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# A randomized clinical trial on the effects of progestin contraception in the genital tract of HIV-infected and uninfected women in Lilongwe, Malawi: Addressing evolving research priorities

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## Abstract

Hormonal contraception is central in the prevention of unintended pregnancy; however there are concerns that certain methods may increase the risk of HIV acquisition and transmission. Hormonal contraceptives may modify the genital mucosa in several ways, however the mechanisms are incompletely understood. Few studies have examined genital HIV shedding prospectively before and after initiation of hormonal contraception. The effects of hormonal contraception on genital HIV shedding in the setting of antiretroviral therapy (ART) are also unknown. We designed a pilot clinical trial in which HIV-infected and uninfected women were randomized to either depot medroxyprogesterone acetate (DMPA) injectable or levonorgestrel (LNG) implant in Lilongwe, Malawi. The objectives were to: 1) assess the effect and compare the impact of type of progestin contraception (injectable versus implant) on HIV genital shedding among HIV-infected women, 2) assess the effect and compare the impact of type of progestin contraception on inflammatory/immune markers in the genital tract of both HIV-infected and uninfected women, and 3) assess the interaction of progestin contraception and ART by examining contraceptive efficacy and ART efficacy. An additional study aim was to determine the feasibility and need for a larger study of determinants of HIV transmissibility and acquisition.

As injectable contraception is widely used in many parts of the world with high HIV prevalence, this study will provide important information in determining the need for and feasibility of a larger study to address these questions that can impact the lives of millions of women living with or at risk for HIV.

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**CDC disclaimer**

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

## 1. Introduction

Globally, over 35 million people are living with HIV, including 16 million women [1]. Like most women, HIV-infected women are in need of safe and effective contraceptive methods. There are many benefits to preventing unintended pregnancy among women. Unintended pregnancy is associated with increased morbidity and mortality, and in HIV-infected women it can prevent the birth of children at risk for HIV transmission. Globally, the most common forms of modern contraception used are progestin-containing hormonal contraceptive methods, either alone or in combination with estrogen, with over 150 million users around the world [2].

Despite its contraceptive benefits, there are concerns that certain types of hormonal contraception may increase the risk of HIV acquisition and transmission. Recent systematic reviews on the role of contraceptives in HIV acquisition [3] and female-to-male transmission [4] concluded that the preponderance of data suggest that oral contraceptives do not increase risk and that, while studies are limited, there is also no evidence of an increased risk with contraceptive implants. In contrast, the results are less consistent with injectable contraceptives. Of the nine studies on injectable contraception and HIV acquisition considered of adequate quality [3], four reported a significant increase in risk of HIV acquisition with injectables [5–8]. Among HIV-infected women, the only study of adequate quality [4] that assessed the risk of female-to-male transmission with injectable contraception found an increased risk [5]. Furthermore, of the three studies evaluating the role of injectables on genital shedding of HIV-1 DNA and RNA, all found increased cervical shedding with injectables [5,9,10]. Although these findings have potentially serious public health and policy implications, the inconsistency among studies and methodologic weaknesses limit interpretation [11]. A World Health Organization (WHO) consultation concluded that there was as yet insufficient evidence to support a change in the current guidelines of no restriction on the use of progestin-only injectable contraceptives among women at high risk for HIV acquisition or among HIV-infected women [12]. However, the WHO states that women at high risk of HIV acquisition considering progestin-only injectables should be informed about the uncertainty of whether or not injectables increase acquisition risk and be provided access to HIV preventive measures, including male and female condoms.

Since access to ART is increasing worldwide, there is also a need to evaluate the impact of hormonal contraception on HIV transmissibility among HIV-infected women on ART, an area with a paucity of data. Pharmacokinetic data suggest potential drug interactions between some antiretrovirals and hormonal contraceptives. Currently, the WHO only restricts contraceptive pill, ring and implant use with specific antiretroviral regimens [13]. DMPA is listed as category 1 (no restrictions to use) with the three major classes of antiretrovirals: nucleoside reverse transcriptase inhibitors (NRTI), non-nucleoside reverse transcriptase inhibitors (NNRTI), and protease inhibitors (PI). However, LNG is listed as category 2 (benefits of use outweigh the theoretical or proven risks) when NNRTI or ritonavir-boosted PI are used [13].

## 2. Rationale for the study

The knowledge/understanding of the mechanism by which hormonal contraception may modify sexually transmitted infection (STI) and HIV risk is incomplete. Hormonal contraception may work systemically or locally within the genital tract to alter HIV susceptibility and transmissibility [14–32]. The physiology, cell composition, and immunology of the genital tract, a critical portal for HIV entry and source of transmission to partners and to newborns during childbirth, can be altered by changes in endogenous hormones and hormonal contraception. Changes in gonadal hormonal levels may alter vaginal wall thickness or barrier function [33], vaginal pH, vaginal microbiome [34], cervical cell ectopy [35], cervical mucus [36], and susceptibility to STIs [37–41].

DMPA leads to high levels of systemic progestin concentrations with profound suppression of endogenous estrogen. This high-progestin, low-estrogen state may affect HIV susceptibility. Estradiol and progesterone regulate multiple functions in the genital tract, including homing of immune cells, chemokines and cytokines, [42] mucosal cellular composition, [43–45] and epithelial cell receptor expression [46]. Progesterone and its derivatives can increase infiltration of antigen-presenting cells in the vaginal epithelium, as well as CD4 + CCR5+ lymphocytes and CD8+ T-lymphocytes in genital tract tissues [44,47–54]. Estrogen, on the other hand, has been shown to decrease recruitment of activated T-cells and macrophages [55]. Thus, it appears that progesterone might increase, while estrogen may decrease, the frequency of HIV-1 target cells in the female genital tract.

Another potential pathway by which hormonal contraception may affect HIV infectivity is by increasing the prevalence of other genital infections. DMPA use has been associated with decreased $H_2O_2$-positive *Lactobacillus* and increased acquisition of cervical chlamydial and gonococcal infections, herpes simplex 2 infection, and candidiasis in women [31,35,45,56,57]. Presence of other genital tract infections has been associated with increased genital tract HIV shedding and HIV transmission [58–60]. While some studies have suggested that DMPA does not alter the prevalence of bacterial vaginosis or vaginal candidiasis, the impact on the vaginal microbiome utilizing more sensitive genetic sequencing techniques is not well characterized [56,61–66].

There are several different injectable hormonal contraceptives, but DMPA via the IM formulation (150 mg IM) is by far the most commonly used globally. A factor limiting contraceptive effectiveness is poor adherence to the repeated injections (every 3 months). Efforts have been made to promote longer-term contraceptive options, such as contraceptive implants. There have been several contraceptive implants utilized globally, including Jadelle, Implanon, Nexplanon, Norplant and Sino-implant. Jadelle, a 2 rod-implant, with each rod containing 75 mg of levonorgestrel (LNG), is the most commonly used in Africa with an efficacy for up to 5, and possibly 7, years [67] following insertion. Given the increasing acceptance of LNG implant use in regions with high HIV prevalence [68], its high efficacy in preventing unintended pregnancy, and its longer duration of action, we chose to use the Jadelle LNG implant as the comparison method in this study.

Different progestins could have different effects on the immune system and the genital tract due to variations in dose, methods of absorption, metabolism, pharmacokinetics, bioavailability, and/or binding of serum proteins and enzymes. Different progestin-only methods will also differ with regards to peak serum progestin concentrations, as well as varying degrees of hypothalamic-pituitary-ovarian axis modulation with resultant suppression of estrogen. Currently available progestins include a wide range of progestogenic molecules with variable degrees of estrogenic, androgenic, anti-androgenic, glucocorticoid and anti-mineralocorticoid activity [69]. Medroxyprogesterone (MPA), for example, has potent glucocorticoid activity, higher than any other progestin or endogenous progesterone, and this could theoretically enhance susceptibility to HIV. The effects of different progestins on the genital tract and relative impact on HIV viral shedding may thus be different, but have yet to be explored [70]. Among progestin-only methods, DMPA concentrations will quickly increase after intramuscular injection, with the highest levels occurring within the first month followed by a plateau of serum concentrations at 1.0–1.5 ng/mL for about 3 months, after which blood levels decline slowly. Endogenous estradiol levels and progesterone levels are suppressed for several months after DMPA injection corresponding to suppression of ovulation [71]. Study findings suggest an antiprogestogenic effect on the vaginal mucosa with MPA that may not be shared by other contraceptives [70].

For the levonorgestrel implant, release of levonorgestrel is sufficient to prevent pregnancy within 24 h of insertion, reaching a maximum level 2 days after placement, with release of 100 μg/day of levonorgestrel during the first month, declining to about 40 μg/day at 12 months and stabilizing at 30 μg/day at 24 months and thereafter. Serum concentrations may vary by metabolic clearance rate, body weight and other factors, but they are not necessarily predictive of pregnancy risk. Serum estrogen concentrations are significantly lower with DMPA compared to LNG implant users [70]. Tissue-specific and systemic effects of the LNG implant are poorly characterized.

Although the mechanisms of action, kinetics of hormone release, and impact on endogenous hormonal concentrations differ between different forms of progestin-releasing contraceptives, studies examining the effects of these methods on HIV transmission are limited. To our knowledge, there is only one study that examined HIV RNA shedding among 5 women using Norplant [10] and no studies that have evaluated Jadelle or Sino-implant. This was another knowledge gap we sought to fill with this study.

In 2012, the Centers for Disease Control and Prevention (CDC) and the University of North Carolina at Chapel Hill (UNC) began designing a randomized controlled trial to fill in these knowledge gaps by evaluating the effect of two different progestin contraceptive methods on HIV genital shedding among HIV-infected women and on the inflammatory/immune/microbial changes in the genital tract of HIV-infected and uninfected women. This paper describes the process of designing the study protocol, as well as the evolution of this protocol to reflect changes that were implemented in light of new emerging knowledge leading to evolving research priorities in a rapidly moving field.

# 3. Methods

## 3.1. Study objectives

To address the study aims we specified the following objectives: **1.** To assess the effect and compare the impact of type of progestin contraception (injectable versus implant) on HIV viral shedding in the genital tract of HIV-infected women**; 2.** To assess the effect and compare the impact of type of progestin contraception (injectable versus implant) on inflammatory/immune markers in the genital tract of both HIV-infected and HIV-uninfected women; **3.** To assess the interaction of progestin hormonal contraception and ART by examining: i. contraceptive efficacy (measured by systemic hormone levels and pregnancy rate during follow-up) and ii. ART efficacy (by drug concentrations in blood and genital tract and HIV viral load response in the plasma in women on ART).

## 3.2. Study design

This study was a randomized trial of the effect of progestin contraception on HIV shedding and mucosal immune activation in the genital tract. HIV shedding and other study outcomes were evaluated by: 1) a within-subject assessment comparing the time periods before and after randomization to a progestin contraceptive, 2) an across-study arms comparison of the difference between the study interventions (DMPA and LNG implant), and 3) an across-study arm comparison of immune genital tract changes between HIV-infected and HIV-uninfected women using progestin contraception. Antiretroviral use was evaluated for an independent effect on HIV shedding and also to determine if it modified the effects of menstrual cycle or progestin contraception on HIV shedding. To analyze the effects of progestin contraception on genital inflammatory/immune markers, HIV-infected and uninfected women on progestin contraception were compared separately and combined, with HIV status treated as a potential effect modifier, within each study arm (before and after initiation of contraception), as well as between the two contraceptive arms.

The study aimed for potential subjects to be screened for entry until 100 HIV-infected and 30 HIV-uninfected women were recruited and randomized to receive either DMPA injections ($n$ = 50 HIV-positive, 15 HIV-negative) or the LNG implant ($n$ = 50 HIV-positive, 15 HIV-negative). HIV-infected women may or may not have been on ART to treat their HIV infection. Evaluation was conducted in two stages: the pre-intervention stage to assess baseline status by stage of menstrual cycle; and the intervention stage where the variables of interest were measured while on the contraceptive intervention.

## 3.3. Study population, location and personnel

The study took place at Bwaila Maternity Hospital in Lilongwe, Malawi. With a population of approximately 16.3 million people, Malawi has a per capita income of $750 and per capita total expenditure on health of $91 ($ amounts are Purchasing Power estimates by WHO at the international $ rate) [59]. The estimated HIV prevalence among adults ages 15–49y is 10.8% [1]. Forty-two percent of all married women use contraception, and there is a 26% unmet need for family planning [72].

Since 1990, UNC has been working in Malawi with local partners conducting research with activities centered in Lilongwe. Bwaila Maternity Hospital was also the site of the CDC-sponsored Breastfeeding, Antiretrovirals, and Nutrition (BAN) clinical trial [73].The CDC institutional review board (IRB), the UNC IRB, the Malawi National Health Sciences Research Committee, and the Malawi Pharmacy, Medicines & Poisons Board approved the initial protocol and all amendments prior to implementation.

The participants for this study were recruited from clinic patients at Bwaila Maternity Hospital (Bwaila) Family Health Unit and from other clinics based at the Bwaila Maternity Hospital and in the surrounding area. Public radio announcements about the study were also broadcast during the first two weeks of study enrollment.

Women who desired to start hormonal contraception were informed of the study and counseled on the progestin contraceptives available within the study. Eligible women who provided informed consent and agreed to randomization to either DMPA or the LNG implant were enrolled. To address the primary outcomes of HIV shedding and mucosal immune activation, we quantified genital tract HIV DNA/RNA and inflammatory/immune markers (cytokines/chemokines, activation markers) at two time points before and several time points after randomization of the HIV-infected and HIV-uninfected women to DMPA or LNG implant. The two time points prior to randomization were chosen within the prior menstrual cycle, with one visit in the follicular phase and the other visit in the luteal phase of the cycle. Assessments after initiation of contraception occurred on days 3, 30, 90, 6 months, and every 3 months thereafter until a follow-up time of up to approximately 2 years and 9 months was completed. Antiretroviral concentrations in the blood and genital tract were also assessed at these time points.

### 3.4. Sample size

HIV-infected women were enrolled to address the primary objective of comparing genital tract HIV shedding using DMPA injections or LNG implants. The sample size of 100 was primarily chosen for this pilot trial because of logistical considerations, in part to evaluate the feasibility of randomization to two different contraceptive methods, and in part to determine whether a larger trial would be feasible and/or necessary. Based on our statistical methods described below, a sample size of 100 women is sufficient to detect a 0.5 $\log_{10}$ difference in viral shedding before and after initiation of progestin contraception, a difference that is clinically meaningful, as each $\log_{10}$ increase in HIV genital shedding has been estimated to lead to an approximate two-fold increase in transmission risk [95]. Based on previous studies [74,75], we also determined that a sample size of 30 HIV-uninfected women would be sufficient to explore differences in distributions of several different immunologic markers in women randomized to DMPA and LNG implant.

### 3.5. Statistical considerations

For the first primary study objective, we hypothesized that there will be an increase in the magnitude of genital HIV shedding following initiation of progestin contraception. There was no information on the direct comparability of genital HIV shedding with DMPA and LNG implant and if the shedding would be more or less notable for HIV RNA or DNA.

The difference in HIV shedding before and after starting progestin contraception in this trial depends on the magnitude of genital HIV detection before starting progestin contraception, the assumed correlation coefficient between a participant's repeated measurements at follow-up visits, the Type I error rate, and the power to detect a statistically significant effect. Data from studies of HIV RNA cervical shedding of women not on ART [76–78], as well as women on ART [10,79–81], formed the basis for estimating this detectable difference in genital HIV shedding in this study given our sample size of 100 HIV-infected women. We estimated that approximately one third of our HIV-infected ($n = 33$) study population would not be on ART yet due to their CD4+ T cell count (following criteria for treatment initiation at the time), while two thirds ($n = 67$) would be on ART. Assuming a mean 2.0 $\log_{10}$ cervical HIV RNA level prior to initiation of contraception in our study [10, 78], we would be able to detect a 0.51 $\log_{10}$ difference after initiation of progestin-based contraception with a power of 80%, alpha = 0.05, a 10% loss-to-follow-up rate, and a within-subject correlation coefficient of 0.20. We would also be able to detect a 0.50 $\log_{10}$ difference in viral shedding between the LNG-implant arm and the DMPA arm after initiation of contraception.

### 3.6. Recruitment, screening, and enrollment

The study recruited women who expressed an interest after hearing a brief overview of the study and its eligibility criteria. Interested women received comprehensive family planning education from a Study Nurse using the Malawi Ministry of Health's family planning counseling flipbook, known as "Kulera" that reviews all available family planning methods in Malawi and includes information on both DMPA and the implant.

Women who after completion of this education session were still interested in the study and who provided informed consent for screening procedures were screened to determine eligibility. The inclusion criteria were: 1) females aged 18–45 years; 2) known HIV status, as documented by at least two concordant rapid tests; 3) at least two regular, monthly cycles (~21–35 days) in the three months preceding study enrollment; 4) off hormonal or intrauterine contraception for at least 6 months (if previously using DMPA, last injection must have been ≥6 months ago); 5) at least 6 months postpartum; 6) interested in initiating a family planning method, specifically DMPA or the LNG implant; 7) willing to be randomized to receive either DMPA or the LNG implant; 8) willing to wait 4–6 weeks after enrollment to receive the method and to use non-hormonal and non-intrauterine methods (such as abstinence or condoms) consistently during this period.

Exclusion criteria were: 1) pregnancy (by clinical history or a positive urine test at screening); 2) current use of any hormonal contraception method; 3) desire to become pregnant within the next 12 months; 4) untreated visible genital ulcers or lesions on initial pelvic examination; 5) known or suspected genital tract cancer (by clinical history or noted during initial pelvic examination); 6) any contraindications to DMPA or LNG implant per the WHO medical eligibility criteria [82] or judgment of clinician (contraindications include lactation within first 6 weeks postpartum, acute deep venous thrombosis or pulmonary embolism, lupus, migraine with aura, unexplained vaginal bleeding, current or history of breast cancer, severe cirrhosis, liver tumors, history of stroke, current or history of ischemic

heart disease); 7) acute HIV infection (as documented by a known negative HIV test 6 months or less prior to screening).

Women who met all eligibility criteria and were good candidates for study participation based on the assessment of the site investigator or designee were enrolled into the study after providing informed consent for enrollment.

## 4. Schedule of study visits

Enrolled women were followed for two study visits during the month preceding randomization; after randomization, women were followed for up to two and a half years. The schedule of the visits was designed to collect samples from both the follicular and luteal phases in the menstrual cycle prior to contraceptive intervention and to coincide with key times in the progestin concentration pharmacokinetic curve for those on DMPA (peak, decreasing, trough), as well as the time of repeat injections (every 3 months) (Tables 1 and 2). For comparability, participants on the implant arm were followed at the same time points. Both history and hormonal assessment were used to confirm phase of menstrual cycle. If a participant was experiencing menstrual bleeding during a follow-up visit, pelvic evaluations were deferred until after menses were complete.

At the enrollment visit, a detailed assessment including questions about demographic, medical, gynecologic and sexual history was conducted. At each follow-up visit an interval history assessment and physical exam were conducted. Specimen collection included blood and genital samples (Tables 1 and 2). Condoms were provided to all participants at each visit as well as contraceptive and HIV/STI risk-reduction counseling.

Specimens collected at follow-up visits were tested for rapid plasma reagin (RPR) (with confirmatory Treponema Pallidum Hemagglutination Assay testing if positive), Herpes Simplex Virus-type 2 (HSV-2), *Chlamydia trachomatis, Neisseria gonorrhoeae,* and *Trichomonas vaginalis*. Testing was conducted because sexually transmitted infections could cause inflammatory genital tract changes, which are potential confounders for the HIV viral shedding and inflammatory/immune markers outcomes and will be accounted for in analyses.

In HIV-infected women, Tear-Flo strips were used to collect cervicovaginal fluid for HIV-1 RNA quantitation and Weck-Cel sponges were used to collect cervicovaginal secretions for ART drug level testing (if the woman was on ART). Concentrations of the antiretroviral agents were measured in the plasma and the upper layer of packed cells (ULPC) [83].

For both HIV-infected and HIV-uninfected women, a cervicovaginal lavage (CVL) sample was collected. The fluid portion was used for testing of immune markers, and centrifuged cell pellets were tested for HIV proviral DNA in HIV-infected women.

The genital tract HIV-1 DNA and RNA levels obtained from CVL were compared to the HIV-1 RNA levels obtained by cervical Tear-Flo strips. There are advantages and disadvantages to assessing HIV concentrations with each specimen type (CVL vs. Tear-Flo). The use of progestin is expected to induce changes in the thickness and other characteristics

of cervical mucus, which has the theoretical potential to affect viral concentrations. On the other hand, the effect of dilution of mixed cervicovaginal secretions by the method of obtaining CVL may also affect ability to detect virus in the genital tract more so than direct collection. For these reasons a direct comparison of CVL vs. Tear-Flo was performed, thus advancing the field methodologically. In addition, both HIV RNA and proviral DNA were evaluated in this study, given lack of consensus about the relative contribution of cell-free vs. cell-associated virus in transmission risk [84].

For HIV-negative women only, a cervical cytobrush specimen for cellular immune activation testing was collected after the CVL collection. In addition, for HIV-negative women, blood was also collected to evaluate for cellular activation of the same markers in whole blood.

For all women, blood was collected for hormonal assessment (estradiol, progesterone, medroxyprogesterone, or LNG were also assessed as appropriate). In HIV-infected subjects, blood was obtained to test for CD4+ T cell count, HIV RNA viral levels, HIV resistance mutations and antiretroviral concentrations.

## 5. Provisions for protecting privacy and confidentiality

Participants' privacy and the confidentiality were protected through formal training of interviewers and other study staff in good clinical practices, study ethics, human subjects research and protocol procedures. All interviews and physical examinations were conducted in private, and all study materials were stored in a locked room. Only a coded study participant identification number (PID) was used on study documents containing participant data. Participants' names were stored separately from documents containing participant data in a locked file in a locked office. Linkages between PIDs and participants' identifying information were maintained on a paper log kept locked and only accessible to limited on-site study staff.

## 6. Protocol amendments

Since study initiation, some new information became available, as some studies suggested there might be an interaction of ART and progestin implants resulting in reduced contraceptive effectiveness: A literature review [85] noted a few case reports of women on ART regimens who conceived pregnancies while on Implanon® (etonorgestrel implant) after 24 months of use. A retrospective chart review suggested that efavirenz (EFV) may decrease the efficacy of LNG implants [86]. The mean time between implant insertion and pregnancy was 16.4 months for the women who became pregnant in that study [86]. Results from a pharmacokinetic study suggested that LNG levels in 20 women receiving EFV were lower compared with women not on EFV [87]. Updated results from this small prospective cohort study showed that 3 women on EFV became pregnant between 36 and 48 weeks after LNG implant insertion, compared to none in the control group [88]. Two of the pregnant women had LNG levels >180 pg/mL at their prior visit, which had previously been thought to be the minimum drug concentration needed to prevent pregnancy. While additional prospective studies are needed to assess the clinical significance (actual increase in pregnancies due to decreased hormonal levels) among HIV-infected women taking EFV [89], these findings

raised concern about use of LNG implants in women taking EFV. In 2014, USAID provided guidance for counseling women who are on ART and progestin implants, taking into account the limited information available [89].

Given this new concern, a protocol amendment was implemented in December 2014 to provide additional counseling regarding the possible decreased effectiveness of implants in preventing pregnancies for women on EFV. Prior to screening, all potential participants were provided with this additional counseling in conjunction with the standard family planning counseling. For those women already enrolled into the study, counseling was provided to all HIV-infected participants not yet randomized to a contraceptive method and to all HIV-infected participants randomized to the LNG implant arm. Two subsequent amendments were implemented to increase the amount of follow-up time from 6 months post-contraceptive initiation to up to about 2 years and 9 months post-contraceptive initiation, in order to evaluate the frequency of occurrence of breakthrough pregnancies; and to obtain qualitative data on women's understanding of the counseling messages and reasons for continuing or discontinuing their contraceptive methods longer-term. The testing schedule was simplified after the initial 6 month period to only cervical swabs for HIV RNA to evaluate genital shedding and no further cytobrush specimens in the HIV-uninfected women.

## 7. Current status

Recruitment and retention in the study has been robust and randomization to a contraceptive method was acceptable and feasible. Of the 97 women randomized to a contraceptive method, 96 have already completed 6 months of follow-up and extended follow-up is ongoing.

## 8. Discussion

The study seeks to address several knowledge gaps that affect HIV-infected and HIV-uninfected women in resource-limited and resource-rich settings: the safety of hormonal contraception with regard to HIV acquisition and transmission, as well as the effectiveness of certain forms of progestin contraception when given in conjunction with some ART agents. Progestin contraception is particularly important to study for several reasons. First, due to the lack of pharmacokinetic interactions with ART, DMPA is widely used among HIV-infected women on ART in many settings, including resource-limited settings with high HIV prevalence. Second, these forms of contraception do not require daily administration (e.g. pills) or per-coital act (e.g., condoms) adherence, and are thus more effective in preventing pregnancy. Promotion of longer-acting reversible contraceptive (LARC) methods, such as the implant, is particularly desirable, due to their high effectiveness and less reliance on the woman's memory and adherence. This study will be able to examine the effects of these forms of contraception on genital HIV shedding (both RNA and DNA) and on immune markers, compare genital HIV shedding and immune markers before and after initiating contraception among HIV-infected and uninfected women, and also compare the two forms of contraception in a randomized design.

Plasma viral load has been shown to be a very strong predictor of heterosexual transmission [79,80], and ART effectively decreases viral load in the blood and in the genital tract [90]. However, several reports have documented that HIV-1 may remain present in the cervicovaginal fluid of patients on ART even if the individual has undetectable plasma viral load [13,80,91–93]. Furthermore, although transmission is markedly reduced by ART, it does not appear to be completely eliminated [84,94–97], as low-level viral replication may persist within the genital tract, even with undetectable plasma viral load. Studies of women on ART have found cervical HIV RNA detectable in 3% to 33% of women studied [10,79,80,98], depending on whether the woman has undetectable plasma viral load (VL) or not [10]. Median genital viral load has been estimated to decrease by about 2 log by 6 months after initiation of ART (from 4.0 to 1.7 log) [10]. It is thus important to address potential modulating factors that can alter genital viral loads in the setting of ART [99,100].

From studies among ART-naïve populations, genital tract HIV RNA load correlates significantly but imperfectly with plasma viral load level ($r = 0.56$) [77,101–103]. Genital HIV RNA has been shown to be an independent risk factor for heterosexual HIV transmission, even after adjustment for plasma viral load, with each 1 log increase in cervical HIV RNA levels associated with a 2.2-fold increased risk of transmission to the male partner (adjusted hazard ratio of 1.67 after adjustment for plasma viral load) [101]. Genital tract HIV shedding can be intermittent and may be upregulated by pregnancy, HSV reactivation, alterations in vaginal flora, time in the menstrual cycle, and even nutritional deficiencies [104–106]. Thus, measurement of genital HIV quantity is a strong surrogate marker of HIV transmission risk, suggesting that the potential impact of new interventions aimed at reducing HIV transmission can be assessed through studies of genital HIV RNA [101].

As mentioned, prior studies have evaluated the impact of hormonal contraception on HIV genital tract shedding, but results are contradictory about DNA or RNA shedding; studies have also used different genital specimens, resulting in difficulty of interpreting or generalizing the information. Our study offers the methodologic advantage of using outcome measures of both HIV RNA and DNA from two genital specimen types (cervicovaginal lavage and cervical swab), obtained at the same time from each woman, at several time points before and after initiation contraception, at times to coincide with different phases of the menstrual cycle, as well as critical points to construct pharmacokinetic curves of progestin concentrations. Also, this is the first study to compare different progestins in a randomized fashion, thus circumventing the potential problem of confounding associated with self-selection of contraceptive method, as well as recall bias when based on self-reported contraceptive use.

The results of our study will add to the evidence base regarding the comparative safety of two different progestin contraceptives for women with and at-risk for HIV and the effectiveness of progestin implants with concomitant use of EFV. This study will add to the understanding of immunologic and genital tract changes that occur with progestin-only contraception and how these changes may relate to risk of HIV acquisition among HIV-uninfected women. In addition, prospective information on risk of contraceptive failure of LNG implants with concurrent use of EFV that can aid in formulating evidence-based

recommendations for hormonal contraceptive use among women on ART. Finally, this study will help increase local state-of-the art clinical research and laboratory capacity at the study site. Based on the findings of this study and the size of the effects observed, the need for a larger study to obtain a more definitive answer to these questions will be determined.

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**Table 1**

Schedule of study visits (initial study).

| Study activities | S/E Visit | Visit 1[a] | Visit 2[b] | Visit 3[c] | Visit 4 | Visit 5 | Visit 6 | Visit 7 |
|---|---|---|---|---|---|---|---|---|
| Weeks from contraceptive initiation | | *a* | *b* | 0 | Day 3 | 4 | 13 | 26 |
| Informed consent | X | | | | | | | |
| Detailed clinical assessment | X | | | | | | | |
| Interval history assessment | | X | X | X | X | X | X | X |
| Randomization and contraceptive initiation | | | | X | | | | |
| Follow-up DMPA injection (if applicable) | | | | | | | X | X |
| Lab tests and exams | | | | | | | | |
| Urine for pregnancy test | X | | | X | | X | X | X |
| Urine for GC/CT | | X | X | | X | X | X | X |
| Urine for schistosomiasis, *T. vaginalis, M. genitalium* | | X | | | | | | |
| Fingerstick for HIV screening | X | | | | | | | |
| Blood for RPR/HSV | | X | | | | | | |
| Blood for serum hormone levels | | X | X | | X | X | X | X |
| Speculum pelvic examination | | X | X | | X | X | X | X |
| Vaginal swabs for wet mount, vaginal microbiome, PSA | | X | X | | X | X | X | X |
| CVL for immune markers[+] | | X | X | | X | X | X | X |
| For HIV-positive women only: | | | | | | | | |
| Blood for CD4 | | X | | | X | | X | X |
| Blood for HIV RNA | | X | X | | X | X | X | X |
| Blood and CVL ART levels | | X | X | | X | X | X | X |
| Cervical and CVL HIV RNA/DNA | | X | X | | X | X | X | X |
| HIV resistance testing | | X | | | | | | |
| For HIV-negative women only: | | | | | | | | |
| Fingerstick for follow-up HIV test | | | | | X | | X | X |
| Cellular activation markers in blood and cervical cytobrush | | X | X | | X | X | X | X |

[+]Immune markers include IL-6, IL-1, IL-Ra, IFN-a, TNF-a, IP-10, MCP-1, MIP-1a, MIP-3a, CCL22, sCD14, sCD163, sE-selectin, SP-selectin, IL-10, TGF-b, SLPI, HBD-2, HBD-3.

Abbreviations: S/E — Screening/Enrollment; DMPA — Depot Medroxyprogesterone Acetate; GC — Neisseria gonorrhoeae; CT — Chlamydia trachomatis; RPR — rapid plasma re-agin; HSV — herpes simplex virus; PSA — prostate-specific antigen (a marker of recent semen exposure); CVL — cervicovaginal lavage; RNA — ribonucleic acid; ART — antiretro-viral therapy; DNA — deoxyribonucleic acid.

[a]Visit 1 occurs within 3 days after completion of the 1st menses following the Screening Visit, during the follicular phase.

[b]Visit 2 occurs 2 weeks after Visit 1, in the luteal phase.

[c]Visit 3 should occur within 7 days of the onset of the 2nd menses following the Screening Visit.



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**Table 2**

Schedule of study visits (study extension).

| Study activities | Visit 8 | Visit 9 | Visit 10 | Visit 11 | Visit 12 | Visit 13 | Visit 14 | Visit 15 | Visit 16 |
|---|---|---|---|---|---|---|---|---|---|
| Weeks from contraceptive initiation[a] | 39 | 52 | 65 | 78 | 91 | 104 | 117 | 130 | 143 |
| Informed consent | X[b] | | | | | X[±] | | | |
| Interval history assessment | X | X | X | X | X | X | X | X | X |
| Follow-up DMPA injection (if applicable) | X | X | X | X | X | X | X | X | X |
| Lab tests and exams[c] | | | | | | | | | |
| Urine for pregnancy test | X | X | X | X | X | X | X | X | X |
| Blood for serum hormone levels | | X | | X | | X | X | X | X |
| For HIV-positive women only: | | | | | | | | | |
| Blood for HIV RNA | | X | | X | | X | X | X | X |
| Blood for ART levels (if on ART) | | X | | X | | X | X | X | X |
| Blood for CD4+ T cells | | X | | X | | X | X | X | X |
| Speculum pelvic examination[c] | | X | | X | | X | X | X | X |
| Cervico/vaginal Weck-Cel sponge for ART levels | | X | | X | | X | X | X | X |
| Cervical Tear-Flo for HIV RNA | | X | | X | | X | X | X | X |
| For HIV-negative women only: | | | | | | | | | |
| Fingerstick for follow-up HIV test | X | X | X | X | X | X | X | X | X |

[±]Informed consent for study extension visits (14–16) may be obtained at any visit after Protocol Amendment version 5.0 is approved.

Abbreviations: DMPA – Depot Medroxyprogesterone Acetate; RNA- ribonucleic acid; ART – antiretroviral therapy.

[a]Participants on the DMPA arm may have received injections from outside providers from the time they completed Visit 7 and the time they begin the extension study visits. Study staff will determine the optimal timing of the extension study visits to coincide with the current clinically indicated schedule of DMPA administration for these participants using guidance provided in the study Manual of Operations.

[b]Depending on timing of IRB approvals for Amendment version 4.0 and when a participant is able to report to the clinic, the first visit during the extension phase may come after Visit 8, and informed consent will be obtained at that time.

[c]Speculum pelvic examination may be performed for HIV-negative participants if clinically indicated based on responses to Interval History Assessment. Additional testing may be performed for both HIV positive and negative participants if clinically indicated.