LCCC 1905: A novel cervical cancer screen-and-treat demonstration project with HPV self-testing and thermocoagulation for women in Lilongwe, Malawi

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Investigator (PI) Name: Lameck Chinula

PI Signature: _____

Date: _____

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1.0 BACKGROUND AND RATIONALE

1.1 Study Synopsis

We propose a prospective study to assess completion and performance of the following novel invasive cervical cancer (ICC) screen-and-treat algorithm among 625 HIV-positive women and 625 HIV-negative women in Lilongwe, Malawi: 1) rapid testing of self-collected vaginal brush for primary high risk (hr)-human papillomavirus (HPV), 2) same-day visual inspection with acetic acid (VIA) for women who are hr-HPV positive, and 3) thermocoagulation for VIA positive/ablation-eligible (by cervical colposcopy) women. This innovative strategy links two new technologies (Xpert HPV and thermocoagulation) with self-collected brush. It has not been rigorously evaluated among women, including HIV-positive women at highest risk for ICC. We hypothesize that >90% of women who are HPV-positive will have VIA performed same-day and that >90% of women who are VIA-positive/ablation-eligible (by colposcopy) will have thermocoagulation performed the same day.

1.2 Background

Invasive cervical cancer (ICC) is the 2nd most common cancer among women living in low-and-middle-income countries (LMICs)[1], where 84% of the 445,000 new cases diagnosed in 2012 occurred.[2] That same year, 270,000 women died from ICC, with >85% of these deaths occurring in LMICs. ICC is preventable with HPV vaccination, cervical cancer screening, and treatment of cervical dysplasia.[3] However, LMICs have relatively poor utilization of these prevention strategies because they are difficult to access and implement, and require trained providers in settings where major shortages of skilled healthcare workers already exist. These challenges are compounded in Sub-Saharan Africa (SSA), the epicenter of the HIV epidemic, since HIV-positive women have >20-fold increased risk of ICC than HIV-negative women.[4] ICC is the most common cancer among women in SSA and Malawi, with the burden disproportionately borne by HIV-positive women.[2,5]

Malawi has the world's highest ICC mortality and is one of the world's poorest countries.[2,6] Malawi's estimated HIV prevalence is 11% among females age 15-49 years, and HPV prevalence among HIV-positive women is estimated at 39%.[7,8] Due to the challenges of implementing an HPV vaccination program, the HPV vaccine is not readily available in public programs in Malawi. Even if widespread HPV vaccination of girls is achieved in Malawi, millions of Malawian females will remain unvaccinated or age ineligible since only girls aged 9-13 years will be vaccination-eligible,[9] and a subsequent decline in ICC incidence will not occur for decades, leaving millions of Malawian women vulnerable to developing ICC.

1.3 Purpose and Rationale

For LMICs, the World Health Organization (WHO) preferentially recommends an ICC screening approach of: 1) hr-HPV testing, 2) triage of hr-HPV positive women by VIA, and 3) cryotherapy treatment for VIA positive/ablation-eligible women (see definition below).[10] Malawi adopted an approach of VIA screening and cryotherapy treatment in 2004, since rapid and inexpensive hr-HPV testing was not yet available.[11] Cryotherapy has been challenging to implement, with <25% VIA centers with established cryotherapy and >50% of women who require treatment not receiving it, largely due to the high cost of refrigerant gas and heavy gas cylinder, which are difficult to obtain and transport.[12] Loss-to-follow-up for VIA-positive women referred to centers with cryotherapy has also been a challenge. A screen-and-treat approach, i.e. same day screening and treatment at the same site, greatly reduces loss-to-follow-up.

Cervical thermocoagulation (aka cold coagulation) is an alternative ablative treatment for high-grade cervical dysplasia, known as cervical intraepithelial neoplasia (CIN) grade 2+ (i.e. CIN2 or more severe), which includes CIN2/3, carcinoma in situ (CIS), the precursors to ICC. It was first introduced in 1966 and used in the United Kingdom in the 1980s.[13–15] Thermocoagulation uses thermosound probes, which are applied serially to destroy cervical lesions, using pre-selected temperatures of up to 120°C. In contrast to electrodiathermy, it produces no smoke, and therefore, no smell. A meta-analysis on the efficacy of thermocoagulation showed that cervical dysplasia cure rates were comparable to other excisional and ablative treatment methods in use worldwide.[16] Despite reports of efficacy against all grades of cervical dysplasia and infrequent minimal side effects, thermocoagulation has been infrequently used since the 1980s because it was replaced by excisional methods that are readily available in high-income countries, and its use was never expanded to LMICs.[16] In the same meta-analysis,[16] none of the studies were from SSA, and all studies evaluated the use of thermocoagulation among physicians, mostly gynecologists, which is impractical for countries such as Malawi, which has less than 20 trained gynecologists for a country of 19 million.[6] However, thermocoagulation is the most implementable treatment modality for CIN2/3 for LMIC, due to ease of use by mid-level providers, and the previously-mentioned challenges faced with cryotherapy.[17] Currently, second-generation Liger Thermocoagulator (Lehi, UT), a handheld and battery-operated thermocoagulation device that can treat around 20–30 women per battery life and which takes 20-40 seconds per treatment making it mobile and attractive for use in LMICs is available. However, there are limited data on the efficacy of thermocoagulation in SSA, where treatment is typically done by mid-level providers (rather than gynecologists), and where HIV prevalence is high. Despite this, SSA countries including Malawi have started adopting thermocoagulation for treatment of cervical dysplasia, due to challenges with cryotherapy, which are limiting availability of treatment for women who screen positive.

The Xpert HPV Assay (Cepheid, Inc., Sunnyvale, CA), is a qualitative real-time polymerase chain reaction assay that uses disposable cartridges to detect DNA from 14 different hr-HPV strains.[18,19] The assay is run on the Cepheid GeneXpert System, which is a scalable instrument platform that can run simultaneous but asynchronous assays for the same or different assays, depending on the cartridge. Unlike previous HPV assays, which required skilled laboratory personnel, batching of multiple samples, and several hours to complete, the Xpert HPV Assay allows for a single sample to be run, with results within 1-2 hours of testing.[18] The Cepheid GeneXpert System can be used for point-of-care testing for HPV, tuberculosis (TB), trichomonas, gonorrhea, chlamydia, and group B streptococcus. Given its multiple uses and rapid turnaround time, the Cepheid GeneXpert machines have recently become widely available in SSA including Malawi, where it is currently used for point-of-care TB testing. Performance of the Xpert HPV Assay in both general screening populations and HIV-positive populations is comparable to other commercially-available HPV assays, with similar sensitivity and specificity, even among self-collected brush.[17-19, 19–21]. Furthermore HPV testing on self- or clinician-collected vaginal samples is more sensitive than VIA for the detection of CIN2+, and ICC.[22] Introduction and scale-up of HPV screening tests is now one priority action in the International Federation of Gynecology and Obstetrics (FIGO) Global Declaration on cervical cancer elimination.[23] Combining the use of Xpert HPV rapid testing and novel strategy of self-collected vaginal specimen for HPV testing could improve women's screening participation as it avoids an initial pelvic exam, allows for same-day HPV results, with similar sensitivity and specificity as provider-collected vaginal specimen for detection of CIN2+,[22,24] and is in line with the FIGO global declaration on cervical cancer elimination.[23]

Other novel screening methods may also allow for same-day screen-and-treat approaches. Recently, HPV DNA testing of urine samples has been investigated as a possible screening modality. Collecting urine samples is less invasive than cervicovaginal samples and urine HPV results seem to be correlated with cervical HPV results and pathology findings.[25,26] There is limited experience with Xpert HPV testing of urine samples.[27,28] Beyond DNA testing, methylation levels of several human genes and HPV genotypes have been found to be strongly associated with the development of CIN2/3 or ICC.[29] Therefore, measuring methylation levels has potential use as both a primary screening method as well as a triage method to improve the specificity and positive predictive value (PPV) for CIN2+ detection in HPV-positive women.[29] The S5 DNA methylation classifier combines testing for methylation of target regions of the human tumor suppressor gene EPB41L3 and the viral late gene regions of oncogenic HPV types 16, 18, 31, and 33.[30] To date, the S5 has been validated as a triage method for HPV-positive women among largely HIV-negative women, with promising findings.[29-32] The S5

classifier has not been validated with urine samples, which as discussed are less invasive to collect.

Mobile ODT EVA system developed by Mobile Optical Detection Technologies combines the functionalities of a colposcopy and digital camera. The EVA system is used for visual examinations and documentation in multiple clinical settings including examination of the cervix. The EVA system allows the storage of information captured by the device in an online secured, and Health Insurance Portability and Accountability Act (HIPAA) (HIPPAA)-compliant cloud storage which is hosted by Amazon web services. The EVA system is encrypted upon set-up, and a PIN number or password is used to access the phone. The system provides a platform for external quality assurance for findings identified during visual examination of the cervix.[33]

We therefore plan to evaluate a novel screen-and-treat algorithm among women, including HIV positive women, in Lilongwe, Malawi for the detection and treatment of high-grade cervical dysplasia with the following combination of strategies: 1) rapid testing of self-collected vaginal brush for hr-HPV, 2) same-day VIA for women who are hr-HPV positive, and 3) thermocoagulation for VIA-positive/ablation-eligible (by colposcopy with Mobile ODT EVA system) women. This innovative approach has not yet been rigorously evaluated among women, including those living with HIV. Therefore, this study will provide essential data to inform national policy in Malawi and other LMICs where both HIV and ICC prevalence are high. If successful, our approach is arguably the most broadly-scalable strategy to optimize ICC screening in countries where the proportion of women screened remains low and ICC prevalence is high.

2.0 STUDY OBJECTIVES AND ENDPOINTS

2.1 Primary Objectives

2.1.1. To assess completion of a novel ICC screen-and-treat strategy among women in Lilongwe, Malawi, using self-collected vaginal brush for hr-HPV testing, followed by same-day VIA and thermocoagulation for HPV-positive/VIA-positive/ablation-eligible (by colposcopy) women.
2.1.2. To determine the 24-week efficacy of thermocoagulation among women, including HIV-positive women, with CIN2/3.

2.2 Secondary Objectives

2.2.1. To evaluate performance of the proposed ICC screen-and-treat strategy among women, including HIV-positive women, by estimating overtreatment for women who are HPV-positive/VIA-positive/ablation-eligible, and undertreatment among HPV-positive/VIA-negative women.

2.2.2. To explore women's experiences with the proposed ICC screen-and-treat strategy.

2.3 Exploratory Objectives

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

The study population will consist of female participants who meet the following eligibility criteria for the study:

3.1.1 Females ≥ 25 years of age at study entry and ≤ 50 years of age (per current Malawi National Cervical Cancer Control Program guidelines for screening[11]).

3.1.2 Ability and willingness of participant to provide written informed consent.

A total of 625 of the study participants will be HIV-positive and must meet the additional criterion:

3.1.3 Females with known HIV-positive status, verified as follows:

3.1.3.1. Rapid HIV test (e.g. Unigold Recombigen, Oraquick, Alere Determine™ Combo)

And confirmed by one of the following:

3.1.3.2. Second antibody test by a method other than the initial rapid HIV and/or enzyme/chemiluminescent immunoassay (e.g. Unigold Recombigen, Oraquick, Oraquick, Alere Determine™ Combo) or

3.1.3.3. Plasma HIV-1 RNA viral load (Abbott RealTime HIV-1) or

3.1.3.4. Bio-Rad Geenius

3.2 Exclusion Criteria

All female participants meeting any of the following exclusion criteria at baseline will be excluded from study participation:

3.2.1. Current or prior history of cervical, vaginal or vulvar cancer or dysplasia.

3.2.2. Current symptomatic sexual transmitted infection requiring treatment (women will be allowed to be in the study upon successful treatment)

2.2.3. Prior HPV vaccination.

3.2.4. Participants with known allergy to acetic acid.

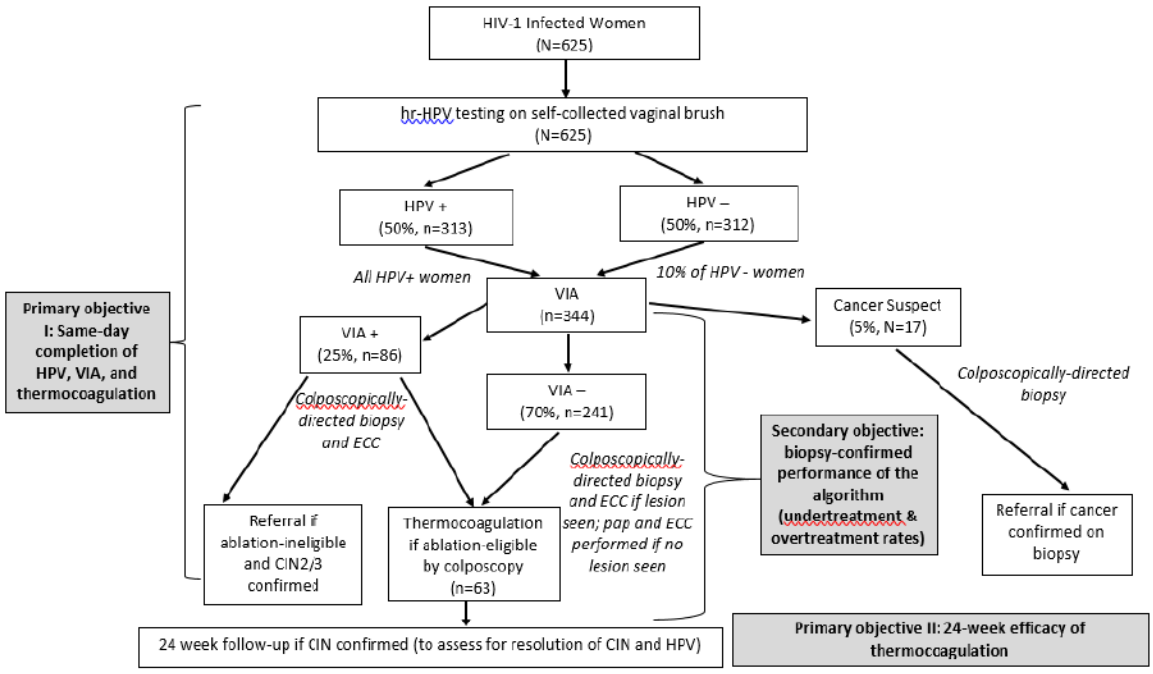
3.2.5. Participants with a history of total hysterectomy.

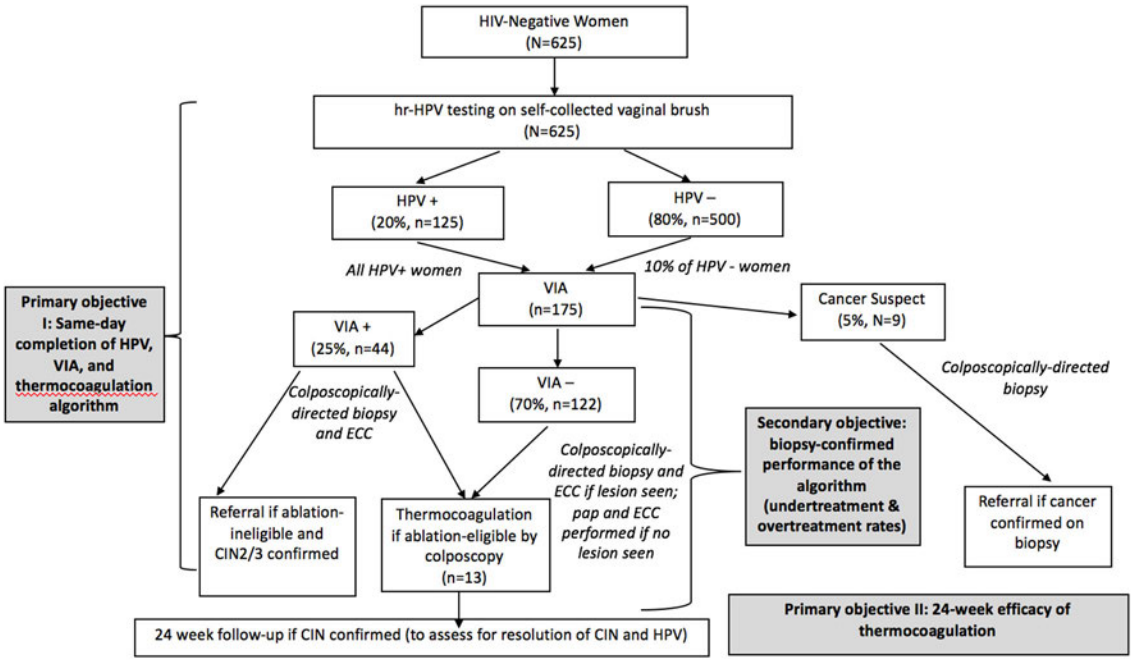
3.2.6. Participants who are pregnant or plan on becoming pregnant during the study period.

3.2.7. Participants who are less than 12 weeks postpartum.
3.2.8. Participants with other illnesses that would limit compliance with study requirements or in the opinion of the investigator or designee, have a problem that would make participation in the study unsafe, or complicate interpretation of study findings.

4.0 STUDY PLAN

4.1 Schema





NB: In these figures, N=625 is the only number that is fixed. The rest of the numbers are assumptions based on very limited data where available.

This is a single arm, prospective study of 1,250 women, comprising of 625 HIV-positive and 625 HIV-negative women, in Lilongwe, Malawi. The primary purpose of this study is to assess completion and performance of a novel ICC screen-and-treat strategy among women in Lilongwe, Malawi, using self-collected vaginal brush for hr-HPV testing, followed by same-day VIA and thermocoagulation for HPV-positive/VIA-positive/ablation-eligible (by colposcopy) women. We anticipate that all participants will be enrolled over 12 months and that active participation will continue for up to 30 weeks at most.

4.2 Duration of Study

Study participants will have four scheduled study visits: Screening/Enrollment, Week 4, Week 24, and Week 28. At Screening/Enrollment, women will complete an interviewer-administered baseline questionnaire, urine pregnancy testing, HIV rapid finger stick testing, and blood for CD4+ T cell count and HIV-1 RNA testing for HIV positive participants, and will self-collect a vaginal brush for hr-HPV testing. Urine collected for pregnancy testing will also be sent for hr-HPV and S5 methylation testing (for research purposes only). HPV-positive women and every 10th consecutive HPV-negative woman will complete same-day VIA with colposcopically-directed cervical biopsy and endocervical curettage (ECC) (if lesion seen), and thermocoagulation if ablation-eligible by colposcopy. If no lesion is seen on colposcopy, the woman will have a cervical pap smear and ECC collected, and no thermocoagulation will be performed. Finally, women who are suspicious for cancer at VIA will undergo colposcopically-directed cervical

biopsies. All women who undergo speculum exam will undergo provider-collected cervical swab, which will be sent for hr-HPV and S5 methylation testing (for research purposes only).

All women who had cervical biopsy or cervical pap smear and ECC done at Screening/Enrollment will return at Week 4 for histology and/or cytology results. All women who had CIN1, CIN2, or CIN3 and underwent thermocoagulation will be asked to return for a follow-up visit at Week 24 post-treatment to assess for resolution of their HPV and cervical dysplasia. At Week 28 visit, women will receive their histology and/or cytology results and be referred as necessary.

4.3 Study Details

Study site

The study will be conducted at UNC Project-Malawi Tidziwe Centre.

Recruitment

We will recruit women from Family Planning clinics, Under-5 Clinics, Outpatient Departments, and ART clinics (for HIV-positive participants) at facilities in Lilongwe by conducting educational talks about cervical cancer and the study in their waiting areas. We will schedule a screening and enrollment visit for potential participants interested in joining the study at UNC Project-Malawi, which is within a 15-30 minute drive from the targeted clinics. There, women will be asked to provide written informed consent and screened for eligibility to participate in the study. Up to thirty women will be recruited for in-depth interviews (IDIs) about their experiences with the proposed screen-and-treat strategy. The HPV-negative women will be recruited at the Screening/Enrollment visit while the HPV-positive women may be recruited at the Screening/Enrollment visit or the Week 4 visit. Transport reimbursement will be provided to participants attending study visits to mitigate participants' expenses for the study-related visits.

Screening & Enrollment

Women will be enrolled in Lilongwe, Malawi, a city with a population of 900,000.[34] Participants will complete an interviewer-administered baseline questionnaire to collect socio-demographic data and cervical cancer risk factors. Baseline evaluation will include urine pregnancy testing, HIV rapid finger stick testing, and blood for CD4+ T cell count and HIV-1 RNA testing for HIV-positive women. Urine samples collected for pregnancy testing will also undergo Xpert HPV testing and S5 methylation testing. These urine tests are only for research purposes and results will not influence patient management.

Women will receive simple instructions on how to self-collect a cervicovaginal sample with vaginal brush for hr-HPV testing in the research clinic, using well-validated, field-tested illustrated instructions that are comprehensible to low-literacy women.[35,36] The self-collection kit will contain a vaginal brush and vial of

sample preservation solution. Participants will then collect this brush, which will be tested onsite using Xpert HPV testing. HPV-negative women will be advised to continue with routine ICC screening per Malawi Cervical Cancer Guidelines.[11] HPV-positive women will complete same-day VIA, using 3-5% acetic acid on the cervix for at least 1 minute, then observing for color changes indicating the presence of a lesion, as described in the WHO IARC Manual.[37] We will also perform same-day VIA on every 10th consecutive woman found to be HPV-negative to assess for possible underscreening with self-HPV testing.

All women who undergo speculum exam will have a provider-collected swab performed, which will be sent for hr-HPV and S5 methylation testing. The results from these tests will be used to help evaluate the validity of urine hr-HPV and S5 testing; they will not be used to influence patient management.

For all women who undergo VIA, we will determine if a woman is VIA-positive and ablation-eligible using colposcopy with Mobile ODT EVA system. Women who are VIA-positive will undergo colposcopically-directed biopsies of the acetowhite lesion, with at least 2 and up to 4 biopsies taken, as well as an ECC. If the woman is still considered to be ablation-eligible by colposcopy, she will then undergo thermocoagulation. Women who are VIA-negative will also undergo colposcopy, and at least 2 and up to 4 cervical biopsies and ECC if a lesion is seen at colposcopy. Thermocoagulation will also be performed on these women if they are found to have an ablation-eligible lesion on colposcopy. If no lesion is seen on colposcopy, the woman will have cervical pap smear and ECC collected, and no thermocoagulation will be performed. Women who are suspicious for cancer at VIA will undergo colposcopically-directed cervical biopsies. Whenever biopsy is indicated, we will perform at least 2 cervical biopsies on women because previous studies have shown that cervical biopsy is most sensitive when at least 2 biopsies are taken.[33] During colposcopy, images of the cervix may be captured with the EVA system. These images will be stored only in password-protected and de-identified study databases, linked only to the study participant PID. The images will be used for internal quality assurance and may also be shared with the sponsor or other parties for research purposes.

For women to undergo thermocoagulation, we will consider them to be ablation-eligible if the following criteria are met at colposcopy: 1) cervical lesions are not suspicious for ICC; 2) cervical lesions are located entirely on the ectocervix; 3) cervical lesions are no more than 2-3 mm into the endocervical canal; 4) the entire squamocolumnar junction (SCJ) can be visualized; and 5) all visible lesions are deemed appropriate for thermocoagulation by the treating provider.[10] Women with suspected endocervical dysplasia, CIS, or ICC will be referred for treatment at the Kamuzu Central Hospital (KCH), a public tertiary and teaching hospital Colposcopy Clinic on which campus UNC Project-Malawi is situated.

For the IDIs, up to 30 women will be enrolled at either the Screening/Enrollment visit or Week 4 visit (if they are HPV-positive). HPV-negative women will only be enrolled if they were not selected to undergo VIA. Participants will be consented for the IDIs separately from their main consent for the study. Women will primarily be recruited at check-out and all interviews will be conducted after women have completed the screen-and-treat algorithm. The IDIs will be conducted in the local language, Chichewa, in private rooms at UNC Project Malawi. Women will be offered the choice to have the interview conducted off site (e.g., in the comfort of their home or some private off site place, if they prefer). The interview will include use of an interviewer-administered questionnaire evaluating women's rating of their privacy, embarrassment, discomfort, pain, and confidence during the screening/treatment process. The IDIs will cover prior knowledge of cervical cancer, experience with the self-sampling, understanding of the results, and intention to share their screening experience with others.

Week 4

All women who had cervical biopsies or cervical pap smear, and ECC done at Screening/Enrollment will return for histology and/or cytology results at Week 4. HPV-positive/VIA-negative women without CIN or ICC on histology and those with normal cytology will be advised to continue with routine ICC screening per guidelines. Women who underwent thermocoagulation will be evaluated for interval medical history or post-treatment adverse events during this visit. HPV-positive/VIA-negative women with CIN2+ on ECC or abnormal cytology on cervical pap smear will be referred to KCH Colposcopy Clinic for further management.

HPV-positive/VIA-positive women who had CIN detected at baseline cervical biopsy, and underwent thermocoagulation will be followed up at Week 24 post-treatment. Women who had CIN and visible cervical lesion that was not ablation-eligible by colposcopy or had ICC will be referred to KCH Colposcopy clinic for further management.

Women who are HPV-positive may be enrolled in and consented for IDIs at their Week 4 visit. These interviews will be conducted similarly to those done at the Screening/Enrollment visit but they will also cover women's experiences sharing their screening results with others and abstaining from sex for the recommended interval after biopsy/thermocoagulation.

Week 24

All women treated by thermocoagulation and had CIN1+ detected by baseline cervical biopsy will return at Week 24 for colposcopy to assess for resolution of cervical dysplasia after thermocoagulation. HPV testing on a provider-collected vaginal brush will also be performed at Week 24 to assess for HPV resolution after thermocoagulation. If the colposcopy shows no visible lesions at this Week 24 visit,

cervical pap smear and ECC will be collected. If a lesion is observed during colposcopy, at least 2 but not more than 4 directed biopsies and ECC will be performed. Images may be captured via the EVA System. These images will be stored only in password-protected and de-identified study databases, linked only to the study participant PID. The images will be used for internal quality assurance and possibly shared in de-identified form for future research. All women who still have CIN2/3 or have developed CIS or ICC at Week 24 will be referred to KCH for additional management. Women will also be evaluated for changes in their interval medical history and post-treatment adverse events during this visit. Finally, all women who were referred to KCH Colposcopy Clinic will be followed up at this visit to ensure that they received the care they required.

Week 28
All women who had cervical biopsy or cervical pap smear, and ECC done at Week 24 will be asked to return at this visit for histology and/or cytology results and any necessary follow-up referrals to KCH Colposcopy Clinic.

Quality Assurance
Dr. Siobhan O'Connor, a pathologist at UNC-Chapel Hill, will re-review all biopsy specimens obtained during the study as a quality assurance measure. She will hold calls as needed with pathology staff at UNC Project-Malawi to discuss discrepancies between their specimen reviews.

Sample storage
Participants' study-associated samples will be stored in the research laboratory at UNC Project-Malawi for future research on HPV infection and other biomarkers of cervical cancer to improve way to screen women for cervical cancer for a maximum period of 15 years. Samples will be stored de-identified using only study identification number with no personal information.

4.4 Expected Risks

The following are possible risks due to participant enrollment into our study:

4.4.1 Sample collection: Risks to study participant include the physical risks from blood draw, vaginal and cervical sample collection, cervical biopsies, and ECC. Participants can experience pain, bleeding, and sometimes infection, and damage to tissue at the site of the sample collection. However, all these sample collections are done as part of routine clinical evaluations when indicated in women of reproductive age and are considered to be minimal risks.

4.4.2 Treatment with thermocoagulation: Risks include pain, infection, and damage to tissue at the site of treatment application, i.e., the cervix. These risks are considered minimal. Thermocoagulation is not associated with future fertility compromise or adverse pregnancy outcomes in future pregnancies.

Thermocoagulation is used for treating VIA-positive women in some cervical cancer screening programs including Malawi.

4.4.3 Psychological risks might arise if a participant's peer or partner finds out about her study participation and does not approve of it. In addition, participants might also have some embarrassment or discomfort providing urine samples; or answering sensitive questions during the survey, but they can opt not to answer such questions. Women who participate in the IDIs may have further risk of embarrassment or discomfort about answering sensitive questions, but they can opt not to answer and they will be assured that doing so will not affect their ability to remain in the study or receive necessary health care.

To address these risks, informed consent forms will be administered in Chichewa, the local national language that participants and study staff are fluent in, and only participants who demonstrate comprehension through a standard comprehension checklist will be enrolled in the study after providing written informed consent. During the informed consent process, participants will be counseled about the potential risks, benefits and side effects of study procedures. Only study staff trained in the Biomedical Research and Good Clinical Practices will be involved in the study. Study procedures, risks, and benefits will be discussed with study participants, and study staff will answer all questions prior to obtaining consent. Each woman will be fully informed regarding the reasonably foreseeable impact of the research on her circumstances.

Physical risks will be minimized by having qualified and experienced staff conducting study procedures. Sterile techniques will be applied to minimize risks of infection. The Study Coordinator/Physician and the PI, will also be available to manage any complications that may arise from study procedures. Psychological risks will be minimized by encouraging potential participants to disclose their study participation to their partners and by minimizing physical tracing of participants unless it is unavoidable, in which case we will only be using information that we received permission from the participants to use, particularly when there are safety concerns. Prior to the interviewer-administered questionnaire, participants will be instructed that they can choose not to answer any questions they are not comfortable with. We will also provide appropriate counseling for HIV, sexually transmitted infections, and urine pregnancy testing to minimize any risks associated with these. Referral for further services will also be used where necessary.

In addition, the confidentiality of all study records will be safeguarded to the extent legally possible. All laboratory specimens, reports, study data, and data collection forms will be identified by a participant ID number only to maintain participant confidentiality. All databases will be secured with password-protected access systems, and computer entries will be identified by participant ID only. Consents, the participant ID log link, and any other data forms that link participant ID

numbers to other identifying information will be stored in a separate, locked cabinet. The IDIs will be audio-recorded after obtaining informed consent from the participant for the interviews to be recorded. The recordings will be uploaded to a password-protected computer, and Data Associates will transcribe and translate the recording into a Microsoft Word document, which will also be password-protected. Any digital cervical images captured during colposcopy will be stored only in password-protected and de-identified study databases, linked only to the study participant PID. As mentioned above, these images may be shared with sponsors or partners for future research into cervical lesion diagnostics.

All data analysis will be done on datasets which have only the participant ID as a unique identifier. Clinical information with individual identifiers will not be released without the written permission of the participant. We expect these procedures to adequately protect participant confidentiality. However, it is possible that a participant's study participation, HIV or cancer screening status could become known to people in the community and may result in stigma or discrimination. Should that occur, study staff will work with the participant and his/her family as appropriate to resolve the situation in whatever manner is preferred. All study procedures carry minimal risks.

Data collection forms, electronic databases, and printed data will only be supplied to appropriate study staff on an as-needed basis. Any publication about this research study will omit names and any other personally identifiable information. However, individually identifiable private information may be reviewed by the following groups to monitor the conduct of the study and participant safety: The Ministry of Health of Malawi, the Malawi National Health Sciences Research Committee (NHSRC), and the UNC IRB.

4.5 Participant Retention

Once a participant is enrolled, study staff will make every effort to retain the participant for the protocol-specified duration of follow-up thereby minimizing potential biases associated with loss to follow-up. Only participants who demonstrate understanding through the comprehension assessment conducted during the informed consent process, including what will be expected of them during study participation, will be enrolled in the study.

To minimize loss-to-follow-up, all participants who require follow-up visits will be provided with an appointment card with a date of their next visit and the contact numbers of our study staff. We will continuously counsel participants about the importance of adhering to their scheduled visits in the study. To facilitate adherence, we will also provide transport reimbursement (in accordance with NHSRC requirements) and refreshments in the waiting area at the study clinic. During their waiting time at the research clinic, we will also provide some educational materials and entertainment through the television that is in the

corridors of our research clinics. Over the years, we have noted that this minimizes boredom among participants while waiting for their study visit and results.

We plan to contact participants by phone or physical tracing a few days before their scheduled follow-up visits to remind them of their visits. We will use a standardized locator form to record essential participant tracing information (with participant permission), including phone numbers for the participant and family members/friends, landmarks close to the participant's home (churches, schools, shops, etc.), drawn maps of the participant's home and village, the name of the village chief and other contacts, and GPS coordinates (if a participant is traced and found to be at home). For missed scheduled follow up visit, a study community nurse or educator will make every effort to bring back the participant for the follow up visit except where participant withdraws consent or where it is not feasible due to unplanned relocation. The study community nurse or educator will contact participant within 1-3 days of missing their visit, through phone or physical tracing to find out the reason for missing the visits and offer re-scheduling of the visits. We will keep track of our retention rates on a weekly basis and discuss any retention issues experienced during the conduct of the study during the study team's weekly meeting.

4.6 Removal of Patients from Protocol

Regardless of the participant retention procedures referenced above, if a participant indicates that she no longer wishes to participate in the study, she will be allowed to withdraw from the study without losing any of her access to standard care. Participants may also be withdrawn from the study by the investigator or designee under the following circumstances:

- Participant re-locates away from the study site or is otherwise determined to be lost-to-follow-up after exhausting tracing efforts
- Investigator or designee determines that continued participation in the study would be unsafe or otherwise not in the best interest of the participant
- The study is stopped or canceled by the sponsors, government or regulatory authorities, or site IRBs/ECs

For any participant who is withdrawn from the study prior to scheduled completion of follow-up, study staff will document the reason for the withdrawal in detail and will make every effort to complete final evaluations as is allowable by the participant at the time. In the event that the circumstances that led to a participant's withdrawal change while the study is still underway (e.g., she returns to the study site area after having re-located previously), the site investigator or designee might consider options for resumption of follow-up.

5.0 TIME AND EVENTS TABLE

5.1 Schedule of study procedures

Study Visit	**Screening & Enrollment**	**Week 4**	**Week 24**	**Week 28**
Women who will attend the study visit	**All women**	**Women who had cervical biopsy or cervical pap smear, and ECC done at Screening & Enrollment**	**Women who had CIN1+ at Screening & Enrollment and underwent thermocoagulation**	**Women who attended the Week 24 Visit**
Evaluations				
Informed consent	X			
Baseline survey	X			
Urine pregnancy testing	X	X	X	
HIV-1 Rapid Test (2 kits)	X			
HIV-1 Confirmatory Test[1]	X			
CD4+ T cell count[2]	X			
HIV-1 RNA (plasma)[2]	X			
Self-collected vaginal brush	X			
hr-HPV testing of self-collected samples	X		X	
hr-HPV and S5 methylation testing of urine sample	X			
Provider-collected cervical brush	X[3]		X	
hr-HPV testing of provider-collected cervical sample	X[3]			
S5 methylation testing of provider-collected cervical sample	X[3]			
VIA[3]	X			
Colposcopy and cervical biopsy or cervical pap smear, and ECC[4]	X		X	
Thermocoagulation[5]	X			
Thermocoagulation-related adverse events evaluation		X		
Provide results and any referrals		X		X
Follow-up questionnaire[6]		X		
Enroll for IDIs	X	X		

[1]For those with discordant HIV rapid tests.
[2]For HIV-positive participants only
[3]For all hr-HPV+ and for every 10th woman who is hr-HPV-.
[4]Cervical biopsy and ECC if lesion seen at colposcopy; Cervical pap smear and ECC if no lesion seen at colposcopy
[5]For women with ablation-eligible cervical lesion at colposcopy.
[6]For follow-up of cervical histology and/or cytology results from Screening/Enrollment and to assess for any change in history or adverse events from thermocoagulation (if thermocoagulation was performed).

6.0 STATISTICAL CONSIDERATIONS

6.1 Study Design

This is a single arm, prospective study of 1,250 women (625 HIV-positive and 625 HIV-negative) recruited from outpatient clinics in Lilongwe, Malawi. The primary objectives of this study are to assess completion of a novel ICC screen-and-treat strategy among women, including HIV-positive women, in Lilongwe, Malawi, using self-collected vaginal brush for hr-HPV testing, followed by same-day VIA and thermocoagulation for HPV-positive/VIA-positive/ablation-eligible (by colposcopy) women, and to determine the 24-week efficacy of thermocoagulation among women with CIN2/3. The secondary objectives will be to evaluate the performance of the ICC screen-and-treat strategy by estimating overtreatment for women who are HPV-positive/VIA-positive/ablation-eligible, and undertreatment among HPV-positive/VIA-negative women, and to explore women's experiences with the proposed screen-and-treat algorithm.

6.2 Sample Size and Accrual

We anticipate enrolling 625 women over approximately 260 days. This enrollment target assumes a screening to enrollment ratio of 10 to 9, with 2-3 women enrolled/day, which is very feasible and allows adequate time for informed consenting, same-day screening with hr-HPV testing, triage with VIA, and thermocoagulation for VIA positive, ablation-eligible (by colposcopy) women, and also sufficient time for scheduled follow-up visits for already-enrolled women. Based on prior studies done among HIV-positive women in SSA, we assume that at least 50% (n=313) of the 625 HIV-positive women will be positive for hr-HPV[4] and that at least 20% of these 313 women will have CIN2/3,[39,40] which gives us at least 63 HIV-positive women with CIN2/3 in our cohort. Among HIV-negative women, we assume that at least 20% (n=125) of the 625 HIV-negative women will be positive for hr-HPV[41,42] and that at least 10% of these women will have CIN2/3,[42] which gives us at least 13 HIV-negative women with CIN2/3 in our cohort.

We assume that 5% of the 76 women with CIN 2/3 will be lost-to-follow-up after 24 weeks (95% is the typical rate of retention for studies done at UNC Project-Malawi at 6-month follow-up). Therefore, we will be able to determine the 24-week efficacy of thermocoagulation among approximately 72 women, including 60 HIV-positive women, with CIN2/3. This sample size of 72 CIN2/3 women, 60 of whom are HIV-positive, is comparable with other studies included in a meta-analysis that evaluated the efficacy of thermocoagulation for treating CIN2/3.[16] Assuming treatment efficacy of 90% over 24-weeks follow-up, we should have >95% confidence to measure the efficacy within ± 8% for the overall sample and the HIV-positive sub-group.

For the IDIs, up to 30 women will be recruited at Screening/Enrolment Visit or Week 4 Visit. The IDIs will explore participants' experiences with the proposed ICC screen-and-treat strategy among women. We expect to reach thematic saturation with this sample size, based on prior work showing that few new themes are established after the first 18 interviews.[43] We expect significant overlap in themes between the HPV-positive and HPV-negative groups.

6.3 Data Analysis Plans

Primary objective I: To assess completion of a novel ICC screen-and-treat strategy among women in Lilongwe, Malawi, using self-collected vaginal brush for hr-HPV testing, followed by same-day VIA and cervical thermocoagulation for HPV-positive/VIA-positive/ablation-eligible (by colposcopy) women.

Data analysis for primary objective I: We will calculate the following proportions with corresponding 95% Confidence Intervals (CIs) for these two primary outcomes: a) the proportion of women who are HPV positive who have VIA performed same-day, and b) the proportion of women who are VIA positive/ablation-eligible (by colposcopy) who have thermocoagulation performed the same-day. We hypothesize that >90% of women who are HPV-positive will have VIA performed same-day and that >90% of women who are VIA-positive/ablation-eligible (by colposcopy) will have thermocoagulation performed the same day.

We will also systematically monitor and analyze indicators for the completion of this novel ICC screen-and-treat strategy, as follows:

Number (and %) of women who received self-collection kit and who returned a self-sample
Number (and %) of HPV positive, negative, insufficient, and missing results from self-collection
Number (and %) of women who receive their HPV results the same day as collection
Number (and %) of HPV-positive women invited for VIA
Completion point 1: Number (and %) of women with HPV-positive results who receive VIA same-day

Number (and %) of HPV-positive women who receive VIA on another day (instead of same-day)
Number (and %) of HPV-positive women who never receive VIA (loss to follow-up)
Number (and %) of HPV-positive women with VIA positive results
Number (and %) of HPV-positive women with VIA-positive results who are eligible for thermocoagulation by colposcopy; not eligible for thermocoagulation but LEEP eligible; or referred for suspected cancer
Completion point 2: Number (and %) of women with VIA positive results and are ablation-eligible by colposcopy who receive thermocoagulation same-day
Number (and %) of women with VIA positive results and are ablation-eligible who receive thermocoagulation on a following day
Number (and %) of HPV-positive women with cervical biopsy or cervical pap smear, and ECC performed, and N (%) with histology and/or cytology read
Number (and %) of CIN2+, CIN1, or normal at Screening/Enrollment
Number (and %) of treated women who return for Week 24 follow up visit.
Number (and %) of treated women who return for Week 24 follow up visit and are found to have resolved versus persistent hr-HPV
Number (and %) of treated women who return for Week 24 follow up visiting that are found to have resolved CIN1
Number (and %) of treated women who return for Week 24 follow up visit that are found to have resolved CIN2+
Number (and %) of treated women who return for Week 24 follow up visit that are found to have persistent CIN1+
Number (and %) of treated women who return for Week 24 follow up visit that are found to have persistent CIN2+
Number (and %) of treated women who return for Week 24 follow up visit that are found to have resolved CIN1
Number (and %) of women referred for further management who have received appropriate management by Week 24

We will also calculate the median time needed to complete the proposed HPV testing, VIA triage, and treatment cascade by recording the times for each of the following steps.

1) Time that the participant received the self-HPV collection brush
2) Time that the participant returned the self-HPV collection brush
3) Time that the HPV test was initiated in the Xpert machine
4) Time that the HPV test was completed in the Xpert machine
5) Time that the participant received her HPV result
6) Time that the participant had her VIA completed (if applicable)
7) Time that the participant had her colposcopy completed (if applicable)
8) Time that the participant had cervical biopsies or cervical cytology, and ECC completed (if applicable)
9) Time that the participant had her thermocoagulation completed (if applicable)

Logistic regression models will be used to assess factors associated with completing the proposed testing and treatment cascade for both HPV-positive and HPV-negative women, such as HIV status, length of time on ART, travel time to

attend in-clinic appointments, knowledge of someone with any cancer, HIV-1 RNA load, and CD4+ T cell count. This objective is largely descriptive and, thus, formal power calculations are not made.

Primary objective II: To determine the 24-week efficacy of thermocoagulation among women, including HIV-positive women, with CIN2/3. Women who undergo thermocoagulation will have follow-up at 24-weeks post-treatment for colposcopy, provider collected cervicovaginal brush for hr-HPV testing, ECC, colposcopy-directed cervical biopsy and ECC (if lesion noted) or cervical cytology and ECC (if no lesion noted at colposcopy). We hypothesize that >90% with CIN2/3 on pre-treatment biopsy will have ≤CIN1 at their 24-week biopsy.

Data analysis for primary objective II: For efficacy of thermocoagulation, we will compare our 24-week cure rates for CIN 2/3 with thermocoagulation to historic cure rates for CIN 2/3 with cryotherapy (85-95%),[44] the recommended ablative treatment modality for CIN 2/3 for resource limited countries.[10] We will perform exploratory analysis to determine the following:
a) 24-week efficacy for CIN1, by calculating the proportion of women who had CIN1 at Screening/Enrollment and then were found to have no cervical dysplasia at Weeks 24 after thermocoagulation treatment;
b) hr-HPV clearance in HPV-positive women treated with thermocoagulation, by calculating the proportion with no hr-HPV at Weeks 24; and
c) Risk factors for CIN2+ among HPV-positive women (i.e., age, age at first sexual intercourse, number of sexual partners, HIV status, length of time on ART, HIV-1 RNA load, CD4+ count) via multivariable logistic regression.

With an expected 76 CIN2/3 lesions among our participants and 90% hr-HPV positivity at time of treatment, we estimate that 68/76 women who undergo thermocoagulation at Screening/Enrollment will be hr-HPV positive. Accounting for 5% loss to follow up by week 24, we will have a sample of 65 women who were hr-HPV positive at time of treatment present for repeat hr-HPV testing. With these 65 women, we will have 99.9% power to detect a difference between hr-HPV positivity pre- and post-treatment, assuming 100% hr-HPV positivity pre- and 35% hr-HPV positivity at 24 weeks post-treatment. We assume that 35% will still be hr-HPV-positive based on a recent systematic review, finding that 35% of women were still hr-HPV-positive after cryotherapy; no such estimates are available for thermocoagulation.[45] In addition, no estimates are currently-available for the 24-week for CIN1 as thermocoagulation has not been used to treat low-grade dysplasia. Hr-HPV results at the Week 24 visit for non-CIN-2/3 cases at enrollment will be investigated in exploratory analyses, stratified by baseline CIN1 and normal/cervicitis status.

Secondary objective I: To evaluate performance of ICC screen-and-treat strategy among women, including HIV-positive women, by estimating overtreatment for

women who are HPV positive/VIA positive/ablation-eligible, and undertreatment among HPV positive/VIA negative women. All HPV positive women and every 10th consecutive HPV-negative woman will undergo VIA. During VIA, we will determine if a woman is VIA-positive and ablation-eligible. Women who are VIA-positive will undergo colposcopically-directed cervical biopsies and endocervical currettings (ECC). If the woman is still considered to be ablation-eligible by colposcopy, she will then undergo thermocoagulation. Women who are VIA-negative will also undergo colposcopy, but they will only have cervical biopsies and ECC performed if a lesion is seen on colposcopy. Following VIA, we will collect directed cervical biopsies and ECC from VIA positive women prior to thermocoagulation treatment if ablation-eligible by colposcopy, or cervical pap smear and ECC from VIA negative women if no lesion is seen at colposcopy. If a lesion is seen at colposcopy, VIA negative will also undergo colposcopically-directed cervical biopsy and ECC. We hypothesize that <25% of HPV positive/VIA positive women would have been overtreated (no CIN2+, but treated) and that <30% of HPV positive/VIA negative women would have been undertreated (CIN2+, but untreated).

Data analysis for secondary objective I: Overtreatment will be calculated as the proportion of HPV positive/VIA positive/ablation-eligible women who would have been treated with thermocoagulation based on their HPV and VIA findings alone (without colposcopy), but were found to have no CIN2+ on colposcopically-directed cervical biopsy or ECC at Screening/Enrollment. This proportion will represent the women with no lesions or low-grade lesions (CIN1) who would have been unnecessarily treated if we based the decision to perform thermocoagulation on HPV and VIA testing alone.

Undertreatment will be calculated as the proportion of women with CIN2+ at Screening/Enrollment who were not treated or referred via our screening algorithm due to a negative HPV and/or VIA result. This proportion will represent the women with CIN-2+ who were missed by our HPV-and-VIA-based algorithm. For undertreatment, we will also evaluate the proportion of women who underwent thermocoagulation but were ECC positive for endocervical CIN2+, and therefore needed to be referred for further management since thermocoagulation does not adequately treat endocervical dysplasia. We have based our hypotheses on a recent review, which found that the pooled estimates for VIA sensitivity and specificity were 0.69 (95% CI 0.54-0.81) and 0.87 (95% CI 0.79-0.92), respectively,[46] as well as from the high false positive rate for VIA found in our pilot study with nurses and clinicians in Malawi. These performance measures, however, were for primary screening, rather than as referral to treatment among HPV positive women.

In addition, to assess the potential for undertreatment of HPV-positive women, we will conduct VIA at Screening/Enrollment for every 10th women who is hr-HPV

negative. This sub-set analysis of HPV negative women undergoing VIA will allow us to control for potential verification bias. As all women will not be referred for verification by colposcopy, using only the histology results on HPV positive women to estimate disease burden could lead to biased inference. To correct for this verification bias, we will utilize the colposcopy results among the random sub-set of HPV-negative women to employ the maximum likelihood method proposed by Zhou.[47] Estimates using this method are valid provided that the histology data are missing at random. This assumption means that for women with the same test results, those who were referred to colposcopy were similar to those that were not. Corrected CIN2+ burden will be estimated using maximum likelihood estimators (MLEs). This secondary objective is largely descriptive and, thus, formal power calculations are not made.

Secondary objective II: To explore women's experiences with the proposed ICC screen-and-treat strategy. Up to 30 women will undergo IDIs in their local language. They will first complete a questionnaire asking them to rate their privacy, embarrassment, discomfort, pain, and confidence during the screening/treatment process. Then, they will participate in in-depth interviews exploring their prior knowledge of cervical cancer, experience with the self-sampling, understanding of the results, and their intention to share screening experience with others. For women who are interviewed at Week 4, they will additionally be asked about their experience following the recommendation to abstain from sex for a specified interval.

Data analysis for secondary objective II: All IDIs will be recorded, and then the recorded interviews will be transcribed and translated into English. The translated interviews and any notes taken during the IDIs will then be analyzed for themes, subthemes, and codes. Modifications may be made to the interview guide throughout the qualitative study as new subthemes and ideas are uncovered which require further investigation.

Exploratory objective I: [redacted]

(b) [redacted]

Exploratory objective II: [redacted]

[redacted]

6.4 Data Management/Audit

During all study visits, the Study Nurse and Data Associate will be doing real-time data entry into the study database via tablets, which will use Open Data Kit (ODK) software, an open-source mobile data collection tool used by many studies at UNC Project. The Study QA/QC Officer will also clean data in real-time by reviewing all forms uploaded to the database within 24 hours of submission to the database. Once a month, Study QA/QC Officer will perform data cleaning by running statistical code in Stata (College Station, TX) to ensure that there are not any aberrations in the data that require further clarification and/or changes to the study forms or database.

Study records will be accessible only to study staff. The records will be held at UNC Project, Tidziwe Center at KCH, Lilongwe, Malawi and kept in lockable cabinets. Data collected will be entered in a database that will be on a password-protected computer accessible to data officers and the study principal investigator (PI). The link between personal identity and unique identifier (PID) will be kept separate from study records and will not be included in the analytic database.

As an investigator initiated study, this trial will also be audited by the UNC Lineberger Cancer Center audit committee every six or twelve months, depending on the participation of affiliate sites.

7.0 STUDY MANAGEMENT

7.1 Institutional Review Board (IRB) Approval and Consent

Prior to study initiation, the PI will ensure review and approval of this protocol and the informed consent form as required, by the Malawi NHSRC, which is responsible for oversight of research conducted in Malawi, and the UNC IRB, in accordance with 45 CFR 46. Before beginning the study, approval will also be sought from local authorities and the Lilongwe District Health Office. Participants and study staff members will take part in a thorough informed consent process. The study staff will make efforts to minimize risks to participants.

Subsequent to initial review and approval, IRBs/ECs must review the study at least annually. The PI will provide annual progress reports as required to the IRB/EC. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, any changes in the research activities, and all unanticipated problems involving risks to human subjects.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form will include all the relevant elements in accordance with 45 CFR 46 and any local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a patient's participation in the trial, the written informed consent form will be signed and personally dated by the patient and by the person who conducted the informed consent discussion. The informed consent process will also address the following:

- The purpose of the study
- The potential psychological and social harms associated with study participation (and what to do if such harms are experienced)
- The possible benefits to taking part in this study
- The right to withdraw or not answer some questions in the study at any time

7.2 Required Documentation

Before the study can be initiated at any site, the following documentation will be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- CVs and medical licensure for the principal investigator and any co-investigators who will be involved in the study

- A copy of the IRB-approved consent form

7.3 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

7.3.1 Emergency Modifications

UNC investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC IRB approval or respective institution's IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, a UNC IRB modification form will be completed by UNC Research Personnel within five (5) business days of making the change.

7.3.2 Single Patient/Subject Exceptions

Eligibility single subject exceptions are not permitted for Lineberger Comprehensive Cancer Center Investigator Initiated Trials under any circumstances. Other types of single subject exceptions may be allowed if proper regulatory review has been completed in accordance with Lineberger Comprehensive Cancer Center's Single Subject Exceptions Policy.

7.3.3 Adverse Event and Protocol Deviations/Evaluations Reporting

7.3.3.1. Definitions:

a) Adverse event (AE) - any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). Each AE will be assessed for relatedness, severity and attribution to the study intervention or participation. The CTEP Version 5.0 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Version 5.0 of the CTCAE is identified and located on the CTEP website at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

b) Serious Adverse Events (SAEs) - any event temporally associated with the subject's participation in research that meets any of the following criteria:

- Results in death
- Is life-threatening at the time of the event (places the subject at immediate risk of death from the event as it occurred)
- Requires inpatient hospitalization or prolongation of existing hospitalization

- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect or
- Any other AE that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of outcomes listed above.

c) ***Unexpected AEs*** – any AE occurring in one or more subjects participating in a research protocol, the nature, severity, or frequency of which is not consistent with either:

- The known or foreseeable risk of AEs associated with the procedures involved in the research that are described in:
  - the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent, and
  - the expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event.

d) ***Related to the research*** – an incident, experience, or outcome that is likely to have resulted from participation in the research study.

- Possibly Related to the research – the reasonable possibility that the AE, incident, experience, or outcome may have been associated with the procedures involved in the research. Reasonable possibility means that the event is more likely than not related to participation in the research or, in other words, there is a >50% likelihood that the event is related to the research procedures.

e) ***Unanticipated problems involving risk to subjects or others (UPIRSO)*** – any incident, experience, or outcome that is:

- Unexpected (in terms of nature, severity, or frequency) given:
  - the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and the characteristics of the participant population being studied;
- Related or possibly related to a subject's participation in the research; and
- Serious or suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

f) ***Protocol Deviation*** – According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants

- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, a variance from the approved study protocol. Examples include study visits or procedures conducted outside of the visit window, incomplete study visits as a result of error by the study staff, and logistical or administrative problems. Protocol deviations must be documented.

g) Protocol Violations: Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report UPIRSO.

7.3.3.2. Procedures for Reporting Incidents

1. All study staff are responsible for reporting known or suspected adverse events (AE), protocol deviations, or UPIRSO immediately (the same day of the incident or discovery of the incident) to the Study Coordinator.

2. If an incident involves harm or injury to a person, the next step is to eliminate immediate hazard to the subject(s) or others. Immediate corrections do not require IRB approval prior to initiation, but should be described in the Incident Report.

3. Routine reporting of AE: all AE will be source documented. The following will be reported on a case report form:

   a) All genital AE regardless of grade and severity,
   b) All AEs with grade ≥ 3.

4. The Study Coordinator must determine if the incident requires prompt reporting to the IRBs. If so, the Study Coordinator must report the incident immediately (the same day of the incident or discovery of the incident) to the PI so that he can notify the IRBs within 7 calendar days of notification.

5. The following incidents require prompt reporting to the IRBs:

a. AEs that are:
   - Unexpected,
   - Related or Possibly Related to participation in the research and are grade $\geq$ 3, and
   - Serious or suggest that there are new or increased risk(s) to subjects.

b. Interim analysis, data and safety monitoring report, findings from other studies, findings from animal or in-vitro testing, or other finding(s) that indicate: (1) there are new or increased risks to subjects or others, or (2) subjects are less likely to receive any direct benefits from the research.

c. Protocol deviations that harmed subject(s) or others or placed subject(s) or others at increased risk of harm.

d. Protocol deviations that are made to eliminate an immediate hazard to a subject without IRB approval.

e. Intentional or unintentional failure to follow applicable federal human subject protection regulations, the requirements or determinations of the IRB, the IRB-approved study protocol, or University policies when that failure adversely affects the rights or welfare of participants, such as:
   - Conducting human subjects research without an IRB-approved protocol or exemption
   - Starting research prior to meeting the conditions required by the IRB and receiving an IRB notification of approval, or conducting research during a lapse in approval
   - Failure to obtain informed consent
   - Deviating from the informed consent or recruitment process approved by the IRB
   - Failure to provide a participant with new information about study risks or procedures that may affect the participant's willingness to continue/participate in the study (i.e., by not reconsenting participants or by using an old version of a consent document to consent a new participant)
   - Initiating changes to the protocol without IRB approval, including using unapproved materials (e.g., fact or information sheets, recruitment materials, questionnaires, focus group guides, scripts, or other materials provided to participants)
   - Failure to complete IRB- or institutionally-required human subjects protection training prior to engaging in human subjects research

- Enrollment of participants beyond what has been approved by the IRB in a study that is greater than minimal risk

f. Breach or potential breach of subject confidentiality

g. Complaint by or on behalf of a research subject that:

- indicates that the rights, welfare, or safety of the subject has been adversely affected, or
- cannot be resolved by the investigator. Subject complaints about payment should be resolved by the study team.

h. Allegation of noncompliance

i. Institution-, investigator-, or sponsor-initiated hold or early closure as a result of safety concerns.

6. All other incidents do not require prompt reporting to the IRBs and will be submitted as needed to the IRBs during the annual continuing review.

7. All incidents should be promptly recorded in the appropriate log by the Study Coordinator, who will then send the updated log to PI for review.

8. For all Protocol Deviations, UPIRSO and reportable AEs, the Study Coordinator will also send the PI an updated Log or Unanticipated Problem Report about the incident, which should contain the following, in accordance with UNC IRB guidelines:

a) Detailed information about the event of issue, including relevant dates. The report should identify the affected subjects by their PIDs and not by their names or other personal identifiers.

b) An assessment of whether any subjects or others were placed as risk or suffered any harm (e.g., physical, social, financial, legal, or psychological) as a result of the incident.

c) If the events involve noncompliance, describe the root cause analysis, which should answer the following questions:

i. What was the error?
ii. How did the error occur?
iii. How widespread is the error?
iv. Why did the error occur? Keep asking why until you identify the root cause.

d) Any corrective and preventive action (CAPA), planned or already taken, which should include:

i. Action type (corrective or preventive)
ii. Action description
iii. Personnel who are responsible for the implementation of the actions.
iv. Date(s) on which the action(s) were taken or are planned.
v. Plan for effectiveness check
vi. Outcome of effectiveness check
vii. If applicable, amendments to the CAPA

e) If the report cannot be completed in its entirety within the required time period, the report should describe what information is still needed and when the investigator anticipates that a follow-up report will be submitted. A follow-up report will then need to be submitted to the IRBs once all information is available.

9. The Study Coordinator and PI will discuss each reportable incident and assess whether the protocol or study procedures need to be modified. If they decide that the protocol needs modification, the PI or designee coordinates the submission of the protocol amendment.

7.4 Data Safety Monitoring

a) Overview
Given that the trial will involve use of Liger Thermocoagulator that was designed and is used for treating cervical dysplasia, except that that there is limited data of its effectiveness in HIV-positive women, and collecting cervical biopsy and ECC which are routine gynecologic procedures done when clinically indicated, no formal Data Safety Monitoring Board (DMSB) will be established. However, we will adhere to the Data Safety Monitoring Plan (DSMP) as outlined below:

The Principal Investigator (PI), Dr. Lameck Chinula, will provide oversight of all recruitment and study procedures and quality assurance checks will be conducted as specified under the direction of a 7-member DSMP Committee composed of the study's Co-Investigators and Other Significant Contributors: Dr. Jennifer Tang, Dr. Jennifer Smith, Dr. Maganizo Chagomerana, Dr. Tamiwe Tamoka, Dr. Benjamin Chi, and Dr. Siobhan O'Connor. The Committee will meet twice per year and additionally as needed, either in person or via conference call. The committee will discuss the project, review its progress, review study modifications, and monitor compliance with IRB rules and human subjects' procedures, as well as unanticipated problems and adverse events. Thus, there will be ongoing review of the study's protocol and its implementation and biannual reviews performed by the full DSMP steering committee. In addition, oversight of data safety and monitoring will be achieved through written reports presented annually to the two independent and non-overlapping UNC IRB at UNC and the Malawi National Health Science Research Committee (NHSRC).

The PI will maintain a high degree of oversight regarding data management, participant safety, and project integrity. The PI will work closely with the research staff to monitor participants' safety and minimize the risks to participants. In addition, study procedures are designed to maximize the confidentiality and integrity of participants' data.

7.5 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. When an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required, the amended consent form, will be sent to both UNC's IRB and Malawi NHSRC for review and approval prior to implementation.

7.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. Study documents will be kept on file until three years after the completion and final study report of this investigational study.

7.7 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study patients. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case

Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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9.0 APPENDICES

9.1 Informed Consent Form: LCCC 1905: A Novel Cervical Cancer Screen-and-Treat Demonstration Project with HPV Self-testing and Thermocoagulation for Women in Malawi Version 4.0 dated 06 Feb 2020 (English and Chichewa versions).

9.1 Stored Specimens Informed Consent Form Version 4.0 dated 06 Feb 2020 (English and Chichewa versions)

9.2 In-Depth Interview Informed Consent Form Version ~~5.0~~ dated 18 Dec 2020 (English and Chichewa versions)

9.3 In-Depth Interview Guide Version 1.0 dated 06 Sept 2019 (English and Chichewa versions)

9.4 In-Depth Interview Questionnaire Version 1.0 dated 06 Sept 2019 (English and Chichewa versions)