexal mass, diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution
Gonorrhea	Negative	NA	Positive culture or other diagnostic test for Gonorrhea, asymptomatic or with mild uterine or cervical motion tenderness (no signs of peritoneal irritation)	Positive test for Gonorrhea with abdominal or uterine or adnexal tenderness on examination, with or without adnexal mass, diffuse tenderness, any signs of peritoneal irritation, or indications for hospitalization	Tubo-ovarian abscess or surgery required for resolution or disseminated gonococcal infection
Urinary tract infection (by urinalysis and urine culture)	Negative	5-10 WBC/hpf on urinalysis with a negative culture per protocol definition (with or without symptoms)	> 10 WBC/hpf on urinalysis OR a positive culture per protocol definition (with or without symptoms)	Pyelonephritis	Sepsis (septicemia) due to urinary tract infection

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INFECTIONS AND DYSPLASIA					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Syphilis	Negative treponemal or non-treponemal test or both positive with known treatment and stable titers (< 4 fold increase)	NA	Syphilis diagnosed by a positive treponemal test along with a positive non-treponemal test and no previous treatment or a four-fold rise in titer on the non-treponemal test after previous treatment regardless of symptoms or non-oral lesions positive by darkfield exam for treponemes	Criteria for Grade 2 Syphilis in the presence of neurologic symptoms or a positive CSF VDRL or FTA-ABS	NA
GENITAL DYSPLASIA					
Condyloma (specify site: cervical, vaginal, vulvar, perianal)	None	Condylomata causing no or mild interference with daily function	Condylomata causing moderate interference with daily function	Condylomata causing severe interference with daily function, secondary infection, or hospitalization indicated	NA
Intraepithelial Neoplasia by biopsy (VIN, CIN, VAIN)	None	Intraepithelial Neoplasia 1 (IN1)	Intraepithelial Neoplasia 2 (IN2)	Carcinoma in situ (CIS)	Invasive carcinoma
Pap (use this category only if treatment performed without diagnostic testing, otherwise use biopsy category above)	nl PAP	ASCUS or LSIL	HSIL	Carcinoma in situ or Carcinoma	NA

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UTERINE BLEEDING AND PREGNANCY COMPLICATIONS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
ABNORMAL UTERINE BLEEDING UNRELATED TO PREGNANCY					
Menorrhagia [2] (prolonged and/or heavy menstrual bleeding)	Participant report of normal bleeding relative to her baseline	Increase from usual with no or minimal interference with usual social & functional activities (including sexual functioning)	Increase from usual with moderate interference with usual social & functional activities (including sexual)	Incapacitating or severe interference with usual social & functional activities (including sexual functioning), transfusion indicated	Life threatening hemorrhage with or without shock
Metrorrhagia [2] (intermenstrual or frequent bleeding)	None or any expected nonmenstrual bleeding	Increase from usual with no or minimal interference with usual social & functional activities (including sexual functioning)	Increase from usual with moderate interference with usual social & functional activities (including sexual)	Incapacitating or severe interference with usual social & functional activities (including sexual functioning), transfusion indicated	Life threatening hemorrhage with or without shock
Unexplained infrequent bleeding (excludes expected absence of menses due to hormonal contraception or pregnancy/postpartum)	Participant report of normal or expected bleeding frequency	No menses for 1-3 months (missed menses)	No menses for > 3 months (oligomenorrhea/ amenorrhea)	NA	NA
Postcoital bleeding	None	Occasional (< 25% of coital acts) OR Increase from usual with no or minimal interference with usual social functioning (including sexual functioning)	Frequent (25-75% of coital acts) OR Increase from usual with moderate interference with usual social functioning (including sexual)	Consistent (> 75% of coital acts) OR Incapacitating or severe interference with usual social functioning (including sexual functioning), transfusion indicated	Life threatening hemorrhage with or without shock

[2] If both Menorrhagia and Metrorrhagia are present, a single adverse event should be reported as "Menometrorrhagia" and graded per the Menorrhagia grading scale.

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UTERINE BLEEDING AND PREGNANCY COMPLICATIONS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
COMPLICATIONS OF PREGNANCY					
First trimester bleeding	None	Spotting or bleeding less than menses with continuation of pregnancy	Bleeding like menses or heavier with continuation of pregnancy	Spontaneous abortion, or profuse bleeding with dizziness or orthostatic hypotension, transfusion indicated	Spontaneous abortion with profuse bleeding and/or shock
Postabortal endometritis/salpingitis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, requiring ≤ 3 days of parenteral antibiotics	Severe symptoms requiring > 3 days of IV antibiotics or development of tubo-ovarian abscess	Ruptured TOA or diffuse peritonitis or severe uterine infection for which operative intervention indicated
Postpartum hemorrhage	EBL < 500 cc for vaginal delivery or < 1000 cc after CS or reported as normal	EBL 500-1000 for vaginal delivery or 1000-1500 for CS or reported as slightly increased	EBL > 1000 for vaginal delivery or > 1500 for CS, with or without mild dizziness, no transfusion required	Hemorrhage at a level for which transfusion of 1-2 units of packed cells, but no other blood products indicated	Hemorrhage with shock or coagulopathy, for which transfusion of > 2 units of packed cells or any amount of other blood components is indicated
Postpartum endometritis	None	Low grade fever and uterine tenderness, resolved with oral antibiotics	Moderate symptoms, treated by ≤ 3 days of parenteral antibiotics	Severe symptoms treated with > 3 days of IV antibiotics or addition of heparin	Severe infection or infection for which operative intervention is indicated
Chorioamnionitis	None	Fever (38°C – 38.4°C or 100.4°F – 100.9°F) with two or more: FHR > 160 BPM, maternal HR > 120, uterine tenderness between contractions or purulent AF or preterm labor	Same as Grade 1 plus fever 38.5°C – 40°C or 101°F – 104°F	Criteria for Grade 2 plus fetal distress or fever > 40°C or 104°F	Criteria for Grade 3 plus either fetal demise or maternal symptoms of shock

DIVISION OF AIDS TABLE FOR GRADING THE SEVERITY OF ADULT AND PEDIATRIC ADVERSE EVENTS
PUBLISH DATE: DECEMBER 2004
Addendum 1
Female Genital Grading Table for Use in Microbicide Studies

UTERINE BLEEDING AND PREGNANCY COMPLICATIONS					
PARAMETER	**GRADE 0 NORMAL**	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
Episiotomy infection	None	Mild erythema, edema, and tenderness of wound	Fever > 38°C or 100.4°F with erythema, edema, and tenderness of wound	Fever with wound dehiscence or debridement required	Fever with signs of wound infection and shock or necrotizing fasciitis
Second/third trimester bleeding	None	Bleeding less than menses	Bleeding like menses or greater, but not requiring intervention	Bleeding requiring delivery or other intervention, e.g., transfusion	Bleeding with fetal demise or coagulopathy
Preterm rupture of membranes	None	NA	Preterm rupture with hospitalization but not resulting in delivery at less than 37 weeks' gestation	Delivery at 33-36 weeks' gestation or 1501-2500 grams birth weight	Delivery < 33 weeks' gestation or ≤ 1500 grams birth weight
Preterm contractions	None	Preterm contractions which resolve without medical intervention	Preterm contractions with cervical change which result in medical intervention but not resulting in preterm delivery	Delivery at 33-36 weeks' gestation or 1501-2500 grams birth weight	Delivery < 33 weeks' gestation or ≤ 1500 grams birth weight
Poor fetal growth	At or above 10th percentile	Fetal growth < 10th percentile but ≥ 3rd percentile for gestational age by ultrasound or newborn exam	NA	Fetal growth < 3rd percentile for gestational age by ultrasound or newborn exam	NA

APPENDIX C
TOBACCO ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	**BASELINE**	__ __/__ __/__ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number

Section A. Basic Cigarette Use Information

1. Have you smoked at least 100 cigarettes (5 packs = 100 cigarettes) in your entire life?

☐ Yes
☐ No → **Skip to Section B**
☐ Don't know/Not sure → **Skip to Section B**

2. How old were you when you first smoked a cigarette (even one or two puffs)?

_____ Years old

3. How old were you when you first began smoking cigarettes regularly?

_____ Years old
☐ Check here if you have never smoked cigarettes regularly.

4. How many total years have you smoked (or did you smoke) cigarettes? Do not count any time you may have stayed off cigarettes.

_____ Years (If you smoked less than one year, write "1.")

5. On average when you have smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

6. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to question 8**

7. How soon after you wake up do you smoke your first cigarette?

☐ Within 30 minutes
☐ After 30 minutes

8. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

Section B. Use of Other Forms of Tobacco

9. Have you ever used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No → **Skip to Section C**

10. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

11. Which of the following products have you ever used regularly?
Check all that apply

☐ Cigarettes
☐ E-cigarettes or other electronic nicotine delivery system
☐ Traditional cigars, cigarillos or filtered cigars
☐ Pipes
☐ Waterpipe
☐ Hookah
☐ Clove cigarettes or kreteks
☐ Bidis
☐ Smokeless tobacco, like dip, chew, or snuff
☐ Snus
☐ Paan with tobacco, gutka, zarda, khaini
☐ Other, Please specify:_____

12. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

Section C. Second-Hand Smoke Exposure

13. Are you currently living with a smoker?

- ☐ Yes
- ☐ No

14. In the past 30 days, have you lived in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

15. In the past 30 days, have you worked in a place where other people smoked cigarettes indoors?

- ☐ Yes
- ☐ No

16. Thinking of all your childhood and adult years, have you ever lived in a place where other people smoked cigarettes indoors?

- ☐ Yes In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

17. Thinking of all the years you have worked, have you ever worked in a place where other people smoked cigarettes indoors?

- ☐ Yes → In total, for about how many years?_____ If less than 1, write "1."
- ☐ No

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____

TOBACCO ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. Do you NOW smoke cigarettes?

☐ Everyday
☐ Some days
☐ Not at all → **Skip to Question 3.**
☐ Never smoked → **Skip to Question 4**

2. On average, when you smoked, about how many cigarettes do you (or did you) smoke a day? (A pack usually has 20 cigarettes in it).

_____ Number of cigarettes per day

3. How long has it been since you last smoked a cigarette (even one or two puffs)?

First check which one of the following choices applies to you. Then, if applicable, write a whole number on the line for how many days, weeks, months, or years it has been since your last cigarette.

☐ I smoked a cigarette today (at least one puff)
☐ 1-7 days → Number of days since last cigarette _____
☐ Less than 1 month → Number of weeks since last cigarette _____
☐ Less than 1 year → Number of months since last cigarette _____
☐ More than 1 year → Number of years since last cigarette _____
☐ Don't know/Don't remember

4. Since your last visit, have you used other forms of tobacco, not including cigarettes?

☐ Yes
☐ No (**End)**

5. How often do you/did you use other forms of tobacco?

☐ Every day → Number of times per day _____
☐ Some days → Number of days _____ per ☐ Week ☐ Month ☐ Year

6. Since your last visit, which of the following products have you used? ***Check all that apply***

- ☐ Cigarettes
- ☐ E-cigarettes or other electronic nicotine delivery system
- ☐ Traditional cigars, cigarillos or filtered cigars
- ☐ Pipes
- ☐ Waterpipe
- ☐ Hookah
- ☐ Clove cigarettes or kreteks
- ☐ Bidis
- ☐ Smokeless tobacco, like dip, chew, or snuff
- ☐ Snus
- ☐ Paan with tobacco, gutka, zarda, khaini
- ☐ Other, Specify_____

7. If you do not currently use other forms of tobacco, but did in the past, how long has it been since you last used other forms of tobacco regularly?

- ☐ Within the past month (0 to 1 month ago)
- ☐ Between 1 and 3 months (1 to 3 months ago)
- ☐ Between 3 and 6 months (3 to 6 months ago)
- ☐ Between 6 and 12 months (6 to 12 months ago)
- ☐ Between 1 and 5 years (1 to 5 years ago)
- ☐ Between 5 and 15 years (5 to 15 years ago)
- ☐ More than 15 years ago
- ☐ Don't know/Not sure
- ☐ Never used other forms of tobacco regularly

The following instructions pertain to questions 8 - 10. During each of the following time frames, please indicate whether you smoked cigarettes every day, some days, or not at all.

8. During study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable

9. After the end of study treatment

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure
- ☐ Not applicable (I have not completed the study treatment)

10. Since your last visit to this clinic

- ☐ Smoked every day
- ☐ Smoked some days
- ☐ Did not smoke at all
- ☐ Don't know/not sure

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____

ALCOHOL ASSESSMENT – BASELINE

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE (MM/DD/YYYY)
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:

For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.

When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. In your entire life, have you had at least 12 drinks of any kind of alcoholic beverage?

   ☐ Yes
   ☐ No **(End)**
   ☐ Refused **(End)**
   ☐ Don't know/Not sure

2. In the past 12 months, on average, how often did you drink any type of alcoholic beverage?

   _____ (Enter the number of days you drank based on the timeframe checked below. Enter 0 if you never drank and skip to Question 6.)
   ☐ Week
   ☐ Month
   ☐ Year
   ☐ Refused
   ☐ Don't know/Not sure

3. In the past 12 months, on those days that you drank alcoholic beverages, on average, how many drinks did you have per day?

   _____ (Enter the average number of drinks per day)

   ☐ Refused
   ☐ Don't know/Not sure

4. In the past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverage?

   _____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

   ☐ Refused
   ☐ Don't know/Not sure

5. Was there ever a time or times in your life when you drank 5 or more drinks of any kind of alcoholic beverage almost every day?

   ☐ Yes
   ☐ No
   ☐ Refused
   ☐ Don't know/Not sure

6. If you do not currently drink alcoholic beverages, but did in the past, how long has it been since you last drank regularly?

☐ Within the past month (0 to 1 month ago)
☐ Between 1 and 3 months (1 to 3 months ago)
☐ Between 3 and 6 months (3 to 6 months ago)
☐ Between 6 and 12 months (6 to 12 months ago)
☐ Between 1 and 5 years (1 to 5 years ago)
☐ Between 5 and 15 years (5 to 15 years ago)
☐ More than 15 years ago
☐ Don't know/Not sure
☐ Never drank regularly

7. At the heaviest point, either now or in the past, on the days when you drank, about how many drinks did you drink a day on the average?

_____ (Enter the number of drinks a day)

☐ Refused
☐ Don't know/Not sure

8. How many years have you been drinking (or did drink) regularly?

_____ years

☐ Refused
☐ Don't know/Not sure

9. At what age did you begin drinking regularly?

_____ years of age

☐ Refused
☐ Don't know/Not sure

10. What type(s) of alcohol do you drink? (Mark ALL that apply)

☐ Wine
☐ Liquor
☐ Beer
☐ Wine cooler

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __
(MM/DD/YYYY)

Investigator Name (please print) _____

ALCOHOL ASSESSMENT - FOLLOW-UP

REGISTERING INSTITUTION	PARTICIPANT ID	VISIT TYPE	VISIT DATE *(MM/DD/YYYY)*
_____	_____	_____	__ __/__ __/__ __ __ __

Instructions:
For the following questions about drinking alcoholic beverages, a drink means a 12 oz. beer, a 5 oz. glass of wine, or one and a half ounces of liquor.
When a number is requested in the response, please enter a whole number (i.e. "4") and not a range or fraction of a number.

1. During the past 30 days, did you drink any alcoholic beverages?

☐ Yes
☐ No **(End)**
☐ Refused **(End)**
☐ Don't know/Not sure

2. During the past 30 days, how many days per week or per month did you drink any alcoholic beverages, on the average?

_____ (Enter number of days you drank based on the timeframe checked below. Enter 0 if you did not drink.)

☐ Week
☐ Month
☐ Refused
☐ Don't know/Not sure

3. On the days when you drank, on average, about how many drinks did you have?

_____ (Enter the average number of drinks you had per day.)

☐ Refused
☐ Don't know/Not sure

4. In the past 30 days, on how many days did you have 5 or more drinks per day?

_____ (Enter the number of days you had 5 or more drinks, or enter 0 if none.)

☐ Refused
☐ Do not know/Not sure

Investigator Signature _____ Date __ __ / __ __ / __ __ __ __ *(MM/DD/YYYY)*

Investigator Name (please print) _____

APPENDIX D
Telephone Consent for Participant Survey

Hello, my name is _____ (name of person calling) and I am from the _____ (insert institution name).

I am calling you because you recently participated in Dr. Rahangdale's cervical study at our site and we would like to see if you would be interested in taking a survey about your experiences in that study. This call will take 10-15 minutes. Is this a good time to talk?

If No: Is there a better time to speak to you about this survey? *(Get time and best phone number to reach them). If they aren't interested in being called again, thank them and disconnect phone contact.*

If Yes: *Continue with the script*
We are interested in your thoughts and experiences while participating in the study. The survey will take about 10 minutes to complete. If you agree to take the survey, I can ask you the survey questions now while we're on the phone, or I will mail or email it to you and you can return it to us either by return mail or email. You will receive $10 for completing the survey. Participation in taking this survey is voluntary. You can decide not to complete the survey without any consequences to any on-going care.

Do you have any questions? ☐ Yes ☐ No

Note questions, if any:
_____
_____

Would you be interested in taking the survey?

☐ **No:** *If Patient DOES NOT agree: Thank her for the call and do not proceed further.*

☐ **Yes:** Would you like to take the survey now over the phone or have it mailed/emailed to you?

☐ *If participant would like to take the survey over the phone now you must obtain verbal consent prior to initiating survey:* Please note that this will be documented as your verbal consent to continue with the survey. *Document date and time of verbal consent given before continuing with the survey. Staff member calling must also sign and date this form and proceed with the survey.*

☐ *If participant would rather receive survey in the mail/email:*
Please note that this will be documented as your verbal consent to provide you with the survey.

Document date and time of verbal consent given. Staff member calling must also sign and date this form.

Verbal consent given at _____ am/pm on _____ (date).

Participant's Name: _____

Address/Email address (for mailing/emailing the survey):

_____
_____
_____

_____ _____

Signature of staff member calling Date of call