Intravaginal artesunate pessaries for treatment of cervical intraepithelial neoplasia 2/3 among HIV-positive and HIV-negative women in Kenya: Study protocol for a pilot trial

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Abstract

Background: Cervical cancer disproportionately affects women in low- and middle-income countries (LMICs), which bear 90% of deaths. Current precancer treatments rely on healthcare workers who may be out of reach for many women. Development of a patient-controlled cervical precancer treatment can significantly improve access in remote areas and promote secondary prevention of cervical cancer.

Methods: This is a phase I trial among 18 HIV-positive and HIV-negative women in Kenya, investigating use of artesunate vaginal pessaries as treatment for cervical precancer among women screening positive for cervical precancer who need excisional treatment. The primary objective will be the safety of self-administered artesunate pessaries. Participants will self-administer 200mg of artesunate vaginally daily for 5 days, followed by a drug-free week, repeated for a total of 4 cycles (artesunate self-administration on weeks 1, 3, 5, 7). The total study duration, including participant follow-up is 48 weeks. Safety and adherence will be assessed through review of symptom diaries and biweekly follow-ups during the treatment phase. Data analysis will include quantitative and qualitative methods. Figure 1 illustrates the study schema.

Discussion: Considering the challenges associated with excisional treatments for cervical precancer in LMICs where access to care is limited, this study proposes an alternative approach using intravaginal Artesunate. This clinical trial will provide important safety and efficacy data on using artesunate as a topical therapy for both HIV-positive and HIV-negative women.

Trial Registration: ClinicalTrials.gov identifier: NCT06165614

Keywords: Artesunate pessaries; Cervical precancer treatment; Human Immunodeficiency Virus (HIV); Cervical Intraepithelial Neoplasia (CIN); Low- and middle-income countries; Human papillomavirus (HPV)

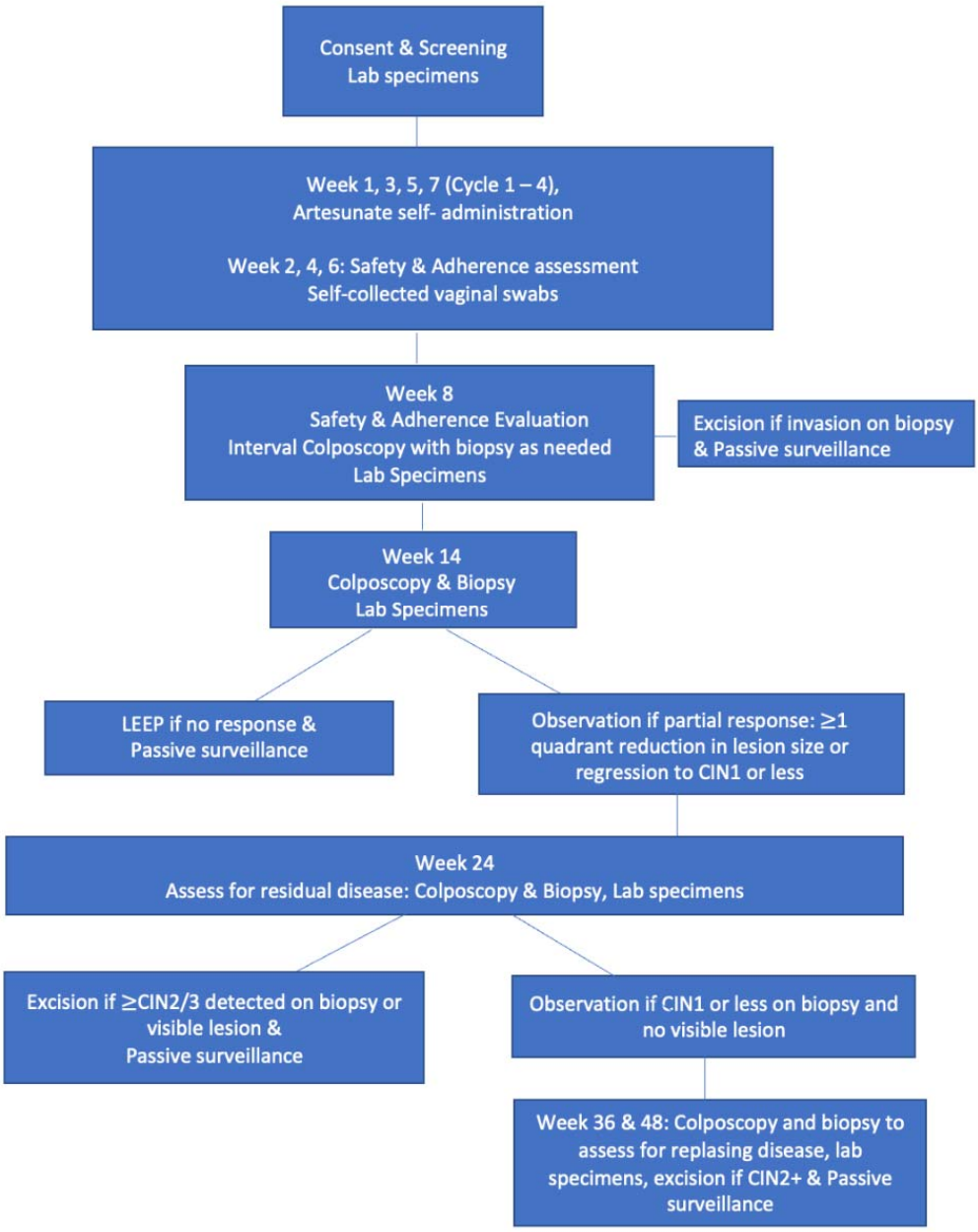

Figure 1. Study schema

Introduction

Although cervical cancer, defined as cancer occurring in cells of the cervix - the lower part of the uterus - is preventable, low- and middle-income countries (LMICs) bear a disproportionate burden, accounting for 85% of an estimated 570,000 incident cases, and 90% of deaths annually.[1] In Kenya, cervical cancer accounts for 5,240 (12.9%) of new cancer cases annually and 3,286 (11.4%) of all cancer deaths annually.[2] Cervical cancer is caused by infection with human papillomavirus (HPV), and primary prevention is obtained through vaccination against the HPV virus. Secondary prevention of cervical cancer is achieved by regular screening, during which time the cervical precancer lesion – cervical intraepithelial neoplasia – can be detected and treated to prevent progression to cervical cancer. Women living with HIV (WLWH), the majority of whom live in LMICs, have increased incidence and persistence of human papillomavirus (HPV) infection,[3] and a six to eightfold increased risk of developing invasive cancer compared to HIV-negative women.[4,5] Cervical precancer is categorized into low grade disease – cervical intraepithelial neoplasia grade 1 (CIN1) – and high-grade disease, cervical intraepithelial neoplasia grade 2 and 3.[6] Both cervical precancer and cancer diagnoses are established through pathology evaluation of cells from the cervix obtained through a biopsy.

The standard of care in Kenya for treatment of cervical precancer, recommended by the World Health Organization, involves ablation using cryotherapy or thermal ablation, or excision, using the loop electrosurgical excision procedure (LEEP) or cold knife cone (CKC).[7] In LMICs including Kenya, ablative treatments are performed by nurses in lower level facilities as they do not require anesthesia or technical expertise, including the ability to control bleeding.[2] However, women who do not meet criteria for ablation, which includes large lesions covering more than 75% of the cervix, are referred for excisional treatment. Excisional treatment is often only

available in tertiary facilities staffed by doctors and consultants, and access to these facilities in LMICs is challenging due to distance and costs associated with referrals, especially for women living in rural regions. In a 2018 study from rural Western Kenya, with a sample of 100 women with abnormal lesions referred to a gynecologist in a tertiary facility, 64% were ultimately lost to follow-up,[8] highlighting significant challenges in the referral process. If women make it to tertiary centers, delays are common due to limited long wait times. In one study from Kenyatta National Hospital, a referral hospital in Kenya, the median time from an abnormal cervical cancer screening result to excisional treatment was 167 days.[9] These delays increase the risk of progression to cervical cancer where curative options are limited.

Unlike ablative treatment, excisional treatment, which involves surgical removal of the diseased part of the cervix, is a surgical procedure which requires local anesthesia and the ability to control bleeding. Complications related to excisional treatments include increased risk of infection and pain associated with the procedure. For women of childbearing age, a significant long term complication of excisional treatments involves future obstetric risks, including premature delivery, premature rupture of membranes, low birth weight, and admission of the newborn to the intensive care unit.[10,11] In comparison to women who undergo no excision, those with a history of loop excision face a 1.56 increased risk of premature delivery, while those with a cold knife cone have 2.70 increased risk of premature delivery.[12] This risk is particularly consequential in LMICs including Kenya where access to neonatal intensive care units – where premature infants are cared for, is severely limited – as a result, many infants born prematurely do not survive.[13] In the context of HIV infection, women develop cervical precancer lesions earlier in age, often before childbearing, increasing their obstetric risks following an excisional procedure.[14] Additionally, women with HIV face up to 18.5% (95% CI 11.6-28.8) rate of high-

grade cervical precancer (CIN2/3) recurrence at 12-months following excision.[3] This recurrence often requires repeat excision, and in a systematic review and meta-analysis which included 65,082 women, the risk of premature birth was increased as a result of this.[11]

To mitigate the complications associated with excisional treatments for HPV-associated precancer, scientists are investigating the use of non-excisional treatments including topical therapies with cytotoxic properties. Recent evidence, primary from studies in high-income countries (HICs), demonstrate that topical therapies – which can both be self- or provider-administered - may be utilized as treatment of HPV-associated anogenital lesions including cervical precancer.[15–20]

Intravaginal Artesunate for Cervical Precancer Treatment

A potential topical therapy for which early studies are available is Artesunate, a semi-synthetic derivative of artemisinin. This World Health Organization (WHO)-approved drug is widely used to treat malaria in LMICs, and there is growing evidence demonstrating cytotoxic effects against numerous cancer cell lines both *in vitro* and *in vivo*.[21,22,23,24] Its proposed mechanisms of action include suppressing cell proliferation by inducing G1 and G2/M phase cell cycle arrest in the human breast, nasopharyngeal, and renal cell cancer. It also modulates key inflammatory pathways characteristic of uncontrolled proliferation and carcinogenesis.[25,26] Ferroptosis – a type of iron-dependent cell death – is thought to be a key anticancer mechanism relevant to HPV-infected cells.[27] Cancer cells are highly proliferative, requiring a heavy iron load which acts as a cofactor in synthesizing deoxyriboses before cell division.[28] Development of both high-grade cervical intraepithelial neoplasia (CIN2/3), the precursor lesion of cervical cancer, and cervical cancer are associated with the expression of two viral proteins in the HPV lifecycle, E6

and E7.[29] Epithelial cells that express either or both of these oncoproteins also overexpress the transferrin receptor, and have been shown to have increased levels of intracellular iron compared with normal cells.[27] This observation has been exploited to investigate whether preinvasive cervical cancer (CIN2/3), can be treated with Artesunate, which contains an endoperoxide bridge that reacts with intracellular ferrous iron to generate free radicals, capable of inducing direct oxidative damage resulting in cell death.[26] Figure 2 illustrates a conceptual framework detailing the proposed mechanism of action of artesunate on HPV-infected cells, specifically targeting E6 or E7 oncoproteins. In vivo studies have shown Artesunate's ability to induce cytotoxicity and apoptosis in HeLA cervical cancer cells.[30] Additionally, preclinical studies in oral mucosa of dogs challenged with canine oral papillomavirus demonstrated that dihydroartemisinin (DHA), the bioactive form of Artesunate, inhibits tumor growth with little to no effect on normal epithelium.[30]

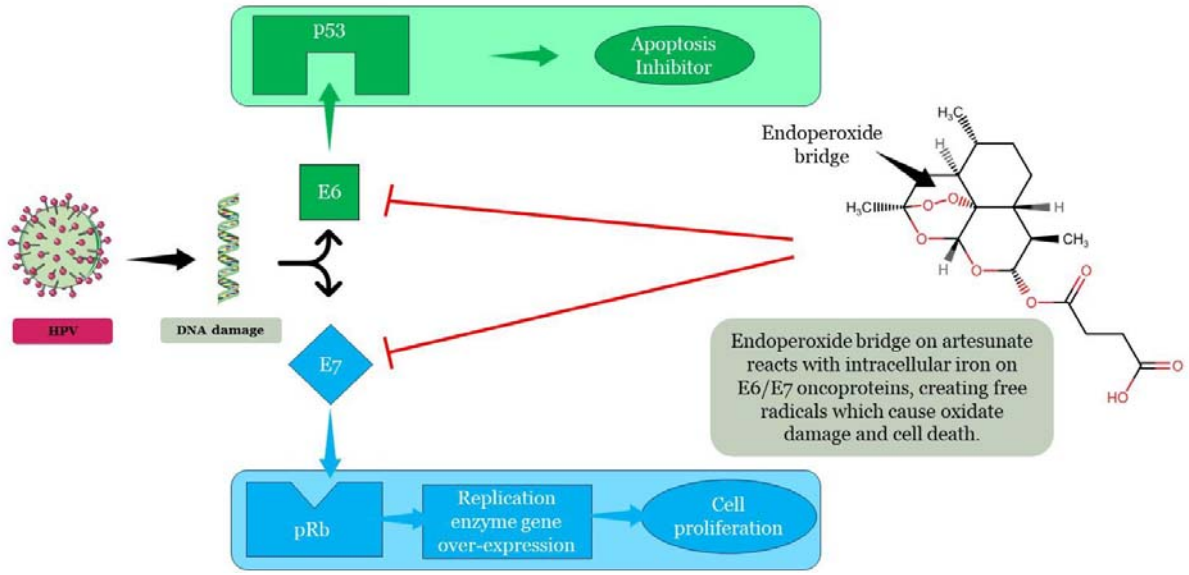

Figure 2. Conceptual framework of artesunate action on HPV-infected cells, specifically targeting E6 & E7 oncoproteins. Image adapted from Bedell et al (2020).

These findings, coupled with Artesunate's favorable safety profile, led to a 2020 proof-of-concept study of intravaginal artesunate suppositories for treatment of CIN2/3 among U.S. HIV-negative women prior to planned standard-of-care excision.[24] In this Phase I trial dose-escalation study involving 28 women with biopsy-confirmed CIN2/3, the self-administration of three five-day cycles of intravaginal 200 mg artesunate pessaries (vaginal inserts) proved to be safe and well-tolerated. Results from this study included the histologic regression of CIN2/3 among 67.9% of participants in an intention-to-treat analysis, with 47.4% experiencing clearance of HPV genotypes detected at baseline.[24] Participants who received three treatment cycles (12.9 weeks) experienced a shorter mean time to CIN2/3 regression compared to those who received one cycle (20.4 weeks). The CIN2/3 regression rate of 67.9% is clinically relevant compared to the 28% spontaneous regression rate observed in a similar group of women with CIN2/3 undergoing close observation for 15 weeks prior to a planned standard of care resection.[24] Furthermore, in this 'first-in-human' study of intravaginal Artesunate for CIN2/3 treatment, treatment was safe, well tolerated, and all reported adverse events (AEs) were grade I or II and self-limited. Reported AEs among participants who used three five-day artesunate cycles included chills and flu-like symptoms (n=3, grade 1), vaginal (yeast) infection (n=1, grade II), dizziness or headache (n=2, grade 1), non-infective cystitis (n=1, grade 2), vaginal pain or uterine cramping (n=9, grade I), vaginal discharge (n=4, grade 1), vaginal pruritis (n=9, grade 1). In summary, 37 drug-related AEs were observed in this Phase I trial, of which 34 (92%) were grade I, and 3 (8%) were grade 2. No grade 3 or 4 AEs were reported, and there were no intolerable side effects that resulted in study withdrawal. Other studies in high-income countries have shown that use of four treatment cycles improves treatment efficacy, particularly in immunocompromised patients. There are no clinical trials of Artesunate among HIV-positive women.

The risks associated with excisional treatments for cervical precancer, coupled with the challenges associated with accessing these treatments in countries like Kenya demonstrate a need for studies on alternative treatments that may be more readily available and have fewer complications for young women. The objective of this study is to build on the evidence from US-based studies which demonstrate safety and early efficacy of artesunate for cervical precancer and perform a Phase I trial in Kenya to evaluate whether artesunate – which is readily available in LMICs and has an excellent safety profile – can be used as a self-administered treatment for cervical precancer among both HIV-positive and HIV-negative women who are referred for excisional treatment. The rationale for performing a Phase I trial in Kenya, despite a previous Phase I trial in the U.S, includes the need to get early safety and efficacy data in WLWH, given their exclusion from the U.S trial. Additionally, this trial aims to obtain data on feasibility, considering the different social contexts in Kenya that may impact the acceptability and adherence to this intervention. Finally, differences between women in Kenya and the U.S., such as variations in the vaginal microbiome which play a key role in HPV acquisition and adherence, may influence treatment responses that are important to establish before a larger trial is conducted.[1–4] These reasons justify a Phase I trial in this setting.

Materials and Methods

Research Objectives

Primary Objective

This study primarily aims to investigate the safety of self-administered artesunate pessaries among HIV-positive and HIV-negative women with cervical precancerous lesions referred for excision in Kenya.

Secondary Objectives

1. Investigate adherence to self-administered artesunate pessaries for cervical precancer treatment among HIV-positive and HIV-negative women.
2. Evaluate change in lesion size following self-administered artesunate pessaries for cervical precancer treatment among HIV-positive and HIV-negative women.
3. Investigate the rate of histologic regression to CIN1 or less following self-administered artesunate pessaries among participants with CIN2 or worse on biopsy, among HIV-positive and HIV-negative women.
4. Investigate acceptability of self-administered artesunate pessaries for cervical precancer treatment among HIV-positive and HIV-negative women in Kenya.

Exploratory objectives

1. Investigate longitudinal changes in Human Papillomavirus (HPV) infection following intravaginal artesunate pessary use among HIV-positive and HIV-negative women.
2. Investigate longitudinal changes in cervical microbiome following intravaginal artesunate pessary use among HIV-positive and HIV-negative women.
3. Investigate longitudinal changes in the expression of biomarkers of local immune activation following intravaginal artesunate pessary use among HIV-positive and HIV-negative women.
4. Investigate changes in artemisinin drug resistance patterns for treatment of malaria, if any, among study participants among HIV-positive and HIV-negative women.

Study Design and Setting

This is a single arm, open-label phase I, non-randomized study. Participants meeting the inclusion criteria will be sequentially enrolled. The study will take place at the Lumumba Sub-County Hospital in Kisumu County, while enrollment will be at local hospitals in Kisumu, Siaya, and Migori counties.

As reported in the 2019 National Consensus, Kisumu County had a population of about 1,155,574 and a land area of 2085.9km[31]. The county has a shoreline on Lake Victoria, occupying northern, western and a part of the southern shores of the Winam Gulf. There is one teaching and referral hospital, 5 County referral hospitals, 14 sub-county hospitals, 74 dispensaries and 18 health centers in the county. While cervical cancer screening is offered in all public hospitals and some health centers, Loop Electrosurgical Excision Procedure (LEEP) treatment is only available at the referral hospital in Kisumu town serving the whole county. All women in Kisumu County who need excision are referred to this referral hospital.

Siaya County has a population of approximately 993,183, based on 2019 data from the National Consensus.[31] In Siaya County, cervical cancer screening is offered at the referral hospital as well as in the approximately 5 sub-county hospitals and a few health centers. While all hospitals and some health facilities in Siaya County which all offer cervical cancer screening, at present, all women who need excision are referred to the teaching and referral hospital in Kisumu County.

According to the same 2019 National Consensus report previously referenced, Migori County has a population of 1,108,950.[31] Public hospitals in Migori include Migori County Referral Hospital and subcounty hospitals including Oyani and Awendo sub-county hospitals. While most hospitals offer cervical cancer screening and ablation for cervical precancer treatment is available in most, no hospital in Migori County offers LEEP treatment. All women

who need LEEP are referred to neighboring counties including Kisii County as well as Kisumu County.

Participant Recruitment and Eligibility

Participants will be recruited from local hospitals in Siaya, Migori, and Kisumu counties where the study team will give educational talks about the study protocol as part of community outreach activities. Women interested in participating will be screened for eligibility and subsequently enrolled and consented if all eligibility criteria are met. Enrollment for this study will be done on a rolling basis at the study sites with no limit to participant enrollment per site.

The study population will include women aged 18 years and older with cervical lesions referred for excision, including those with biopsy-confirmed CIN2/3. Prospective participants must meet additional inclusion criteria to qualify for enrollment in this study. These criteria include a minimum weight of 50 kg at the start of the study, a weight confirmed as safe by comprehensive safety data for the planned artesunate dosing of ≤4 mg/kg. Additionally, participants must express willingness to use contraception (barrier or hormonal) until week 24 if of childbearing age and must possess the capacity and willingness to provide informed consent.

Potential participants will be excluded if they meet any of the following criteria: current pregnancy or breastfeeding; current or past history of invasive cervical cancer; history of total hysterectomy; CD4 count <200 at time of study entry if HIV positive; presence of adenocarcinoma in situ on cervical biopsy; currently undergoing systemic chemotherapy or radiation therapy for another cancer; current use of systemic immunosuppressants or steroids (>10 mg of prednisone or equivalent); have a medical comorbidity that, in the opinion of the investigator, would interfere with study participation; received chemotherapy within <1 month

prior to day 1 of study treatment; identify as male at birth; or current using efavirenz antiretroviral therapy.

Study Procedures by visit

Screening Visits:

Once a participant has been deemed eligible for participation and has provided their informed consent to the study procedures, they will go through various screening activities before they complete their first self-administration of artesunate. During these visits, basic demographic and medical history information will be collected. A comprehensive physical examination will be conducted, including blood collection for HIV testing and malaria surveillance, pregnancy testing, a colposcopy and biopsy, self-collection of vaginal swabs, and the collection of cervicovaginal specimens. Alongside these various medical tests, study staff will collect updated locator information, review concomitant medication, review potential adverse events that could result from this study drug, and will counsel participants on artesunate self-administration and the participant symptom diary.

Week 1, 3, 5, and 7

Weeks 1, 3, 5, and 7 mark the four treatment cycles in this study. Each of these weeks will see the administration of a 200 mg pessary (vaginal insert) of artesunate nightly for five consecutive days, each five-day period marking one treatment cycle. To ensure proper usage, all study participants will receive detailed counseling and instructions on artesunate use prior to administration, including instructions to wash their hands before and after inserting the pessaries.

Following the application of the artesunate pessary using a study-provided applicator, participants will be encouraged to insert a tampon overnight to keep the pessary at the cervix, removing the tampon the next morning. In ensuring the participant's safety, non-superabsorbent tampons will be provided, with clear instructions to not keep them in for longer than 10 hours to minimize the risk of toxic shock syndrome. Participants will also be instructed to abstain from sexual intercourse after applying the pessary to minimize irritation, however, sexual intercourse before pessary insertion is not prohibited.

Literate participants will record artesunate use on a study-provided calendar. Weekly phone calls from study staff will serve to review usage instructions, document any adverse events (AE's), and address participant questions. Participants will be advised against douching during the dosing phase and will be told to refrain from using any ointments, gels, or other types of pessaries. In cases of bacterial or year vaginal infections, participants should use oral medication as prescribed by the study investigator.

The selected dose for this study (200 mg) was chosen based on a combination of published clinical and pharmacokinetic data regarding intra-rectal administration of artesunate suppositories setting along with clinical data demonstrating the safety and tolerability of intravaginal artesunate (the same drug/formulation used in this study) among women with CIN2/3.[24, 32-34]

The frequency of treatment, occurring once every other week, allows for a drug-free week between treatment cycles. This intentional break not only accommodates a break for menstrual bleeding in women with menses but also offers participants relief if they experience local adverse events, in hopes to ensure compliance. Additionally, while sexual activity is not prohibited while using the pessaries, this treatment frequency allows for flexibility in intimacy

schedules, especially for participants who do not want to have sexual intercourse during treatment. The intermittent dosing proposed for this study are also consistent with existing literature on topical therapies for cervical or anal HPV lesions like 5-Fluorouracil (5FU) and Imiquimod.[15,16,19]

Weeks 2, 4, and 6

Weeks 2, 4, and 6 mark the off weeks of artesunate treatment. During these weeks, participants will return to the study clinic for safety and adherence assessments. These evaluations include malaria surveillance tests, pregnancy testing, pelvic exams to assess adverse events, and the collection of self-collected vaginal swabs. Also, during these weeks, the study staff will update participant locator information, re-review concomitant medication, and maintain regular telephone contact with the participants to support adherence.

Week 8

This visit is intended to serve as a built-in safety check for participants. During this visit, participants will return to the study clinic for the final safety and adherence assessments. Similar safety and adherence assessments will be done as in weeks 2, 4, and 6 (malaria surveillance tests, pregnancy testing, pelvic exams to assess adverse events, and the collection of self-collected vaginal swabs), with the addition of a colposcopy and collection of cervicovaginal specimens for HPV testing and microbiome testing. Participants will return their unused pessaries, used applicators, and packaging from used pessaries to the clinic. An acceptability questionnaire will be administered, and an in-depth interview performed as part of acceptability assessment. If a lesion appears larger or more severe compared to baseline, a biopsy will be taken to test for

invasion. If invasion is present – which is unlikely due to the speed at which cervical precancer progresses[35] – the participant will be referred for immediate treatment.

Week 14

Participants will return to the clinic for the following safety assessments: pelvic exam, pregnancy testing, collection of self-collected vaginal swabs and cervicovaginal specimens, a colposcopy and cervical biopsy, and review of adverse events. During this visit, it will be determined whether a participant requires a LEEP or continued surveillance based on the result from the biopsy. If there is regression noticed, the participant will be monitored again in week 24. If the lesion has progressed or remains persistent, then they will receive a LEEP. The rationale behind the 10-week observation period following regression detection is to allow time to monitor the impact of Artesunate. This decision is based on existing data that indicates that participants with partial response may take up to 22 to 38 weeks to achieve regression. This observation period is safe given the known prolonged time to progression to cancer.[24]

Weeks 24, 36, and 48

The exams and specimens to be collected during these weeks will be the same as in week 14. Participants will return to the clinic for the following safety assessments: pregnancy testing, collection of self-collected vaginal swabs and cervicovaginal specimens, and a colposcopy and biopsy. Similar to Week 14, during these visits it will be determined whether a participant requires a LEEP or continued surveillance based on the result from the biopsy. At Week 24, any participant with CIN2 or higher or a visible lesion on colposcopy will have standard of care excision. Participants with regression to CIN1 or less and no visible lesion – which demonstrates

cure – will be monitored for relapse at Week 36 and Week 48 and will have excision if CIN2 or higher is diagnosed. At each follow-up period, at least two (and up to 4) cervical biopsies (in each quadrant) and an endocervical curettage will be done to improve diagnostic accuracy. Participants whose lesions resolve completely will have biopsies at the sites of the lesion.

Statistical Analysis

Being a single-arm phase I study, our design is not meant to demonstrate differences in outcomes. Instead, our goal is to calculate the precision needed to demonstrate the primary endpoint of safety. With our sample size of 18 participants and assuming no serious AEs (grade 3 or higher) are observed (as was the case in a US-based phase I intravaginal artesunate study), we anticipate a one-sided upper 95% confidence bound for the prevalence of serious AEs to be 15%. Assuming a potential 15% drop off, with 15 participants completing the study, the one-sided upper 95% confidence bound for the prevalence of adverse events would be 18%. Based on our prior studies on cervical cancer in this region of Kenya, where we anticipate enrolling 1-2 participants a week, we expect to enroll 18 participants in 9-18 weeks (2.25-4.5 months). The follow-up period for participants with a complete response is expected to be up to 12 months.

Safety will be assessed by evaluating the type, frequency, severity, and duration of adverse events (AEs) using the U.S National Cancer Institute Common Terminology Criteria for Adverse Events, v5.0 (CTCAE 5.0). Adverse event counts for each participant, categorized by severity (grades 1-5), will be tabulated in both HIV status groups. The proportion of participants experiencing severe AEs (grade 3 or higher) within each HIV status group will be reported along the exact (Clopper-Pearson) one-sided upper 95% confidence bounds. Additionally, the proportion of participants reporting severe AEs (if any) will be reported, along with an exact one-sided upper 95% confidence bound. The safety assessment period will begin at study week 1

(first artesunate cycle) and will continue through study week 14 (8 weeks post-artesunate use), or until the last attended safety visit in the case of premature study exit. Safety data will be reported for all participants who start artesunate use.

Secondary endpoints will be analyzed as follows. Adherence will be evaluated based on participant self-report, examination of returned packaging for used pessaries, and inspection of vaginal applicators under ultraviolet light for evidence of intravaginal insertion. A participant will be considered adherent if both methods substantiate the use of 80% (16 of 20) of the pessaries provided. The proportion of participants meeting this 80% adherence criterion will be reported along with a precise two-sided 95% confidence interval (CI), stratified by HIV status. Additionally, the difference in the proportion of participants who meet the adherence criterion between the HIV-positive and HIV-negative groups will be reported, along with an exact two-sided 95% CI.

To evaluate the change in lesion size, colposcopy images of cervical lesions from baseline and time of excision, or week 24 (whichever comes earlier), will be compared. This evaluation will note the changes in number and size of lesions as well as the cervical quadrants involved. Blinded evaluations of pre and post treatment images will be done by two gynecologists not involved in the study to determine evidence of reduced lesion size. If the two gynecologists do not agree, then a third will be used as a tiebreaker. The proportion of participants with a reduction in lesion size will be reported with an exact two-sided 95% CI, stratified by HIV status. The difference in proportions with reduction in lesion size between the HIV-positive and HIV-negative groups will be reported, along with an exact two-sided 95% CI.

Histologic regression will be evaluated by comparing the initial diagnostic biopsy at study entry with the exit biopsy or excisional specimen final diagnosis. Participants with

complete regression at follow-up will have a confirmatory biopsy at the lesion site which will be used for final diagnosis, defining regression as CIN1 or less in the resection or biopsy specimen. All specimens will be reviewed by an in-country pathologist and slides scanned for further review by a second pathologist at UNC. A third pathologist will be used for consensus if the first two pathologists disagree. The proportion of participants with histologic regression will be reported with corresponding 95% CI, stratified by HIV status and differences in proportions with histologic regression between the HIV groups will be estimated with corresponding exact two-sided 95% CIs.

Acceptability will be assessed using a close-ended structured questionnaire and in-depth interviews with study participants performed at the end of artesunate use in week 8. Responses to the acceptability questionnaire will be summarized, including means and standard deviation for responses to questions graded on a Likert scale, and proportions and 95% CIs for yes/no questions. In-depth interviews will be transcribed, coded, and analyzed using thematic analysis.

Exploratory outcomes will include longitudinal changes in HPV infection, markers of local immune activation, and the cervical microbiome throughout the study period. Specimens will be collected and stored for future analysis. This includes the measurement of changes in the expression of biomarkers associated with local immune activation, such as IFN-α2, IFN-Y, IL-10, IL-12, IL-1α, TNF, CD8 (effector T cells), CD71 (transferrin receptor), and cleaved caspase 3 (apoptotic cell death) The analysis of these biomarkers will be stratified based on HIV status.

Changes in the cervical microbiome will be assessed using several techniques that include evaluating the diversity of the bacterial taxa by identifying community state types (CSTs) and evaluating changes in Lactobacillus-dominant environment. To quantify changes in

artemisinin resistance patterns between baseline and week 8, genotypic and phenotypic changes in isolated parasites will be compared during two specific time periods.

Discussion

This pilot trial will examine the safety and early efficacy of artesunate – a widely available WHO-approved drug used to treat malaria in LMICs – as a self-administered therapy for cervical precancer among women referred for excisional treatment, including both HIV-positive and HIV-negative women. This study will build upon existing research in HICs but will expand its scope to obtain safety data of this drug in WLWH, as studies in the U.S. did not include participants with HIV. Additionally, this research will explore the feasibility of implementing this topical therapy in Kenya where social contexts for women are notably different than in the U.S. Existing data on artesunate indicates a favorable safety profile and cytotoxic effects against numerous cancer cell lines both in vitro and in vivo. For these reasons, it is anticipated that self-administered artesunate pessaries will be safe and effective for both HIV-positive and HIV-negative women with cervical precancer in LMICs such as Kenya.

Current Status

The study opened for enrollment in March 2024.

Trial registration: The trial is registered under U.S Clinical trial registry (clinicaltrials.gov, NCT06165614).

List of abbreviations

LMIC:	low- and middle-income countries
HIV:	human immunodeficiency virus
HPV	human papillomavirus
WLWH:	women living with HIV
CIN2/3:	cervical intraepithelial neoplasia grade 2 and 3
LEEP:	Loop Electrosurgical Excision Procedure
CKC:	Cold Knife Cone
DHA:	Dihydroartemisinin
AEs:	adverse events
5FU:	5-Fluorouracil
CTCAE	U.S National Cancer Institute Common Terminology Criteria for Adverse Events
CI	Confidence Interval
CSTs	Community state types
WHO:	World Health Organization

Data Availability Statement

Data

Ethics Statement

This clinical trial has full ethics review board approval from the University of North Carolina Chapel Hill and Amref Health Africa. Written informed consent will be obtained from all study participants.

Competing interests

"The authors declare they have no competing interests."

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Authors' contributions:

CM, LR, and JT conceived and designed the study, providing subject matter expertise and overseeing all aspects of protocol development. JO provided guidance on protocol development and will lead protocol implementation in country. LR (Co-Investigator) provided expertise on study design and protocol development. JO (Co-Investigator) contributed subject matter expertise, study design, protocol implementation, and capacity building for providers. KS and CH contributed to manuscript writing. All authors, in their respective roles, contributed to study and manuscript preparation and have collectively approved the final manuscript.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA A J Clin.* 2018;00(00):1-31. doi:10.3322/caac.21492.
2. Ministry of Health, Nairobi K. Kenya National Cancer Screening Guidelines. Published online 2018:1-122.
3. Smith JS, Sanusi B, Swarts A, et al. A randomized clinical trial comparing cervical dysplasia treatment with cryotherapy vs loop electrosurgical excision procedure in HIV-

seropositive women from Johannesburg, South Africa. *Am J Obstet Gynecol*. 2017;217(2):183.e1-183.e11. doi:10.1016/j.ajog.2017.03.022

4. Dryden-Peterson S, Bvochora-Nsingo M, Suneja G, et al. HIV infection and survival among women with cervical cancer. *J Clin Oncol*. 2016;34(31):3749-3757. doi:10.1200/JCO.2016.67.9613
5. Peter IM, Deraney RN, Orango O, Philip T, Rosen B, Cu-Uvin S. Radical hysterectomy for operable early cervical cancer in HIV-positive and HIV-negative women in western Kenya. *Int J Gynecol Obstet*. 2020;148(3):403-404. doi:10.1002/ijgo.13004
6. World Health Organisation. Cervical Cancer. Accessed March 18, 2020. https://www.who.int/cancer/prevention/diagnosis-screening/cervical-cancer/en/
7. World Health Organisation. WHO guidelines for screening and treatment of precancerous lesions for cervical cancer prevention. Published online 2013:1-40. www.who.int/about/licensing/copyright_form/en/index.html
8. Kiptoo S, Otieno G, Tonui P, Mwangi A, Orango P, Itsura K, Muthoka J, Ogunda B, Rosen P, Loehrer P C-US. Loss to Follow-Up in a Cervical Cancer Screening and Treatment Program in Western Kenya. *J Glob Oncol*. 2018;4(Supplement 2):97s-97s.
9. Muruka Kays, Mugo R. Nelly, Wanyoike Gichuhi, Kihara Anne-Beatrice, Cheserem J. Eunice KJR. Same day colposcopic examination and loop electrosurgical excision procedure (LEEP) presents minimal overtreatment and averts delay in treatment of cervical intraepithelial neoplasia in Kenyatta National Hospital, Kenya. *Open J Obstet Gynecol*. 2013;3(3).
10. Arbyn M, Kyrgiou M, Simoens C, et al. Perinatal mortality and other severe adverse pregnancy outcomes associated with treatment of cervical intraepithelial neoplasia: meta-analysis. *BMJ*. 2008;337:a1284. doi:10.1136/bmj.a1284
11. Kyrgiou M, Athanasiou A, Paraskevaidi M, et al. Adverse obstetric outcomes after local treatment for cervical preinvasive and early invasive disease according to cone depth: systematic review and meta-analysis. *BMJ*. 2016;354:i3633. doi:10.1136/bmj.i3633
12. Kyrgiou M, Athanasiou A, Kalliala IEJ, et al. Obstetric outcomes after conservative treatment for cervical intraepithelial lesions and early invasive disease. *Cochrane database Syst Rev*. 2017;11(11):CD012847. doi:10.1002/14651858.CD012847
13. Masaba BB, Mmusi-Phetoe RM. Neonatal Survival in Sub-Sahara: A Review of Kenya and South Africa. *J Multidiscip Healthc*. 2020;13:709-716. doi:10.2147/JMDH.S260058
14. Castle PE, Einstein MH, Sahasrabuddhe V V. Cervical cancer prevention and control in women living with human immunodeficiency virus. *CA Cancer J Clin*. 2021;71(6):505-526. doi:10.3322/caac.21696
15. Fonseca BO, Possati-Resende JC, Salcedo MP, et al. Topical Imiquimod for the Treatment of High-Grade Squamous Intraepithelial Lesions of the Cervix: A Randomized Controlled Trial. *Obstet Gynecol*. 2021;137(6):1043-1053. doi:10.1097/AOG.0000000000004384
16. Desravines N, Miele K, Carlson R, Chibwesha C, Rahangdale L. Topical therapies for the treatment of cervical intraepithelial neoplasia (CIN) 2-3: A narrative review. *Gynecol Oncol reports*. 2020;33:100608. doi:10.1016/j.gore.2020.100608
17. Palefsky JM, Lee JY, Jay N, et al. Treatment of Anal High-Grade Squamous Intraepithelial Lesions to Prevent Anal Cancer. *N Engl J Med*. 2022;386(24):2273-2282. doi:10.1056/NEJMoa2201048

18. Rahangdale L, Lippmann QK, Garcia K, Budwit D, Smith JS, Le L Van. Topical 5-flourourcil for treamtent of cervical intraepithelial Neoplasia 2□: a Randomized Controlled Trial. *Am J Obstet Gynecol*. 2014;210(4):314.e1-314.e8. doi:10.1016/j.ajog.2013.12.042
19. Maiman M, Watts DH, Andersen J, Clax P, Merino M, Kendall MA. Vaginal 5-fluorouracil for high-grade cervical dysplasia in human immunodeficiency virus infection: A randomized trial. *Obstet Gynecol*. 1999;94(6):954-961. doi:10.1016/S0029-7844(99)00407-X
20. van de Sande AJM, Koeneman MM, Gerestein CG, Kruse AJ, van Kemenade FJ, van Beekhuizen HJ. TOPical Imiquimod treatment of residual or recurrent cervical intraepithelial neoplasia (TOPIC-2 trial): A study protocol for a randomized controlled trial. *BMC Cancer*. 2018;18(1):1-6. doi:10.1186/s12885-018-4510-7
21. World Health Organisation. *World Health Organization. Guidelines for Treatment of Malaria. 3rd Edition*.; 2015.
22. Ellis T, Eze E, Raimi-Abraham BT. Malaria and Cancer: a critical review on the established associations and new perspectives. *Infect Agent Cancer*. 2021;16(1):33. doi:10.1186/s13027-021-00370-7
23. Ma Z, Woon CY-N, Liu C-G, et al. Repurposing Artemisinin and its Derivatives as Anticancer Drugs: A Chance or Challenge? *Front Pharmacol*. 2021;12:828856. doi:10.3389/fphar.2021.828856
24. Trimble CL, Levinson K, Maldonado L, et al. A first-in-human proof-of-concept trial of intravaginal artesunate to treat cervical intraepithelial neoplasia 2/3 (CIN2/3). *Gynecol Oncol*. 2020;157(1):188-194. doi:10.1016/j.ygyno.2019.12.035
25. Jeong DE, Song HJJ, Lim S, et al. Repurposing the anti-malarial drug artesunate as a novel therapeutic agent for metastatic renal cell carcinoma due to its attenuation of tumor growth, metastasis, and angiogenesis. *Oncotarget*. 2015;6(32):33046-33064. doi:10.18632/oncotarget.5422
26. Efferth T, Sauerbrey A, Olbrich A, et al. Molecular modes of action of artesunate in tumor cell lines. *Mol Pharmacol*. 2003;64(2):382-394. doi:10.1124/mol.64.2.382
27. Siegel EM, Patel N, Lu B, et al. Circulating biomarkers of iron storage and clearance of incident human papillomavirus infection. *Cancer Epidemiol biomarkers Prev a Publ Am Assoc Cancer Res cosponsored by Am Soc Prev Oncol*. 2012;21(5):859-865. doi:10.1158/1055-9965.EPI-12-0073
28. Daniels TR, Bernabeu E, Rodríguez JA, et al. The transferrin receptor and the targeted delivery of therapeutic agents against cancer. *Biochim Biophys Acta*. 2012;1820(3):291-317. doi:10.1016/j.bbagen.2011.07.016
29. Schiffman M, Doorbar J, Wentzensen N, et al. Carcinogenic human papillomavirus infection. *Nat Rev Dis Prim*. 2016;2:16086. doi:10.1038/nrdp.2016.86
30. Luo J, Zhu W, Tang Y, et al. Artemisinin derivative artesunate induces radiosensitivity in cervical cancer cells in vitro and in vivo. *Radiat Oncol*. 2014;9:84. doi:10.1186/1748-717X-9-84
31. Statistics KNBo. *2019 Kenya Housing and Population Census*. Nairobi, Kenya: Kenya National Bureau of Statistics; 2019.
32. World Health Organisation. The use of rectal artesunate as a pre-referral treatment for severe P . falciparum malaria. 2022;(January):2020-2022.

33. Gomes MF, Faiz MA, Gyapong JO, et al. Pre-referral rectal artesunate to prevent death and disability in severe malaria: a placebo-controlled trial. *Lancet (London, England)*. 2009;373(9663):557-566. doi:10.1016/S0140-6736(08)61734-1
34. Gomes M, Ribeiro I, Warsame M, Karunajeewa H, Petzold M. Rectal artemisinins for malaria: a review of efficacy and safety from individual patient data in clinical studies. *BMC Infect Dis*. 2008;8:39. doi:10.1186/1471-2334-8-39
35. Silverman MH, Hedley ML, Petry KU, Weber JS. Clinical trials in cervical intraepithelial neoplasia: balancing the need for efficacy data with patient safety. *J Low Genit Tract Dis*. 2002;6(4):206-211. doi:10.1097/00128360-200210000-00002