**Linking HIV Prevention and Postpartum Care: Safety, Efficacy and Feasibility of Cabotegravir-LA PrEP in a High-Risk Breastfeeding Population in Botswana**

 **(‘Tshireletso’ Study)**

**Phase IV**

**Coordinating Centre:**

Botswana-Harvard School of Public Health Partnership for HIV Research and Education

Princess Marina Hospital

Private Bag BO 320, Bontleng Gaborone, Botswana

Tel: +267-390-2671, Fax: +267-390-1284

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Principal Investigator: Rebecca Zash

Co-Investigators:

 Ellen Caniglia

 Edmund Capparelli

 Tendani Gaolathe

 Jessica Haberer

 Michele Hacker

 Joseph Makhema

 Shahin Lockman

 Rodgers Moeng

 Chelsea Morroni

 Sikhulile Moyo

 Dinah Ramaabya

 Roger Shapiro

 Alice Sehurutshi

 Emily Shava

 Marcella Yoseph

**Linking HIV-prevention and postpartum care: Safety, efficacy and feasibility of cabotegravir-LA PrEP in high-risk breastfeeding population in Botswana**

**(‘Tshiriletso’ Study)**

**Version 2.0**

**Dated June 19, 2023 PROTOCOL SIGNATURE PAGE**

I will conduct the study in accordance with the provisions of this protocol and all applicable protocol- related documents. I agree to conduct this study in compliance with United States (US) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Conference on Harmonization Guideline for Good Clinical Practice (E6); Institutional Review Board/Ethics Committee determinations; all applicable in-country, state, and local laws and regulations; and other applicable requirements (e.g., US National Institutes of Health) and institutional policies.

**Signature of Investigator of Record:**

**Date signed: (dd/mm/yy)**

**Name of Investigator of Record (printed):**

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# Study Team Roster

**Principal Investigator:**

Rebecca Zash, MD

Division of Infectious Disease

Beth Israel Deaconess Medical Center

Botswana-Harvard Partnership

110 Francis St, Suite GB

 Boston, MA 02115

Phone: 617-275-6630 (cell)

Fax: 617-632-7706

Email: rzash@bidmc.harvard.edu

**Co-Investigators:**

Ellen Caniglia

University of Pennsylvania Perelman School of Medicine

Department of Biostatistics, Epidemiology and Informatics

Philadelphia, PA

 Phone: 717-330-6790

Email: ellen.caniglia@pennmedicine.upenn.edu

Edmund Capparelli, PharmD

Division of Clinical Pharmacology and Developmental Therapeutics

University of California, San Diego

San Diego, CA

Phone: 858-246-0009

Fax: 858-246-0025

Email: ecapparelli@ucsd.edu

Tendani Gaolathe, MD

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: 267-3902671

Fax: 267-3901284

Email: tgaolathe@bhp.org.bw

Jessica Haberer, MD

MassGeneral Hospital

Boston, MA

Phone: 617-724-0351

Fax: 617-724-1637

Email: jhaberer@partners.org

Shahin Lockman, MD, MS

Brigham and Women’s Hospital, Boston MA

Harvard T.H. Chan School of Public Health, Boston, MA

Botswana-Harvard Partnership

Phone: 617-771-8780 (cell)

Fax: 617-739-8348

Email: slockman@hsph.harvard.edu

Joseph Makhema, MD

Botswana-Harvard Partnership

Gaborone, Botswana

Phone: +267-3902671

Fax: +267-3901284

Email: jmakhema@bhp.org.bw

Mompati Mmalane, MD

Botswana-Harvard Partnership

Gaborone, Botswana

Phone: +267-3902671

Fax: +267-3901284

Email: mmmalane@bhp.org.bw

Rodgers Moeng

Division of Infectious Disease

Beth Israel Deaconess Medical Center

110 Francis St, Suite GB

 Boston, MA 02115

Phone: 617-632-7626

Fax: 617-632-7706

Email: lmoeng@bidmc.harvard.edu

Chelsea Morroni, MD, PHD

University of Edinburgh

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: 267-3902671

Fax: 267-3901284

Email: chelsea.morroni@gmail.com

Sikhulile Moyo, MPH, PhD

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: +267-3902671

Cell phone: +267 72113640 Email: smoyo@bhp.org.bw

Dinah Ramaabya

Botswana Ministry of Health and Wellness

Gaborone, Botswana

Phone:+267-3632500

Email: dramaabya@

Roger Shapiro, MD, MPH

Department of Immunology and Infectious Disease

Harvard T.H. Chan School of Public Health

Botswana-Harvard Partnership

651 Huntington Ave, FXB 402

Boston, MA 02115

Phone: 617-771-0040 (cell)

Fax: 617-739-8348

Email: rshapiro@hsph.harvard.edu

Alice Sehurutshi

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: +267-3902671

Cell phone: +267 72113640

Email: asehurutshi@bhp.org.bw

Emily Shava

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: +267-3902671

Cell phone: +267 72304005

 Email: eshava@bhp.org.bw

Marcella Yoseph

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: +267-3166625

Cell phone: +267 71789856

 Email: myoseph@bhp.org.bw

**Data Operations and IT**

Coulson Kgathi

Botswana-Harvard Partnership

Gaborone, Botswana

Phone: +267-390-2671

Email: ckgathi@bhp.org.bw

**Pharmacist**

Tshepho Frank, BPharm

Botswana-Harvard Partnership

Gaborone, Botswana

Phone: +267-390-2671

Email: tfrank@bhp.org.bw

**Project Administrator**

Ria Madison

Botswana-Harvard Partnership

Gaborone, Botswana

 Phone: 267-3902671

Cell phone: 267-72109025

Fax: 267-3901284

Email: rmadison@bhp.org.bw

**Study Funder**

National Institutes of Health

Eunice Kennedy Shriver National Institutes Child Health and Development

National Institute of AIDS and Infectious Diseases

ViiV Healthcare

**Program Officer**

Denise Russo

*Eunice Kennedy Shriver* NICHD/NIH
6710B Rockledge, Room 2156
Bethesda, MD 20817
Telephone:  301-435-6871

Fax:  301-496-8678

Mobile: 301-640-6328
Email:  drusso1@mail.nih.gov

**Study Management**

All questions concerning this protocol, including issues regarding:

Clinical medical management, toxicity management, concomitant medications, laboratory tests, and/or forms development should be sent via e-mail to rzash@bidmc.harvard.edu

Document history

|  |  |
| --- | --- |
| **Version** | **Date** |
| 1.0 | March 29, 2022 |
| 2.0 | June 19, 2023 |

Summary of major CHanges

|  |  |  |
| --- | --- | --- |
| **Section of study protocol** | **Amendment or update** | **Reason** |
| 1.0 Protocol summary | * Revised CAB-LA dosing schedule from 4 weeks then 8 weekly to 1 month then 2 monthly.
* Follow up visits were revised to “months” for consistency
* Mother and infant hair samples were incorporated.
 | * Weeks were changed to months to align with the revised Apretude package insert
* Hair samples were included per protocol LoA #1 dated
 |
| 2.8 Background: Pharmacokinetics of CAB-LA | * Background information and studies conducted using hair sample
 | * Justification for adding hair samples to current protocol
 |
| 4.0 Study design | * Revised weeks to months
* Revised the language for consenting for PK substudy
 | * Aligning with Apretude package insert
* Clarification on the consenting process for the PK substudy
 |
| 7.0 Study product and interventions; figure 1 | * Revised figure 1 on study product dosing schedule and handling of missed dosed
 | * Revised per the changes in the package insert
 |
| 8.0 Study Procedures and evaluationsTable 1 and narrative | * Revised the table to include counselling and head circumference evaluations listed in the narrative
* Revised the table to include hair sample collection
* Narrative was revised to explain hair sample collection and specimen handling
 | * Clarification of previously approved study evaluations
* Per LoA#1
 |

# Glossary

AE adverse event

AIM acceptability of intervention measure

ART antiretroviral therapy or treatment

BHP Botswana-Harvard AIDS Institute Partnership

BHHRL Botswana-Harvard HIV Reference Laboratory

CBV Combivir

CAB-LA long-acting cabotegravir

CRF case report form

DAIDS Division of AIDS, National Institutes of Health

 DBS dried blood spot

EAE Expedited adverse event (reporting)

FDA Food and Drug Administration
FIM feasibility of intervention measure

FTC emtricitabine

IAM intervention appropriateness measure

INSTI integrase strand transfer inhibitors

IRB institutional review board

IV intravenous

3TC lamivudine

LC-MS/MS liquid chromatography/tandem mass spectrometry

LFT liver function test

MTCT mother-to-child transmission

NIH US National Institutes of Health

OHRP US Office of Human Research Protection

 PBMC peripheral blood mononuclear cells

PCR polymerase chain reaction

PI protease inhibitor or Principal Investigator

PK pharmacokinetics

PMTCT prevention of MTCT

POC point-of-care

PrEP pre-exposure prophylaxis

RCT randomized controlled trial

SES socioeconomic status

SMC Safety Monitoring Committee

STI sexually transmitted infection

TDF tenofovir disoproxil fumarate

WLWHIV women living with HIV

ZDV zidovudine

# PROTOCOL SUMMARY

**Title:** Linking HIV-Prevention and Postpartum Care: Safety, Efficacy and Feasibility of Cabotegravir-LA PrEP in a High-Risk Breastfeeding Population in Botswana

**Short title:** Cabotegravir PrEP Postpartum

**Sample Size:** 500 women and their infants

**Study Population:** Women without HIV in the immediate postpartum period

**Participating Sites:** Gaborone (Princess Marina Hospital, DHMT clinics) and Molepolole (Scottish Livingstone Hospital, DHMT clinics)

**Study Design:** This is a hybrid implementation/safety study of long-acting cabotegravir (CAB-LA) as pre-exposure prophylaxis (PrEP) to prevent HIV infection in a post-partum cohort in Botswana where breastfeeding is common. We will enroll 500 women at high risk for HIV while they are admitted to the postpartum maternity ward after delivery at up to 4 government-run health care facilities in Botswana and follow them for 24 months. The first CAB-LA injection will occur within 14 days of delivery and generally before discharge from the maternity ward. Follow up injections at 1 month, and then every two months, administered at clinics where the women and their infants receive routine care or at research study sites. We will measure uptake, adherence, and implementation metrics using a mixed methods approach. We will also measure HIV incidence and safety outcomes, including postpartum depression, weight gain, and infant growth. Pharmacokinetics of CAB-LA in lactation (women, infants and breastmilk) will be evaluated in 30 mother-infant pairs enrolled in the main trial.

**Study Duration:** 24 months

**Study Intervention:** Following a negative HIV test, CAB-LA 600mg will be administered as a 3mL intramuscular (IM) injection in the gluteal muscle at enrollment, 1 month, and every two months, for a maximum of 13 injections over 24 months of follow up. The enrollment visit (first injection) will occur most often in maternity wards postpartum. Subsequent injection visits will coincide with routine pediatric, maternal or other outpatient clinical visits whenever possible.

**Primary Objectives**

**Specific Aim 1:** To evaluate the uptake, adherence, implementation metrics, acceptability and effectiveness of a CAB-LA PrEP program for women at high risk of HIV enrolled immediately post-partum, with follow up co-located with routine postpartum/pediatric care whenever feasible

**Specific Aim 2:** To evaluate the safety of CAB-LA PrEP in postpartum women and their breastfed infants

**Specific Aim 3:** To evaluate the pharmacokinetics of CAB-LA PrEP in postpartum women and their breastfed infants

**Secondary Objectives**

-- To describe maternal morbidity and mortality through 24 months and compare with existing post-partum cohorts enrolled in studies in Botswana that did not receive CAB-LA

-- To describe infant morbidity and mortality through 24 months and compare with existing post-partum cohorts enrolled in studies in Botswana that did not receive CAB-LA

-- To evaluate different HIV testing strategies for determination of breakthrough infections and development of integrase inhibitor (INSTI) resistance

-- To evaluate HIV drug resistance and health outcomes in women and infants who acquire HIV during the study

-- To describe pregnancy and birth outcomes among participants who become pregnant while taking CAB-LA PrEP

-- To evaluate HIV risk self evaluation as a predictor of incident HIV,PrEP adherence and acceptability

-- To perform a mixed methods survey to better understand reasons for declining CAB-LA PrEP

-- To perform a mixed methods survey to better understand participant preferences for HIV prevention during periods without CAB-LA coverage, including missed injections and during the pharmacologic tail

-- To investigate hair levels of CAB in mothers on CAB-based PrEP and their breastfeeding infants as a metric of maternal and infant exposure.

# BACKGROUND

***2.1.*** Preventing HIV among young women is key to ending the HIV epidemic. Every week, more than 6000 women aged 15-24 are infected with HIV, most in sub-Saharan Africa where 60% of new HIV infections are in women aged 15 to 24.1,26 Despite significant progress in treatment of HIV, and declines in overall adult HIV prevalence, young women continue to be disproportionately impacted. In Botswana, with implementation of the ‘test and treat’ strategy, we found that HIV prevalence in pregnant women declined from 25.8% in 2015 to 22.7% in 2019 in the Tsepamo study (>50% of all births in the country).27 The largest decline was seen in older women aged 35-39 (8.7%), but only a very small decline was observed in young women 15-49 (0.1%). Overall there was a 69% lower relative decline in HIV prevalence among women <25 compared to women >25.27 This persistently high HIV incidence in adolescent girls and young women has also been described throughout sub-Saharan Africa.2,28

PrEP is a highly effective intervention to address the high HIV incidence in young women, but its success depends directly on adherence. The efficacy of oral PrEP approaches 100% among those with excellent adherence, including in several studies among African women.3-10 However, uptake, adherence and persistence challenges have led to poor results for oral PrEP in women in multiple studies11 and in programmatic settings.12 For example, in the Partners PrEP study of serodiscordant couples, where adherence was ~80%, there was 72% protection against HIV in seronegative women with a seropositive partner.4 However, in the Fem-PrEP and VOICE studies, where adherence was closer to 25%, oral PrEP was not found to be protective against HIV in women.9,29 The adherence challenges of women in these studies were not based on an altered risk perception, but had more to do with stigmatization, relationship dynamics, lack of daily routines and organization, and general inexperience with the need for taking a daily medication.30 Innovative strategies to overcome PrEP-related stigma and structural barriers, such as adherence feedback and digital reminders are currently being developed and tested but these challenges are difficult to overcome.30-32

Despite progress in the rollout of PrEP, young women are underrepresented among PrEP users.13,33 Implementation of national PrEP programs in Africa, where >70% of new HIV infections among women occur, have been slower than in North America and Europe. Currently only a third of all PrEP users live in Africa.13 Within African settings, not all programs recognize young women and girls as a priority group, and have focused more on female sex workers.34 Additionally, several African countries have delayed widespread implementation of PrEP in young women because of a perceived lack of safety data in pregnancy and lactation, knowing that a majority of women who access PrEP will be of reproductive potential.2

* 1. **Women who become pregnant are at high risk of HIV acquisition, particularly in the post-partum period.** Unprotected sex that leads to pregnancy can also lead to HIV infection. Women who have multiple pregnancies are at particularly high risk of acquiring HIV.35 Among pregnant women in Botswana (Tsepamo), HIV prevalence was 10% in the first pregnancy, 21% in the second pregnancy and 39% in the third (or greater) pregnancy.27 Although the prevalence of HIV is lower in women 15-24 (11.7%) vs. women 25-34 (28.6%), multiparity remains highly (and independently) associated with HIV .27 The post-partum period is a time of particular vulnerability to incident HIV.18 A meta-analysis of 19 African studies found the incidence of HIV in the postpartum period was 2.9 per 100 person-years, a rate comparable to WHO-defined ‘high risk groups’, including female sex workers.16,36 A secondary analysis of two randomized controlled trials (RCTs) found that the increased risk may have a biologic basis, as HIV acquisition per condomless sex act was 4 times higher in post-partum women than non-pregnant/non-post partum women.17 Additionally, male partners of pregnant women may be more likely seek out new sexual partners, increasing the risk of HIV through concurrent partnerships and increased risk of incident sexually transmitted infections (STIs).37-39 Other factors including change in the frequency and type of sexual activity increase intimate partner violence.
	2. **CAB-LA in the postpartum period could have a large public health impact.** Given the high HIV prevalence (22.7% in 2019)27 and excess risk of HIV associated with having multiple pregnancies in Botswana, a substantial proportion of post-partum women are at high risk for HIV and could benefit from CAB-LA PrEP. The effectiveness of CAB-LA outside of a clinical trial is not known, but with excellent implementation and adherence, the incidence of HIV among post-partum women could approach 0.21% (the incidence in HPTN084).22  We know from a large community based trial that HIV incidence is 0.92% per year in the general population in Botswana, and more than twice as high in young women (1.87% per year).46,47 Post-partum women in Botswana are likely to have even higher incidence than all young women, given the increased risk with parity and the estimated 2-4 fold risk in the post-partum in general.16 If the true incidence of HIV in post-partum women in Botswana is 1.8%-3.8% per year without PrEP and CAB-LA is only half as effective in the real-world setting as in HPTN084 (0.42%) this is still a 4- to 9-fold decrease in HIV acquisition. An unpublished Tsepamo analysis estimates a ~5-7% absolute reduction in HIV prevalence among pregnant women in Botswana if CAB-LA were to be taken by 50% of women without HIV immediately after their first pregnancy and continued through childbearing--double the decrease in HIV prevalence of 3% seen over 5 years after the implementation of ‘HIV test and treat’.
	3. **Preventing HIV in the postpartum among women who are breastfeeding is key to eliminating pediatric HIV infection.** Increased HIV incidence in the post-partum period helps to drive the pediatric HIV epidemic through vertical transmission of HIV during breastfeeding. Incident HIV among breastfeeding women is the second leading cause of vertical HIV transmission worldwide, accounting for up to a third of new pediatric infections every year.19 Not only is the risk of breastfeeding transmission almost 3-fold higher with acute HIV infection due to high viral load, but diagnosis is often delayed due to low perceived risks and results in poor maternal and infant outcomes.16 Modeling from South Africa suggests that in a conservative scenario, where 1/3 of high-risk pregnant women took oral PrEP, there could be a 13% reduction in the number of infant HIV infections and up to 41% reduction with more optimistic uptake.49
	4. **The post-partum period is an ideal time to start PrEP because women are accessible and motivated.** Increasing PrEP uptake in young women, who are at the highest risk of HIV, has been one of the challenges in global PrEP rollout. Many HIV and PrEP programs utilize existing healthcare infrastructure to recruit and distribute PrEP, but young healthy women often do no not access routine healthcare, contributing to lower PrEP uptake in this group. In contrast to lack of routine primary care, in countries with high HIV prevalence and high fertility, the vast majority of women of reproductive age encounter the healthcare system during pregnancy. Antenatal care services are utilized by more than 90% of pregnant women in most sub-Saharan African countries with near universal integration with PMTCT services.50 By the time of delivery, most women are aware of their HIV status, including 99.5% of pregnant women in Botswana.51 Additionally, many women remain engaged with the healthcare system after delivery during post-natal maternal and pediatric care, which often includes monthly infant visits through 24 months. Taking advantage of existing infrastructure for maternal and child healthcare as an entry point for PrEP in women makes logical sense.

Pregnancy and the post-partum period is a unique time when people have high levels of motivation to get and stay healthy.20,21 Among women living with HIV (WLWHIV), even those with a history of adherence challenges are more likely to achieve viral suppression during pregnancy compared to before or after pregnancy.52-54 Fewer studies exist for PrEP, but in the Partners PrEP study, adherence to PrEP was highest among women who became pregnant (N=267) and approached 100% in the immediate pre-conception period.52 This suggests that the highest motivation to take PrEP and to attend visits where long-acting PrEP is offered may be in young women during periods of risk to themselves and their infants, and when they are in frequent contact with the healthcare system.55,56

***2.6.* Using CAB-LA may be able to overcome adherence challenges to daily oral PrEP in the post-partum period.** While motivation for health may be increased by giving birth, adherence to medications is difficult in the post-partum period. Challenges include unpredictable daily schedules, extreme fatigue, increased stress from new parenting responsibilities and increased financial pressures.56,57 In a pooled meta-analysis of WLWHIV, including 51 studies in both low- and higher-resourced settings, post-partum adherence to ART was significantly lower in the post-partum period compared with pregnancy (53% vs. 76%).58 This translated directly to decreased rate of viral suppression, and vertical transmission via breastfeeding, among women who stopped taking ART.19,54 There are less data on post-partum adherence to PrEP, but given the well-described challenges with adherence for oral PrEP in non-pregnant women, there are likely to be even larger challenges with post-partum daily oral PrEP. Long-acting CAB-LA, with bimonthly injections, has the potential to overcome adherence challenges to daily medication in the post-partum period. In the clinical trial setting (HPTN 084), adherence to CAB-LA was higher than oral tenofovir/emtricitabine (TDF/FTC) and was associated with a 9-fold decrease in HIV incidence in non-pregnant women.22 In a real-world setting, if effective strategies can be identified to facilitate PrEP persistence, then CAB-LA PrEP should be extremely effective. This approach also fits with community preferences. Studies on PrEP acceptability from VOICE and Fem-PrEP study participants found that women wanted PrEP that was low burden, fit in easily with their lifestyle and was effective enough that they could feel peace of mind.59 African women specifically named injectable long-acting formulations as a preferred theoretical strategy for PrEP before this technology was developed.59,60

***2.7.* Using an implementation science framework to evaluate a post-partum CAB-LA program provides an evidence-based approach to measuring the quality and effectiveness of this innovative strategy.** Implementation science can help bridge the divide between the efficacy of an intervention in a clinical trial setting and the effectiveness of the intervention in a real-world setting. A multi-faceted, systematic approach to studying implementation typically incorporates a systems perspective, investigates generalizability, is transparent, and employs practical measures that encourage participation from community and stake-holders.61,62 Among possible approaches, the Proctor framework is well established and has been previously used in African settings and with PrEP implementation.63,64 This framework enables measurement of implementation outcomes in relation to intervention outcomes, as well as impact on clients and is an ideal approach to evaluate a CAB-LA PrEP program.

***2.8.* The pharmacokinetics of CAB-LA may differ in post-partum women and have additional implications for breastfeeding infants.** No current pharmacokinetic (PK) data exists in post-partum women on CAB-LA. When CAB is given to non-pregnant, non post-partum women as a 600mg intramuscular (IM) injection, 80% achieve plasma concentration 4x PA-IC90 after the first injection steady state is achieved after the third injection.65 CAB level is related to the elimination half-life, which ranges from 5.6 to 11.5 weeks and is driven primarily by the rate of absorption from the intramuscular site and secondarily by metabolism via the UGT1A1 pathway.66 The elimination half-life is longer (resulting in higher CAB troughs) when absorption is slower, which has been observed in obesity.65 In the post-partum period, women have higher BMI due to pregnancy weight gain and so the elimination half life may be longer. On the other hand, UGT1A1 may be enhanced by higher levels of progesterone in pregnancy, leading to more rapid elimination of CAB-LA in the early post-partum period before progesterone levels normalize.67 These effects are more likely to lead to higher CAB levels and so should not impact efficacy. However, it remains important to have specific pK data for post-partum women to evaluate changes in CAB levels over time and to determine whether different dosing schedules may be possible in this population. There are also no pK data in infants exposed to CAB during breastfeeding. We expect levels of CAB clinically insignificant given the low level of exposure to other integrase strand transfer inhibitors (INSTIs) via breastmilk (e.g. 3% with DTG) and relatively lower affinity for proteins in breastmilk compared with extremely high affinity for serum protein with CAB (99.7%). Data are needed in infants to determine if CAB levels will be high enough to impact the development of INSTI resistance if a transmission occurs, as this will directly impact treatment decisions as DTG becomes first line for children with HIV.68

Additionally, drug levels in infant hair are highly effective in quantifying long-term post-natal exposure to maternal medications and prior studies have measured ARVs in paired mother-baby hair samples to assess maternal-to-infant transfer during breastfeeding. Drug levels of CAB among mothers will play a critical role in determining the onset of protection, the duration of protection during the PK tail, and assessing PrEP failures. Traditional Pharmacokinetic (PK) studies are important, but they are also labor intensive, costly and rely on invasive blood draws. Hair sampling is less invasive and less expensive, so can be done on a larger population, allowing for an understanding of PK in a broader population. While mother and baby hair samples have been used to assess maternal to infant transfer of a variety of ARV agents during pregnancy and breastfeeding in the US and in Uganda[, but this is the first study to evaluate CAB-LA levels in hair in a breastfeeding population.

***2.9.* Prior safety of INSTIs during lactation is reassuring**. Lactation safety data are needed specifically for CAB-LA in women without HIV, but it is reassuring that other INSTIs appear to be safe during breastfeeding in WLWHIV. INSTI-based DTG-based ART is used in >85% of patients in most high HIV incidence countries supported by PEPFAR, where >1 million WLWHIV per year become pregnant, and breastfeeding is recommended.69 Thus, hundreds of thousands of infants have been exposed to DTG during lactation and there are no reported safety concerns. There were also no safety concerns identified in two RCTs (Dolphin-2 and IMPAACT 2010) when DTG was initiated during pregnancy with close monitoring of mothers and infants during breastfeeding.22,23 Serum concentration of DTG in breastfeeding infants whose mothers take DTG for HIV treatment is approximately 3% of maternal serum concentration.70,71 No similar data exist for CAB-LA. Pre-clinical animal studies did not identify any problems with growth and development of rats exposed to CAB in utero.66 Nine women who became pregnant during an HIV treatment trial with CAB-LA/rilpivirine, but all stopped ART soon after conception (or terminated the pregnancy).72 Transplacental transfer of CAB-LA appears to be lower than DTG, which may be predictive of lower risk of fetal toxicity, but does not necessarily predict concentration of drug in breastmilk or infants exposed to breastmilk.73,74

***2.10.* Studying CAB-LA PrEP in lactating women is ethically sound.** Reassuring data from other INTSIs, the need for effective PrEP in women of reproductive potential, and the large potential benefits of preventing HIV infection during breastfeeding provide the foundation to propose a closely monitored study of CAB-LA in lactating women at high risk of HIV. This approach is driven by three ethical principles, which are highlighted in the recently published findings of the study of the ‘Pregnancy and HIV/AIDS Seeking Equitable Study’ (PHASES).75 The first is ‘equitable protection from drug-related risks’. Typically, safety data would be collected in post-marketing surveillance using passively collected data from patients in routine care. This process is inefficient and requires women to use medications without safety data. By collecting safety data in a closely monitored setting shortly after regulatory approval, this study will quickly be able to identify drug-related risks if they exist. The second ethical principle is ‘equitable access to first line medications’. Without safety data, CAB-LA is unlikely to be included in clinical guidelines for women of reproductive potential and clinicians are less likely to prescribe to pregnant or lactating women. Given that CAB-LA appears to be up to 9-times more effective at preventing HIV, continued use of TDF/FTC in women would be considered second-line. The third ethical principal is ‘equitable respect for pregnant and lactating women’s own health.’ Sufficient data exist for CAB-LA safety and effectiveness in non-pregnant women that it is feasible to design an informed consent process that allows women to weigh risks and benefits of joining a CAB-LA PrEP study.

***2.11.* A strategy of offering women PrEP while on the post-partum ward after delivery is innovative and efficient and will provide the first estimate of real-world effectiveness in this population**. This will be among the first studies to provide data on the effectiveness of CAB outside of an RCT, and the first to look at effectiveness in the post-partum period. This will also be the first study to evaluate implementation challenges and with long-acting PrEP in the post-partum period. Barriers to adherence related to attending every other month injections are likely to be different than barriers to adherence to remembering to take daily oral PrEP. We expect unique challenges with intermittent injections may relate to transportation, travel or relocation for work and perceived stigma during interactions with healthcare workers, but we are likely to uncover many unexpected barriers and challenges in specific sub-populations. Given similarities in healthcare infrastructure for pregnant and post-partum women, this strategy for initiation and follow up of CAB-LA PrEP is expected to be easily scalable in Botswana and other high HIV incidence settings, if found to be beneficial.

***2.12.* Performing a PK study in lactating women and their infants before widespread availability of a drug is unique, and will lead to safer and more rapid rollout of CAB-LA in women of reproductive potential.** For antiretrovirals, there is an average 5-year lag between FDA approval of a medication and pregnancy/lactation PK data.76 CAB-LA received FDA approval at the end of December 2021, and so this study would provide PK data in within approximately one year of approval. Given the clear need and demand for CAB-LA PrEP in women, performing the PK studies now will allow will for more rapid—and safer—rollout of CAB-LA PrEP in settings with high HIV incidence in women of reproductive potential.This approach is in line with recent calls to action from multiple organizations (Pregnancy and HIV/AIDS: Seeking Equitable Study [PHASES], American College of Gynecologists [ACOG], Society for Maternal Fetal Medicine [SMFM], the World Health Organization [WHO]) to include pregnant and lactating people in research. We will also have the unique opportunity to provide the first pK data on CAB levels in infants. While infant toxicity is unlikely given low levels of infant exposure to other INSTIs via breastmilk, it is clinically important to understand whether infant CAB levels will be high enough to cause development of HIV resistance in an infant who becomes infected while exposed to CAB as this will impact empiric infant treatment decisions.

***2.13.* The Botswana-Harvard AIDS Institute Partnership (BHP).** The Botswana-Harvard AIDS Institute Partnership (BHP) was established in 1996 as a signed agreement between the Ministry of Health (MOH) of Botswana and the Harvard School of Public Health AIDS Initiative. BHP now maintains one of the largest research laboratories for HIV/AIDS-related work in Africa. Multiple studies enrolling more than 7,000 participants have been conducted at BHP, including HIV vaccine, prevention of mother-to-child transmission (PMTCT), ART trials (Phase I through Phase III), as well observational studies; most of these studies have been NIH-funded. In 2006, BHP was selected as a Division of AIDS (DAIDS) “Clinical Trials Unit” (CTU) for the AIDS Clinical Trials Group (ACTG) and the International Maternal Pediatric Adolescent AIDS Clinical Trials (IMPAACT) group, and is now also a site of the HIV Prevention Trials Network (HPTN). BHP works in close collaboration with the MOH in the conduct of clinical trials.

# PRIMARY OBJECTIVES AND Study Hypotheses

**Specific Aim 1: To evaluate the uptake, adherence, implementation metrics, acceptability, and effectiveness of a CAB-LA PrEP program for women at high risk of HIV enrolled immediately post-partum, with follow up co-located with routine post-partum/infant care whenever feasible.** This uncontrolled, open-label study will be conducted at two sites in Botswana, using established clinical research infrastructure provided by BHP. In this hybrid implementation and safety study, our primary objectives will be to measure uptake, programmatic retention and acceptability of CAB-LA PrEP in the post-partum period and HIV incidence in our study populations over 24 months of follow up**.**

**Hypothesis:** Post-partum CAB-LA PrEP will have high uptake, acceptability, and adherence, particularly among women who are primary caregivers for their infant, regardless of location and income. Maternal HIV incidence with CAB-LA will be <0.4% per person-year.

**Specific Aim 2: To evaluate the safety of CAB-LA PrEP in post-partum women and their breastfed infants.** The primary objectives will be to measure incident vertical transmission of HIV, drug resistance in women and infants with breakthrough HIV infections, lab abnormalities, treatment limiting adverse effects, weight gain, post-partum depression and infant growth. These outcomes will be compared with an ongoing observational study of post-partum women enrolled at delivery without CAB-LA PrEP exposure.

**Hypothesis:** Benefits of CAB-LA for preventing HIV will outweigh risks resulting from weight gain or other adverse effects.

**Specific Aim 3: To evaluate the pharmacokinetics of CAB-LA PrEP in post-partum women and their breastfed infants.** In 30 breastfeeding women,we will measure CAB-LA levels in maternal serum and paired breastmilk and infant plasma samples at 4 time points (1- and 5-month troughs and peaks 1 week after the 1-month injection and 1 week after the 5-month injection). CAB-LA levels will also be measured in all incident maternal or infant HIV infection.

**Hypothesis:** Concentration of CAB-LA in post-partum women will be in a clinically acceptable range when compared with target values for adults. Infant CAB-LA concentration will be <3% of maternal serum concentration.

# STUDY DESIGN

***Cabotegravir-LA PrEP (all participants)***

Following a negative HIV test, CAB-LA 600mg will be administered as a 3mL IM injection in the gluteal muscle at enrollment (within 14 days of delivery), 1 month, and then every two months, for a maximum of 13 injections over 24 months of follow up. The enrollment visit (first injection) will generally occur in maternity postpartum (but may occur at an outpatient clinic or study clinic in a minority of participants). Subsequent injection visits will coincide with routine maternal/pediatric visits whenever feasible and may occur at clinical visit sites or at the study clinic site.

***PK Sub-study (30 participants)***

A pharmacokinetic (PK) sub-study will occur among 30 breastfeeding women and their infants, who will have a separate place to sign specifically for the PK sub-study on the main study consent form. For these participants, maternal plasma and paired infant plasma and breast milk sample will be collected immediately before (trough) and 1 week after (peak) the second (1 month) and fourth (5 month) injections.

# STUDY SITES AND STUDY POPULATION

## Clinical and Laboratory Sites

**Clinical Sites**

General information about the study and CAB-LA PrEP will be given to groups of women waiting for appointments, and/or during health talks, at antenatal clinics in the catchment area for delivery at our enrollment sites. Individual participants will be approached while they are admitted to the maternity ward either before or after delivery. Targeted screening for the study will occur Princess Marina Hospital (PMH) in Gaborone, Scottish Livingstone Hospital (SLH) in Molepolole and DHMT clinics with maternity wards in greater Gaborone and Molepolole catchment areas. Although most women approached will have delivered at these sites, some deliveries occur prior to admission or at other local clinics, and delivery site is not a criterion for enrollment. Study follow-up will be performed in conjunction with outpatient pediatric or maternal visits at clinics within the greater Gaborone/Molepolole catchment area, or at a designated BHP clinic near each hospital/clinic site.

**Laboratory Sites**

HIV screening will be done for the enrollment visit by trained personnel using point of care 4th generation HIV Ag/Ab combo testing (Alere HIV Combo or Alere Determine HIV-1/2). In rare circumstances where point-of-care (POC) testing is not available at enrollment, HIV testing will be performed at the Botswana-Harvard HIV Reference Laboratory (BHHRL), adjacent to Princess Marina Hospital in Gaborone using 4th generation EIA (Determine HIV Ag/Ab combo) or by HIV RNA or DNA per BHHRL standard protocol. Point of care testing at subsequent injection visits will also use 4th generation HIV Ag/Ab combo and will be performed by trained study staff. If the 4th generation POC test is not available at the injection visit, we will use 3rd generation POC HIV-1/2 test (Determine or Unigold) and collect blood for HIV testing at the BHHRL using a 4th generation EIA.

Laboratory specimens will be shipped to the BHHRL in Gaborone on a regular basis. Hematology, chemistries, confirmatory HIV testing, HIV-1 RNA measurements and CD4 counts and HIV genotyping (for participants who seroconvert) will be carried out at BHHRL. Techniques will be performed according to the most current approved protocols at the BHHRL. Samples may occasionally be sent between BHP sites or to designated back-up laboratory sites.

PK testing will be performed in the lab of Dr. Edmund Capparelli at the University of California, San Diego, California USA (see 8.12 below).

Hair samples will be stored at BHP and later analyzed at the University of California, San Francisco in the lab of Dr. Monica Gandhi (see section 8.3 below).

## Recruitment Process

Trained research staff will review maternity records daily to identify women at substantial risk for incident HIV based on local epidemiologic criteria (age <30 or <3 prior pregnancies) who had at least one prior documented negative HIV test during pregnancy or while admitted for labor and delivery. Potentially eligible participants will be approached using a standard script and given information about the study and about PrEP, including an interviewer-administered questionnaire about socio-demographics and sexual history. If interested, further information on the CAB-LA PrEP study will be provided. Of note, the self-administered questionnaire will be used as a tool to guide discussion on risk and benefits of PrEP as part of the informed consent process, but will not be used to make eligibility determinations. While individual risks will be variable, we do not want to risk excluding people who are vulnerable to HIV but who are unwilling to disclose their risk, or who are at risk for reasons not included in the questionnaire. For participants who decline participation in the overall study, this information will not be stored (except for those who consent to be in the acceptability study). For participants who enroll, we will collect questionnaire responses on HIV risk assessment after the informed consent process.

During the enrollment process, all eligible participants will be offered optional enrollment in the PK sub-study until the limit of 30 participants have been enrolled. The choice of whether to breastfeed an infant will be determined by the mother prior to enrollment in the PK sub-study using information and guidance provided during routine antenatal and post-natal care (from non-study staff). All eligible participants will also be offered optional enrollment in the mixed-method acceptability sub-study at enrollment until the limit of 20 participants have been enrolled. For this sub-study, we will enroll 10 participants from each geographic area (Gaborone and Molepolole). Participants who decline participation in the study after study information has been given will be asked if they would like to share their reasons for declining the study. If they are interested, they will be separately consented until the sample size of 20 participants has been reached, 10 participants from each region (Gaborone and Molepolole).

## Study Consent

If women wish to enroll, written informed consent will be obtained. The protocol-specific consent forms describe the study product to be used and all aspects of protocol participation. Elements of informed consent are included as described in Title 45, CFR Part 46 and Title 21 CFR, Part 50, and in the International Conference on Harmonisation (ICH) E6, Good Clinical Practice: Consolidated Guidance 4.8.

The consent process will describe the purpose of the study, the follow up requirements, the risks and benefits of the study, and alternatives to CAB-LA PrEP available in the public sector (such as oral TDF/FTC PrEP).

There will be a written consent form for the main study of CAB-LA PrEP and the PK sub-study. A separate consent form will be used for an acceptability assessment among a subset of participants in the main CAB-LA study and individuals who decline the CAB-LA study.

# Inclusion / Exclusion Criteria

## Inclusion Criteria

1. Mother 18 years of age or older and willing and able to provide an informed consent
2. < 14 days after delivery (calendar day of birth = day 0)
3. Negative HIV screening test (conducted at the time of enrollment)\*
4. Mother <30 years old or has had < 3 prior pregnancies (Gravida 1, 2, or 3 including this pregnancy)
5. Plan to stay and receive postpartum and pediatric care in the Gaborone or Molepolole region for 24 months

\*Women who test positive at the time of enrollment visit will not continue with the study and will instead be referred to local HIV care.

## Exclusion Criteria

1) Receiving carbemazapine, phenobarbital, phenytoin, oxycarbazepine, rifampin, rifabutin, rifapentine, systemic dexamethasone (>1 dose oral/IV), or St. John’s wort

2) Suspected to have, recently diagnosed with, or on treatment for TB (due to interaction with rifampin)

3) Previous hypersensitivity reaction to CAB or other INSTI

4) Unstable medical or psychiatric condition making it unlikely they will be able to adhere to injections every 2 months

5) Plan for pediatric and post-partum care outside the government system (private clinics)

6) Inflammatory skin condition that compromises the safety of the intramuscular injection

7) Weight <35kg

## Additional Inclusion Criteria for PK Substudy

1. Plan to breastfeed for at least 6 months
2. Able and willing to attend extra study visits

## Additional Exclusion Criteria for PK Sub-study

1) Infant condition that makes 6-month survival unlikely (e.g. extreme prematurity, major congenital abnormality, neonatal sepsis)

2) Contraindication to collection of breastmilk (e.g mastitis or other breast disease)

***6.5 Additional Requirement for Inclusion in the Mixed-Methods Sub-Study of CAB-LA acceptability***

1) Able and willing to answer additional questionnaire and attend interview

2) For participants who are not enrolled in the main CAB-LA study, they must have eligibility criteria but declined enrollment in the CAB-LA study after hearing the details of the study.

\*no additional exclusion criteria

# STUDY PRODUCT AND INTERVENTIONS

## Long-Acting Cabotegravir Description, Supply, and Storage

The study product CAB-LA will be provided by ViiV Healthcare. CAB-LA has been approved for PrEP in the United States for at-risk adults and adolescents weighing at least 35kg who have a negative HIV test. Each injection is 600mg (3mL) given intramuscularly (IM). The package insert indicates that an oral lead-in period is optional. We do not plan to use an oral CAB lead-in period. There were no concerning adverse reactions during the oral lead in phase of HPTN 083 (randomized clinical trial of CAB-LA PrEP in men who have sex with men and transgender people) and HPTN 084 (randomized clinical trial of CAB-LA in women at risk for HIV) and during the FLAIR trial extension when CAB-LA was used without oral lead-in and in combination with rilpivirine for treatment of HIV. Oral lead-in is optional in both CDC guidelines and package insert.82 Our participants will be in a monitored setting while receiving their first injection and so will have close monitoring for immediate adverse effects.

Dosing recommendations without oral lead-in are that the first two injections be given one month apart for 2 doses, and then injections are given every two months, with injections given up to 7 days before or after the scheduled date. If any injection is missed by more than 7 days, daily oral PrEP is recommended until the next dose of IM cabotegravir can be given. If the second injection is less than or equal to 2 months after the first injection or if the third or greater injection is less than 3 months after the previous injection, then injections every 2 months can be restarted. If the second injection is greater than 2 months, or the third and greater injection is greater than 3 months since prior injection, then cabotegravir should be restarted with 2 injections one month apart before resuming the every-2-month schedule of injections(Figure 1).

Figure 1 (from package insert)





CAB LA is formulated as a sterile white to slightly coloured suspension containing 200 mg/mL of CAB LA for administration by IM injection. Each vial is for single use containing 3mL (600MG), and does not require dilution prior to administration. CAB LA injectable suspension will be stored below 30o C and not frozen. CAB-LA will be drawn up into syringes for injection in accordance with pharmacy policy and procedures. After withdrawal of the CAB-LA suspension from the vial into a syringe, we will not exceed 2 hours between withdrawing the contents of the vial(s) into a syringe and administration to the study participant. The prepared CAB LA study product in a syringe may be exposed to temperature up to 30o C (86F). Injections will be performed by trained study staff and injected into the gluteal muscle.

Any empty vials, unused portion of entered vials, unused suspension that contains study product or product that does not meet the above-described storage conditions will be discarded in a biohazard containment bag and incinerated or autoclaved in accordance with institutional or pharmacy policy.

## Schedule of Intervention and Dosing

At enrollment and follow up visits, an HIV screening test will be performed, participants will receive counseling on contraceptive options and STI and HIV screening and prevention, maternal blood sample and infant dried blood spot (DBS) will be taken, maternal weight and blood pressure and infant weight and length will be measured.

At the enrollment visit, the rapid POC 4th generation EIA HIV test will be performed (Alere HIV Combo or Alere Determine HIV-1/2) (or if the rapid POC 4th generation is unavailable, blood will be sent to BHP lab where 4th generation EIA, or HIV RNA or DNA PCR may be substituted) and the result will be available before CAB-LA dosing occurs (in most cases, the injection will occur on the same day as the consent and screening process, but may occur up to 14 days after the consent and screening process; see section 5). Women who test positive for HIV at enrollment will discontinue study participation, and will be referred for prompt confirmatory HIV testing per Botswana guidelines, as well as ART initiation and HIV care if confirmed to have HIV. For all subsequent visits, the CAB-LA dosing will proceed after rapid POC 4th generation test. If the rapid POC 4th generation is not available at the time of injection visit, will use a standard of care POC test in Botswana, currently 3rd generation HIV-1/2 ELISA, Determine or Unigold, and blood will be drawn and sent to BHHRL for 4th generation EIA test, but injection will not be delayed to wait for result of 4th generation EIA ; see section 8.5.2 for procedures in the event of a positive HIV test.

Following a negative 4th generation (or RNA or DNA PCR) HIV test (enrollment visit), CAB-LA 600mg will be administered as a 3mL IM injection in the gluteal muscle. Injections will occur at enrollment, 1 month, and then every 2 months, for a maximum of 13 injections over 24 months of follow up. The enrollment visit (first injection) will be given by trained study staff and will usually occur while still admitted in maternity postpartum and participants will be monitored for 30 minutes after injections. If discharged from maternity, a woman can return to the maternity ward or to a BHP study clinic for the first injection. The subsequent injections will be given by trained study staff and will usually occur at the clinic where the participant attends post-natal, sexual and reproductive health, and/or pediatric care. If the injection cannot be given at the participant’s clinic for any reason, a woman can come to a BHP study clinic for any follow-up injection.

# STUDY PROCEDURES AND EVALUATIONS

## Clinical Evaluations and Procedures

Study follow-up for participants will be performed in conjunction with routine outpatient pediatric or maternal visits at clinics within the greater Gaborone/Molepolole catchment area, or at a designated BHP clinic near each study site. When follow up visits are taking place at the government clinics, the participant will notify our research team when they arrive for their scheduled appointment. If it is feasible for CAB-LA to be given to participants within 2 hours of preparation of the syringe, we will conduct the injection visit at the government clinic. If the study team determines it will not be possible to give the CAB-LA injection within the window (either due to clinic location, lack of available space in the clinic, driver availability, timing of clinic visit, or other logistic constraint) then transportation will be arranged for the participant to come to the BHP offices for the study visit.

[**Table 1**](#_bookmark34) outlines the evaluations at each study visit.

|  |  |
| --- | --- |
|  | **Table 1. Schedule of follow-up and laboratory testing** |
|  |  | **Study Month** |
| **Evaluation/Testing** | **0** | **1** | **1month + 1 week** | **3** | **5** | **5months+1week** | **7** | **9** | **11** | **13** | **15** | **17** | **19** | **21** | **23** | **24\*** |
|  | ***Maternal Evaluations and Procedures*** |
| **CAB-LA Injection** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X |  |
| **HIV POC (4th Gen)** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **Stored plasma and cell pellet** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **Complete blood count**  | X |  |  |  |  |  | X |  |  | X |  |  | X |  |  | X |
| **LFTs**  | X |  |  | X |  |  | X |  |  | X |  |  |  |  |  | X |
| **HgbA1c** | X |  |  |  |  |  |  |  |  | X |  |  |  |  |  | X |
| **Lipids**  | X |  |  |  |  |  |  |  |  | X |  |  |  |  |  | X |
| **Weight, BP** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **Hair Sample**  |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **Depression screen**  | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **Counseling on contraceptive options, HIV prevention options** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **CAB-LA Acceptability questionnaire\*\*** | X |  |  |  |  |  | X |  |  |  |  |  | X |  |  |  |
| **Questionnaires for analysis of implementation metrics+** | X |  |  |  |  |  | X |  |  |  |  |  | X |  |  |  |
|  | ***Infant Evaluations and Procedures***  |
| **Infant weight, length,head circumference** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **Infant DBS** | X | X |  | X | X |  | X | X | X | X | X | X | X | X | X | X |
| **Infant Hair Sample (\*only if breastfeeding)** |  | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| ***PK sub-study (N=30)*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| **Maternal and infant plasma, and breast milk CAB level**  |  | X | X |  | X | X |  |  |  |  |  |  |  |  |  |  |
|  | ***Mixed Methods Acceptability sub-study (N=20 enrolled participants and 20 who declined)*** |
| **Questionnaire and in-depth interview (enrolled)** | X |  |  |  |  |  | X |  |  |  |  |  | X |  |  |  |
| **Questionnaire and in-depth interview (declined enrollment)** | X |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

\*For any participant who comes off study early, there will be an ‘early discontinuation visit’ which will include all evaluations and procedures included in the 24-month study visit.

\*\*Acceptability questionnaire will include 1) Acceptability of Intervention Measure (AIM), 2) Intervention Appropriateness Measure (IAM) and 3) Feasibility of Intervention Measure (FIM). The acceptability questionnaire will also be administered to any participant who chooses to stop taking CAB-LA during the study at their next study visit

+Questionnaires for analysis of implementation metrics include: Demographics, medical and obstetric history, food security, perceptions of PrEP, sexual history and self-assessment of HIV risk, social support, identification of primary infant caregiver, infant feeding method, partner involvement, partner HIV status, availability of transportation and time to travel to clinic.

## Participant Sampling

Samples will be obtained as outlined in **Table 1**. Please see below discussion of blood volume limits. To collect hair samples, up to 2cm of the proximal hair sample (strand) will be cut.

## Specimen Management

All specimens will be sent to the BHP Laboratory in Gaborone, usually on the day of collection. Complete blood counts, chemistries, 4th generation HIV Ag/Ab EIA, confirmatory HIV testing, HIV RNA and/or DNA, and CD4 cell counts will be performed at these or at BHHRL/NIH-designated back-up laboratories. Validated laboratory values will be entered by the study team into the REDCap database.

Stored PK specimens will be shipped periodically to Dr. Capparelli’s lab in California. Initial laboratory data review will evaluate data completeness, data checks against the clinical database (e.g., dates of samples/results), and assessment for outliers.

The proximal 2.0 cm of the hair strand will be cut into 4 equal pieces at the first two visits (month 1 and 3) and into 2 equal pieces for the remainder of the visits. Stored hair samples will be stored at room temperature and shipped periodically to Dr. Gandhi’s lab in California.

## Blood Volumes

The NIH recommends a limit of 5 mL/kg per single blood draw and a limit of 9.5 mL/kg in any 8-week period. We will draw no more than 60 mL at any draw during the study (in the case of a participant seroconversion and need for additional testing) and <16 mL at most visits. If additional clinical blood draws occur, study staff will account for these draws and make adjustments in the volume drawn for the study if needed.

## Schedule of Evaluations

* + 1. **Evaluations in the Setting of Remaining HIV-uninfected:**

All visits will assess for diagnoses, signs and symptoms of any Grade.

Enrollment Month 0 Visit

* + - * Confirmation of signed and dated consent form
			* Confirmation of negative result on HIV 4th generation Ag/Ab POC test (or HIV 4th generation Ag/Ab EIA, HIV RNA or DNA if 4th generation POC test is not available) since admission for labor and delivery\*
			* Counseling on contraceptive options, STI screening options, and HIV prevention (including availability of oral PrEP through public healthcare system)
			* Depression Screening
			* CAB-LA Acceptability questionnaire
			* Questionnaires related to implementation metrics: socio demographics, sexual activity, self-assessed HIV risk, structural, community and individual factors
			* Mixed-method acceptability assessment including qualitative interviews among up to 20 participants who choose to join the study and 20 participants who choose not to join the study (separate consent will be used)
			* Measure maternal weight and blood pressure and infant weight, length and head circumference
			* CAB-LA injection given with monitoring for 30 minutes post-injection
			* Maternal Samples obtained for: CBC, LFTs, HgBa1c, lipids, stored plasma and cell pellet.
			* Infant DBS obtained
			* Set up follow up visit for week 4 injection

\*Women who receive a positive HIV screening test result at enrollment will discontinue study participation.

Follow up visits month 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 (all participants)

* + - * HIV point-of-care screening test
			* Counseling on contraceptive options, STI screening options, and HIV prevention (including availability of oral PrEP through public healthcare system)
			* Depression Screening
			* CAB-LA Acceptability questionnaire (month 7, month 19 only)
			* Questionnaires related to implementation metrics: socio demographics, sexual activity, self-assessed HIV risk, structural, community and individual factors (month 7, month 19 only)
			* Mixes-methods acceptability assessment including qualitative interviews in subset of 20 participants (month 7, month 19 only)
			* HIV risk self-assessment, Depression Screening
			* Measure maternal weight and blood pressure and infant weight, length and head circumference
			* CAB-LA injection given
			* Maternal Samples obtained for: stored plasma and cell pellet (all visits), CBC (month 7,13,19 only) LFTs (month 3,7,13 only), HgbA1c and lipids (month 13 only)
			* Infant DBS obtained
			* Hair sample taken for mothers and breastfeeding infants
			* Set up follow up visit for next injection

Month 1, month 1 + 1 week, month 5, month 5 + 1 week Visit for subset enrolled in PK study only (N=30)

* + - * Samples obtained for: maternal blood, infant blood, and maternal breastmilk CAB levels, maternal albumin, maternal and infant hair samples

Month 24 (final) Visit

* + - * HIV point-of-care screening test
			* Counseling on contraceptive options, STI screening options, and HIV prevention(including availability of oral PrEP through public healthcare system)
			* Depression Screening
			* Measure maternal weight and blood pressure and infant weight, length and head circumference
			* Maternal Samples obtained for: CBC, LFTs, stored plasma, lipids and HGBA1c
			* Infant DBS obtained
			* Hair sample for mothers and breastfeeding infants
			* End of study procedures(including plan for continuation of PrEP or CAB-LA tail coverage if applicable)
		1. **Evaluations in the Event of HIV Seroconversion:** We will use HIV EIA point of care test (4th generation Ag/Ab combo) to screen for HIV infection at study visits. If the 4th generation POC test is unavailable at the time of enrollment, we will substitute a 4th generation EIA (at BHHRL), HIV RNA or DNA PCR test. If 4th generation POC is not available at follow up injection visits, we will substitute the SOC HIV test in Botswana (currently 3rd gen POC HIV ½) and draw blood to test using 4th generation EIA at BHHRL. This HIV testing strategy was chosen to be in line with the most likely way Botswana will implement a future CAB-LA PrEP program for the country. This strategy differs from CDC guidelines updated in 2021 which suggests HIV RNA PCR tests prior to starting CAB and for monitoring incident HIV infection with CAB-LA PrEP. This recommendation is based on a recent report from HPTN 083 that noted that detection of HIV using standard HIV ELISA led to delayed detection of incident HIV up to 16 weeks in 4 patients.91 However, in most high HIV prevalence settings, including Botswana, the resources, logistics and cost of HIV viral load testing with every 2-month injections would effectively eliminate the possibility of using CAB-LA as PrEP. We believe that the potential benefits of preventing a large number of incident HIV infections in women (and associated vertical transmission via breastmilk) using CAB-LA (9-fold higher protection than with oral PrEP) outweighs the potential risks associated with the delay of an HIV diagnosis in a very small number. However, we will carefully monitor for delayed diagnosis in our study by storing plasma at every injection visit. When an HIV infection is identified, we will retrospectively use HIV PCR on stored samples to determine when the infection occurred, and test prior samples with 4th generation HIV Ag/Ab EIA to determine whether a lab-based test (rather than POC) 4th generation test would have identified the seroconversion sooner. Additionally, we will evaluate whether the delayed diagnosis has any adverse effect by looking for INSTI resistance, following participants on ART to ensure viral suppression, evaluating for vertical transmission (and the timing of transmissions, if any) via breastfeeding, and assessing for OIs or other clinical impact. We will evaluate CAB-LA levels from maternal serum and maternal hair samples for all participants who seroconvert.

CAB-LA will be discontinued in any participant with an indeterminate or positive HIV screening test, we will follow Botswana guidelines for confirmation (currently a second HIV screening test). Additionally, blood will be immediately drawn and sent for HIV EIA (Determine HIV ½ Ag/ab combo) and viral load (HIV-1 RNA). If viral load is positive (HIV-1 RNA is detected), we will send a blood sample to BHHRL for HIV resistance testing (and additional infant samples will be sent if < 3 months since cessation of breastfeeding). Given the reports of challenges to confirming HIV infection in HPTN 083 and 084, we may send additional HIV tests (to include Genius, HIV DNA, alternative PCR platforms) when needed for clinical management. If HIV infection is confirmed, the participant (and infant, if infected) will be referred immediately to their local HIV clinic (IDCC) for ART initiation, which is provided at no cost through the government. The study team will work closely with IDCC providers for ART questions and to provide existing data on ART for those who become infected while taking CAB-LA. Participants who seroconvert during the study will continue with scheduled study follow up visits, and all procedures will be performed except for HIV testing, CAB injections and acceptability/implementation metric questionnaires. These participants will be evaluated for viral suppression rates, opportunistic infections, hospitalizations and deaths through the study and through the local healthcare system.

## Reimbursement

We will offer reimbursement for participant transport (or will provide patient transport), and 100 Botswana Pula (approximately $10USD) per attended scheduled visit to compensate for participant time commitment and to cover the cost of text to the study team when they arrive at their local clinic on the day of scheduled injection visit. We will also offer reimbursement for unscheduled visits that are requested of the participant for follow-up tests.

## Participant Retention, Missed Visits, Study Withdrawal

We anticipate that our study will be able to achieve high retention, as has been our experience to date with prior studies in Botswana, with < 5% loss to follow-up 25,95,96 (women who choose to stop taking CAB-LA PrEP will continue to be followed and not considered LTFU unless we are unable to contact them). Resources will be devoted to cohort retention, transport provided as needed, and participants will be compensated fairly for their time at a rate approved by the Botswana IRB. Most women who enroll in BHP studies have a cell phone number or a family member with a cell phone number.

We will perform calls for any missed CAB-LA injection visit and make every effort to reschedule CAB-LA injection within the injection window (7 days before to 7 days after scheduled injection, which is 3 to 5 weeks after the first injection and 7 to 9 weeks after all subsequent injections). If a participant missed a CAB-LA injection visit (outside the injection window), we will contact them by phone, provide counseling about HIV risk, ensure they know the date for their next injection appointment, and refer them to their local clinic for oral PrEP. If a participant wishes to continue CAB-LA injections after a missed visit, we will offer an injection during the window for their next follow-up (injection) visit. If a participant presents in person for a follow up visit after the window for their CAB-LA injection but >4 weeks before their next scheduled follow up visit, we will perform all study procedures for the missed visit except for CAB-injection. In line with product guidelines, any participant who is >2 months late for their second injection or >3 months late for third or greater injections will need to restart CAB with 2 injections 1 month apart, before the every 2 month schedule can restart (see Figure 1). Participants who no longer want CAB-LA injections will continue in the study, with safety evaluations and HIV testing. Counseling during the informed consent process, the final visit, and for participants who miss injection visits or who discontinue CAB-LA, will include information about the ‘pharmacologic tail’,83 and the possibility of INSTI resistance if a participant were to become infected with HIV during this time. Participants who wish to remain on PrEP during the pharmacokinetic tail will be directly referred to a government-run clinic that provides PrEP, currently TDF/FTC, free of charge.

## Medical Care and Referrals

General medical care of participants and their children will be provided through the local healthcare system. Participants and their children requiring further care or hospitalization will be referred appropriately, and clinical decisions during hospitalization will be made by hospital staff. In the case of HIV seroconversion, study staff will refer participants and their children (if confirmed to be infected) to the local HIV treatment clinic and will provide advice on initial HIV treatment regimen if the seroconversion occurred while the participant was taking cabotegravir.

## End of Study Follow-Up

Participants will be followed up for a minimum of 24 months. At the end of the study, participants will be counselled about PrEP options available to them through the government system or through other research studies If they choose to continue PrEP we will provide the appropriate referral.

## Timing of evaluations

Enrollment should occur after delivery while the participant is admitted on the maternity ward. The enrollment visit can occur up to 14 days from delivery (calendar day of delivery = Day 0).

Scheduled evaluations

Every attempt should be made to conduct the visit as close as possible to the target visit date, and barring that, within the target injection window (+/- 7days from scheduled injection). If an injection visit window is missed, counseling will be provided to the patient about HIV risk reduction prior to the next CAB-LA injections visit, including referral to clinic for oral PrEP. Every effort will still be made to conduct the laboratory evaluations (with exception of PK testing if applicable), and clinical evaluations that were required at the missed injection visit, at the time of the next clinical contact, even if this falls outside of the specified target injection window (with the exception of PK testing) as long as it occurs within the visit window (+/- 4 weeks) from scheduled injection. The study team may check on participants by telephone between visits.

Missed visits

If a scheduled visit is missed then the next attended visit should include major monitoring parameters from the missed visit (within allowable blood draw ranges). If a visit is missed, the schedule of visits should not be “reset” but should remain as if the visit were not missed. In the situation where a participant has missed visits and needs to restart CAB injections with 2 injections 1 month apart (because duration since last injection >2 month for second injections and >3 month for third or greater injection), the second of these monthly injections will be scheduled on the date of a previously scheduled visit,

Premature discontinuation of CAB-LA injections

If the participant decided to be discontinue CAB-LA, stops breastfeeding, or if her infant dies while enrolled in the PK substudy, an additional participant will be recruited to the PK substudy in her place. Any participant permanently discontinuing CAB-LA will be counseled about the ‘pharmacologic tail’, referred for oral PrEP and will continued to be followed until study end at 24 months.

Restarting CAB-LA PrAP after missed injection(s)

For participants who missed injection visits or had previously decided to discontinue CAB-LA, we will allow for re-start of CAB-LA at any point in the study up to 21 months. The schedule of visits will not be “reset” and the participant will receive their next injection at the next scheduled study visit. Participants will be counseled about the potential to have lower levels of CAB after restarting injections which could increase risk for HIV infection and counseled to use oral PrEP until after their subsequent injection.

## Laboratory Testing Panels in Botswana

Samples may be stored indefinitely if consent is granted and participants can request that any unused samples be destroyed at any time.

HIV ELISA

Enrollment HIV screening will be done using 4th generation Ag/Ab test point of care test (Alere HIV Combo or Alere Determine HIV-1/2). After enrollment HIV screening will be done using same tests as is standard in the government system in Botswana, currently this is 3rd generation point of care HIV ELISA (currently Determine or Unigold).

Hematology

Hemoglobin, hematocrit, red blood cells (RBC), mean corpuscular volume (MCV), white blood cell count (WBC), differential WBC (neutrophils, lymphocytes, eosinophils, monocytes, and basophils), absolute neutrophil count (ANC), and platelets.

Blood Chemistries / LFTs

Sodium, potassium, chloride, glucose, bicarbonate, BUN, creatinine, ALT, AST, and bilirubin (total and direct).

Diabetes Screening and Lipids

HGBA1C, LDL, HDL, triglycerides

CD4+/CD8+

Determinations of CD4+ and CD8+ cell counts and subset percentage evaluations will be performed at the approved BHP laboratories in Gaborone, throughout the course of the study among participants who seroconvert.

Stored Plasma/Cells

Stored plasma will be performed per laboratory SOP, either from the PBMC separation process or upon direct processing of whole blood for HIV RNA testing. When PBMC processing is not performed, cells will be stored separately after processing for plasma. Stored plasma will be used for HIV viral load testing and PK testing in cases where a participant seroconverts.

HIV-1 PCR

HIV Plasma Viral Load will be tested at the Botswana Harvard HIV Reference Laboratory (BHHRL) at Gaborone using licensed assays that are under external quality assurance monitoring and accredited under ISO 15189. Specifically, the primary assay will be the Abbott m2000sp/m2000rt with a measuring range of 40  to 10 million copies per milliliter of plasma.  All results will be made available to the protocol database after appropriate user validation.  BHHRL is enrolled in the External Quality Assurance with the College of American Pathologists (CAP).

HIV-resistance testing (including INSTI resistance)

Viral mutations associated with HIV drug resistance will be analyzed from sequencing of protease (PR), reverse transcriptase (RT), and integrase strand inhibitor (INSTI) genes using the validated commercial assays and in-house methods. The commercial assay of choice that covers both PR, RT, and INSTI will Thermofisher HIV Drug Resistance Assays. BHHRL enrolled in the External Quality Assurance with the Virology Quality Assurance (VQA) program at Duke University. PR, RT, and INSTI resistance mutations will be analyzed using the Stanford University HIV Drug Resistance Database.

PK Testing

The lab of Dr. Capparelli will perform the PK testing for maternal and infant samples in the PK substudy.

Hair Sample Testing

The lab of Dr. Gandhi will perform the testing of CAB levels on hair samples

## PK Testing

Samples will be frozen and stored in Botswana and sent to University of California San Diego (UCSD) where a standard liquid chromatography/tandem mass spectrometry (LC-MS/MS) assay developed in Dr. Capparelli’s lab will be used to measure CAB in maternal, breastmilk and infant samples. Quality control (QC) samples will be interspersed within analytical runs to ensure accuracy of resulting data per local SOPs. Dr. Capparelli has unique expertise in the study of pharmacokinetics in lactating women and infants and has been involved in many studies involving antiretroviral medications, helping to design and serve pharmacologists on IMPAACT P1026s – a PK study of ARVs in pregnancy that has evaluated over 1000 WLWHIC – the largest PK study in pregnancy. His lab is uniquely positioned to support cabotegravir PK studies because they have state-of-the-art analytical chemistry instrumentation for quantitative determination of drug concentrations in biological fluids (including 2 AB Sciex 4000 LCMS instruments and 1 AB Sciex 5500 LCMS instrument), and have been developing and testing a cabotegravir-specific assay.

In healthy adults, the long-acting formulation of CAB reaches peaks ~7 days after injection, and slowly decreases, remaining relatively stable and >4x the protein-adjusted 90% inhibitory concentation (PA-IC90) over the ensuing 7 weeks after the third injection. Therefore, we will measure the lowest trough at month 1 then one week later (peak), steady state trough at month 5 and 1 week later (steady state peak). Pharmacokinetic tail will not be evaluated in maternal samples as women will be non-lactating by the time they stop CAB-LA, and ample prior PK data from adults has already been collected. Breastfeeding infants ingest CAB (no IM injections), there is no pharmacokinetic tail in infants. Additionally, because CAB-LA is so highly protein bound,66 we will measure albumin in maternal samples to aid in interpretation of CAB levels. In the case of an HIV seroconversion in a participant who is not in the PK sub-study, stored maternal plasma (and infant DBS if breastfeeding) will be used to retrospectively evaluate CAB concentrations before seroconversion.

Hair samples will be stored at BHP and later analyzed at the University of California, San Francisco lab of Dr. Monica Gandhi. CAB-LA levels will be analyzed from all visits in all seroconverters and from visits of randomly sampled controls (3:1) who remain HIV-noninfected. Infants of mothers who seroconvert will have hair analyzed for CAB levels as well, along with appropriate controls. Additionally, hair samples may be analyzed in cases where there is suspected toxicity from CAB-LA. The cabotegravir hair assay will be done by liquid chromatography-tandem mass spectrometry (LC/MS-MS). Results are only for research purposes and will not be available for participants.

## SMC Review of Safety and PK Data from PK study

After the first 10 participants in the PK sub-study have completed the 1 month trough and 1 month peak (1 week after the injection) sample collection, these samples will be sent to UCSD for PK testing. Safety Monitoring Committee (SMC) and the study team will review maternal and infant plasma CAB level among these first 10 participants within 12 weeks of receiving the samples. PK data will be assessed based on pre-specified PK peak and trough targets in non-lactating populations. The study team will summarize the data, determine if mean trough and peak concentrations are within pre-specified targets and present this information in a written report to the SMC. In addition, any outlier concentrations will be presented and reviewed. The SMC and the study team will review any cases of outlier PK concentrations and make individual recommendations about whether a participant with these levels should continue to receive the CAB-LA. The study will continue during the SMC review process.

The SMC will review safety data utilizing pre-defined safety criteria defined in the protocol (see Section 14.8).

# ASSESSMENT OF SAFETY

## Serious Adverse Events (SAEs)

A Serious Adverse Event (SAE) is defined as an adverse event (AE) following any exposure to the study agent that:

* + Results in death
	+ Is life-threatening (the term “life-threatening” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.)
	+ Requires inpatient hospitalization or prolongation of existing hospitalization (hospitalization itself is not an AE, but is an outcome of the event).
	+ Results in persistent or significant disability/incapacity
	+ Results in a congenital anomaly/birth defect, or
	+ Is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above.

Guidelines for reporting SAEs that require expedited reporting (EAEs) are summarized in an EAE SOP.

## Adverse Event Reporting

All adverse events (Grade 1 or higher), including all injection reactions, will be recorded on an Adverse Event Log. Potential injection reactions will also be described on the Injection Reaction form.

**Expedited Adverse Event (EAE) Reporting to NICHD:** SAEs that are both related to the research and unexpected will be reported to NICHD on an expedited basis, within the same timeframe required by the IRBs for events requiring prompt reporting. Documentation should be emailed to NICHDAdverseEventRep@mail.nih.gov. NICHD’s reporting policy is available at https://www.nichd.nih.gov/sites/default/files/inline-files/AdverseEventsReportPolicy2020.pdf

**Grading Severity of Events:** The most current Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) at the time that the study opens will be used, and is available at https://rsc.niaid.nih.gov/clinical-research-sites/daids- adverse-event-grading-tables.

***9.2 Adverse event reporting in case report forms***

All grade 3 or higher adverse events that may potentially be related to study participation will be reported on case report forms.

# CLINICAL MANAGEMENT

## Toxicity Management

Major safety concerns of CAB-LA PrEP in non-pregnant, non-lactating adults have been thoroughly evaluated in HPTN083, HPTN084 and trials of CAB/Rilpivirine, and include hepatotoxicity (<2%), increase in weight (0.4kg excess after initial injections which stabilizes), and possible increase in mood disorders (rare).22,66,94 In this study, safety and toxicity monitoring will be focused on events specific to post-partum women and their breastfeeding infants. This includes post-partum depression, excess weight gain and associated cardiometabolic effects (which may be impacted by breastfeeding), CAB levels and outcomes in infant exposed to CAB-LA in breastmilk (including growth), and infant HIV transmission with evaluation for INSTI resistance. At enrollment and follow up visits, participants will be screened for post-partum depression (using PHQ-9), maternal weight and blood pressure (in duplicate using manual cuff) and infant height and weight will be measured. A complete blood count will be drawn at enrollment and every 6 months, liver function tests will be drawn at enrollment, at 3 months, 6 months, 13 months and 24 months, while HgBA1c and fasting lipids (HDL, LDL and triglycerides) will be tested yearly. Women with depression will be referred for further evaluation and treatment and women with a concern for pre-diabetes/diabetes or hyperlipidemia will also be referred for work up and medical care. A study monitoring committee (SMC) is planned for this study. In addition to 6-monthly reports of HIV incidence, safety outcomes and pK data, the SMC will review maternal and infant plasma CAB level among the first 10 participants in the PK substudy within 12 weeks of completion of the first (1 month trough and peak) samples. Contraception counseling will be provided at study visits, and informed consent will include discussion of risks/benefits of pregnancy while on CAB-LA, but contraception will not be required for enrollment. Women who become pregnant on CAB-LA will be followed every 2 months until delivery. Pregnancy complications and birth outcomes (gestational age, birthweight, congenital abnormalities and neonatal death) will be reported to the SMC.

**AEs of interest to be monitored for include:**

* Constitutional symptoms, such as fever, changes in blood pressure, rigors/chills;
* Injection site reaction/extravasation changes, pruritus, urticaria, erythema, desquamation, ulceration;
* Anaphylaxis; Acute Respiratory Distress Syndrome, bronchospasm/wheezing, facial flushing;

The most current Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table) should be used to grade adverse events and is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse- event-grading-tables.

This Toxicity Management section refers to management of toxicities that occur among participants receiving cabotegravir. Additional details are provided in study-specific SOPs.

* + 1. **Dosage Modification Instructions:** No dosage modification will occur for this protocol.
		2. **Management for Rashes, Urticaria and Hypersensitivity Reactions:** All rashes are graded based on the DAIDS toxicity table (Corrected Version 2.1, July 2017).

Grade 1 or 2 rashes or urticaria

CAB-LA continued with close observation, at the discretion of the study clinician. If an isolated Grade 1 or 2 rash does not resolve within 14 days of onset, further management should be discussed with the study team. Other potential causes of rash/urticaria should be investigated and treated, and potential causative agents (such as lotions/creams/soaps) should be discontinued.

Grade 3 rash with no definitive alternative explanation (Grade 3 rash should have vesicles or limited number of bullae or superficial ulcerations of mucous membrane):

Any products or non-essential medicines that could be causing the rash should be discontinued. ALT and FBC with differential should be drawn, and the participant should be evaluated clinically approximately weekly, until the rash resolves to Grade 1 or lower. If the Grade 3 rash was ultimately deemed very likely due to another diagnosis or drug, then CAB-LA may be continued, in consultation with a study PI/designee. However, if CAB-LA was paused for possible hypersensitivity and no other explanation is apparent, they it should be permanently discontinued and participant counselled about alternative PrEP.

Grade 4 rash (with mucosal involvement, or suspected hypersensitivity):

Immediately refer to the hospital with capacity for dialysis for further clinical care/management and seek guidance from a study PI/designee. Given long-acting formulation, inpatient monitoring with cabotegravir levels may be required.

* + 1. **Guidelines for Managing Local Reactions / Infusion Site Reactions**

Injection site reactions should be managed as follows:

Grade 1-2: Continue administration of CAB-LA. Use alternate injection site until event resolves to less than Grade 1.

Grade 3: Defer further CAB-LA administration until event reviewed by the study team. Contact protocol team within 72 hours. Participant should be followed approximately weekly until resolution to Grade ≤1 or until stabilized and no longer in need of frequent monitoring, as determined by the site investigator.

Grade 4: Provide immediate clinical management and consult with protocol team. Permanently discontinue CAB-LA. Participant should be followed approximately weekly until resolution to Grade ≤1 or until stabilized and no longer in need of frequent monitoring, as determined by the site investigator.

* + 1. **Guidelines for Other Toxicities**

In general, CAB-LA may be continued for other toxicities that are Grade 1 or 2.

For Grade 3 toxicities, CAB-LA may be continued or held, depending on the toxicity and potential relatedness to study drug.

Participants who develop a symptomatic Grade 4 adverse event or toxicity felt to be related to CAB-LA should discontinue further injections.

Participants experiencing adverse events requiring permanent discontinuation of study treatment should be followed approximately weekly until resolution of the adverse event to Grade ≤1 or until stabilized and no longer in need of such frequent monitoring, as determined by the site investigator.

* + 1. **Guideline for managing TB diagnosis**

PK studies indicate that rifampin reduces levels of CAB-LA, potentially below effective levels. Therefore, if any participant is diagnosed with TB during the study,(or any other condition requiring use of rifampin) we will hold CAB injections, counsel regarding HIV risk reduction and oral PrEP until they have completed TB treatment (or until the end of planned study follow up, whichever comes first).

* + 1. **Guideline for managing participant who becomes pregnant**

Contraception counseling will be provided at study visits, and informed consent will include discussion of risks/benefits of pregnancy while on CAB-LA, but contraception will not be required for enrollment. Women who become pregnant during the study will be counseled on PrEP in pregnancy (including oral PrEP) and given the option to continue CAB-LA injections or not. Regardless of their decision to continue CAB-LA or not, pregnant patients will continue study visits as planned. Pregnant patients will be followed every 2 months until the end of pregnancy. Pregnancy complications and birth outcomes (gestational age, birthweight, congenital abnormalities and neonatal death) will be collected.

* + 1. **Guideline for managing participant who has liver toxicity**

The PK of CAB-LA may be altered with liver failure.  We will hold CAB-LA injections if:

-ALT >= 3xULN **AND**bilirubin >= 2xULN (>35% direct bilirubin)

-ALT >=5xULN for more than 2 weeks

-ALT >=8xULN or

-ALT >=3xULN (or >=3x baseline ALT) with symptoms of worsening of acute hepatitis or hypersensitivity such as fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash or eosinophilia

CAB-LA may be restarted once participants LFTs have normalized if the cause of the LFT abnormality is not felt to be due to CAB-LA. LFTs will be checked at 3 consecutive study visits if CAB-LA is restarted.

10.1.8 Guideline for managing participants started on medication that could interact with CAB-LA

In addition to the interaction of rifampin and CAB-LA (see 10.1.5, guideline for managing TB diagnosis), in the case of a participant who is prescribed carbamazepine, phenobarbital, phenytoin, oxycarbazepine, rifampin, rifabutin, rifapentine, systemic dexamethasone, or St. John’s wort during the study (after having

already received CAB-LA injections), the decision about whether to continue CAB-LA injections will be made on an individual bases by study physicians, taking into account the dose and duration of the new medication and also the risk of HIV acquisition with stopping CAB-LA or switching to oral PrEP with truvada.

## Participants Lost to Follow-up

Collecting any intention to move or change of address during each visit will minimize the loss of contact with participants. In case of absence at a scheduled visit, a study team member will attempt to locate the participant by phone or home visit. Efforts will be made to keep all participants in study follow-up. In the event that consent is withdrawn, a final study visit / blood draw will be requested and counseling about the CAB-LA tail will be provided.

## Participant Death

In the case of a participant’s death, a Death Form should be completed within 3 business days of the study team becoming aware of the death. If a participant dies in a health facility, events prior to and at the time of death will be collected from hospital records and a verbal autopsy CRF will be completed. If death occurs outside the hospital setting, a verbal autopsy CRF will be completed upon interview of a household member or next of kin.

* 1. **Criteria for Study Discontinuation**
* Request by the participant to withdraw.
* The participant is judged by the investigator to be at significant risk of failing to comply with the provisions of the protocol as to cause harm or to interfere with the validity of the study results.
* At the discretion of the NIH or local Ministry of Health.
* At the recommendation of the SMC.
* The study may be discontinued at any time by NIH, the FDA, the IRB/ECs, Office of Human Research Protection (OHRP), Botswana Government or other government agencies as part of their duties to ensure that research participants are protected.

# STATISTICAL CONSIDERATIONS

## Sample Size and Power

Our sample size (N=500) was chosen based on Aim 1 objectives to evaluate implementation and effectiveness. Uptake, adherence and implementation metrics are descriptive and we have a sample size similar to or larger than other PrEP implementation studies in Africa. The sample size will allow reasonable power to evaluate effectiveness by comparing HIV incidence in the CAB-LA cohort to the Doris Duke cohort (the Doris Duke cohort are enrolled in the immediate post-partum period from the same sites into an observational study and followed for 18 months). We estimate HIV incidence in the Doris Duke cohort using data from a community-based study in Botswana (Botswana Combination Prevention Project), where the use of PrEP was negligible and the annual HIV incidence was 0.92%.46 Multiple studies suggest a two- to four-fold increased risk in the post-partum period,16-18 which would yield an annual HIV incidence of 1.84%-3.86%. The annual HIV incidence was 0.21% in women treated with CAB-LA PrEP in HPTN084, and we hypothesize that our study will be able to achieve high retention (as been our experience to date with prior studies in Botswana with < 5% loss to follow-up)25,95,96 and adherence such that HIV incidence will be no more than 2 times higher than HPTN084. Assuming HIV incidence in Doris Duke is 1.84% to 3.68% and incidence is 0.21% to 0.42% in the proposed study, with 5% LTFU and two-sided alpha of 0.05, we will have 50% to 93% power to detect a difference between cohorts (**Table 2**). In addition, if incidence in the Doris Duke cohort is lower than expected and power is limited for this direct comparison, the point estimate and 95% confidence limits of incidence in the CAB-LA PrEP cohort will be useful as a single arm study that can be compared with published incidence from the HPTN 084 data. With a sample size of 475 (5% LTFU), we will have good precision (relatively small 95% CI) around the range of estimates of HIV incidence with CAB-LA: 0.21% (95% CI 0.01%, 1.2%) and 0.42% (95% CI 0.12%,1.5%).

|  |
| --- |
| **Table 2. Power to detect a difference in HIV incidence in the DD Cohort vs. CAB-LA PrEP trial, varying HIV incidence** |
| Ongoing observational study without CAB-LA PrEP (Doris Duke) | Proposed CAB-LA PrEP trial | Power |
| Annual HIV incidence | Sample size(5% LTFU) | Annual HIV incidence | Sample size (5% LTFU) |   |
| 1.84% | 285 | 0.21% | 475 | 64% |
| 2.76% | 285 | 0.21% | 475 | 84% |
| 3.68% | 285 | 0.21% | 475 | 93% |
| 1.84% | 285 | 0.42% | 475 | 50% |
| 2.76% | 285 | 0.42% | 475 | 75% |
| 3.68% | 285 | 0.42% | 475 | 89% |

## Data Analyses and Interpretation

**Aim 1:** We will describe implementation outcomes,of uptake (% accepting CAB-LA PrEP), adherence (% of injection visits attended) and persistence (% of participants continuing PrEP at 5,11,17 and 24 weeks. We will consider the intervention 1) acceptable, 2) appropriate, and 3) feasible if 80% of participant agree with 3 of 4 questions on the respective questionnaires 1) Acceptability of Intervention Measure (AIM), 2) Intervention Appropriateness Measure (IAM) and 3) Feasibility of Intervention Measure (FIM). For qualitative interviews, we will use an approach that uses systematic coding to identify, define and map concepts, find associations between concepts and seek explanations from these concepts.97 Coding will be done using the Dedoose program. Inductive and deductive reasoning will be used to identify concepts, patterns and recurring themes. Log (categorical) or linear (continuous) binomial regression will be used to evaluate associations between 1) high adherence (< 2 missed injections) and 2) high persistence (through 24 months) and age, gravida, employment status (employed vs. unemployed), education (none/primary vs. secondary/tertiary), food insecurity (score >4), housing type (formal vs. informal, any use of drugs/alcohol, any depression (score >9), high self-esteem (likert response < 3), perceptions of PrEP (above/below median) and self-assessment of risk (above/below median), social support (above/below mean), planned primary infant caregiver (self vs. other), planned infant feeding method (breastfeeding vs. formula), partner involvement (none vs. some), partner HIV status (infected, uninfected or unknown), geographic location (Gaborone vs. Molepolole), availability of transportation (own car or not) and time to travel to clinic.

The primary measure of effectiveness will be the incidence of HIV infections (# infections per person-year) among enrolled women during the 24-month follow up period, regardless of adherence to CAB-LA. We will compare HIV incidence in the CAB-LA cohort to the Doris Duke cohort using Cox proportional regression to calculate a hazard ratio. While it would be ideal to compare HIV incidence to unexposed participants enrolled in the same study, it would be unethical to do a randomized trial including an arm without CAB-LA PreP in women (due to superiority over oral PreP in HPTN084) and it would be biased to enroll an observational arm in this study of women who did not want PrEP because they are likely to be at lower risk of HIV. While we acknowledge that this comparison is not perfect, the Doris Duke and CAB-LA cohorts should have very similar underlying characteristics and will be enrolled at similar times (no concurrent enrollment will be allowed) (**Table 2**). Doris Duke participants will not be on CAB-LA PrEP because it is not currently available in Botswana outside of a study, and using current Tsepamo data, we predict than <0.5% of the Doris Duke cohort will be taking oral PrEP. Two important differences in inclusion criteria are that the CAB-LA study is limited to those with <3 prior pregnancies (no restrictions in Doris Duke) and the study follow-up period is only 18 months for Doris Duke and 24 months for this study. To account for different follow-up time, we will compare incidence in person-years of follow up. There is no data to suggest that HIV incidence increases or decreases during the 18-24 month post-partum period, so this is unlikely to bias the estimate (but does decrease the power). Parity is a potential confounder as it is associated with both the cohort and HIV acquisition risk; thus we will perform a sensitivity analysis including only those with <3 prior pregnancies. We will adjust for other potential confounders (age, education, employment, region) in regression analysis. We also will describe the difference in HIV incidence in this CAB-LA cohort to results in HPTN084. We expect the underlying risk to be similar to the HPTN084 cohort, which also targeted women at high risk of HIV, in settings with similarly high population HIV prevalence. Demonstrating narrow 95% confidence intervals around a similar incidence as HPTN084 will in essence move the HPTN084 data a step closer to “real world applicability,” and will also provide assurance that the intervention remains robust in a breastfeeding population.

**Aim 2:** Primary maternal safety outcomes will include the proportion of participants with a grade 2 or higher DAIDS-graded adverse event, treatment emergent obesity (pre-pregnancy BMI <25 and 24-month BMI >30), new onset diabetes and pre-diabetes (HgBA1c >5.8 or diagnosis during routine care), hypertension (systolic >140 or diastolic >90 on 2 separate measurements, or diagnosis during routine care) and prevalence of post-partum depression (PHQ-9 score >9). Additionally, we will describe the median change in weight over the follow up period and the median weight gain from pre-pregnancy weight (when available). Infant outcomes will include the number of incident HIV infections, the proportion of incident infant infections with CAB resistance, median length and weight at 11- and 24-months, and the proportion with Z-score <2 standard deviations (SD) below norms for length-for-age and weight-for-age based on WHO growth curves.98 All of these outcomes are also measured in the Doris Duke study (**Table 3**) and so the relative risk of each outcome in the CAB-LA cohort compared with the Doris Duke cohort will be estimated by log-binomial or linear regression, using an intent-to-treat analysis. A secondary analysis will include only those with high adherence to CAB-LA (<2 missed visits). The following prognostic factors may be included in the models; age at baseline, marital status (married, unmarried or unknown), occupation (salaried, unsalaried or unknown), education (none or primary, more than primary, unknown), parity (0, 1 or more, unknown), delivery site and distance from delivery site to antenatal clinic. Assuming the prevalence of adverse outcomes in the Doris Duke cohort the general population in Botswana we will be able to detect clinically meaningful differences in many outcomes (**Table 3**)**.**

|  |
| --- |
| **Table 3. Minimal Detectable Differences in Safety Outcomes in CAB-LA vs. Doris Duke (no CAB-LA) cohort** \*Effect size calculations assume power of 80% and two-sided α=0.05. |
| Outcome  | Estimated value in the Doris Duke Cohort (N=300) | Minimal Detectable Difference in the CAB-LA cohort (N=500) |
| Treatment emergent obesity | 12% | 20% |
| Hypertension | 3% | 8% |
| Diabetes or pre-diabetes | 1.5% | 5% |
| Poor infant growth (<2SD length-for-age) | 5% | 11% |
| Poor infant weight gain (<2SD weight-for-age) | 5% | 11% |
| Postpartum Depression | 3% | 8% |

**Aim 3:** Descriptive analyses of plasma concentration of CAB-LA at the end of the dosing period (trough) and 1 week after injection (peak) in maternal and infant plasma and in breastmilk will be performed and will include range, mean, and standard deviation at each time point (1 month, 1 month one week,5 months, and 5 months 1 week). We will calculate the proportion of maternal participants with plasma CAB concentration >1x PA-IC90 (0.166 micrograms/mL)65 , the proportion with plasma CAB concentration >4x PA-IC90 (0.45 micrograms/mL) and 95% CIs around mean levels. Infant plasma concentration and breastmilk concentration will additionally be reported as a % of maternal plasma concentration. In an exploratory analysis we will evaluate the impact of several factors which may impact CAB levels, including maternal BMI (<18, 18-25, 25-30 and >30), mixed infant feeding (yes/no), albumin level (as a marker for protein) and fat content of breast milk. Maternal results will be compared descriptively to prior PK studies in non-lactating people.

# DATA HANDLING AND RECORD KEEPING

## Data Management

Data will be entered by clinical study staff onto standardized case report forms (either paper or electronic) at the sites. Electronic data capture will occur using REDCap,

A data manager will incorporate basic error checking capability to minimize data entry errors and to allow rapid querying of the sites for illogical or missing data. The data manager will generate monthly accrual reports as well as periodic reports (approximately 3-monthly) that will summarize basic demographic data and reported aggregate diagnoses, to allow real-time review of enrollment/data. Data will be password protected and encrypted. We will keep all links between protected health information and data separate.

## Source Documents

The following data items will be considered source documents:

* **Signed Consent Form**
* **Certified Copies of Clinical records:** photocopies of clinical records, including hospitalization notes (where necessary) will be made at every visit and all copies will be certified as true copies of the original by the person making the photocopy. This will be documented by signing and dating a “certification of copy” stamp on the photocopy and those copies will be filed in the study participant’s file at the site.
* **For any death of a study participant while on study:**
	+ Copies of death certificate when available.
	+ Copies of hospital inpatient or outpatient/clinic records with information thought by the Study Physician to be directly related to the participant’s death.
	+ Copy of the completed verbal autopsy form.
	+ Any laboratory or other clinical investigation/procedure report containing information directly related to the participant’s death.

## Quality Control and Assurance

Keyed data will be reviewed by a second person (either a study physician or study nurse) whenever feasible, to improve quality control.

If an error, inconsistency or incomplete data is identified, the CRF will be tagged “unverified” until the error is resolved. If the original staff member is not available to make corrections, the study physician or other research nurse will make corrections or completions on his/her behalf provided they have access to the correct information.

There are additional levels of quality control, which will occur at the Data Manager level. Error checking is part of the REDCap entry program and assists the keyer in identifying problems. The keyer is notified by the program feature of a logic check function that gives a detailed explanation of the incomplete or inconsistent data. This allows for potential errors to be resolved prior to CRF completion.

# CLINICAL SITE MONITORING

Clinical monitoring will not occur for this protocol.

# HUMAN SUBJECTS PROTECTIONS

Human subjects will be enrolled in this study.

## Characteristics of study population

The study population will consist of postpartum women.

## Sources of Research Material

Research material will consist of data obtained from questionnaires, qualitative interviews, medical records, and from blood draws.

## Participant Recruitment and Study Consent

Participants will be recruited as outlined above. Written informed consent for the study will be obtained by trained study staff. Consent forms will be written in both Setswana and English and will be reviewed verbally by the study team. The consent process will describe the purpose of the study, the nature and duration of study follow-up, and the risks and benefits of participation, and will highlight the freedom to decline or withdraw participation at any point without compromising her or her child’s future medical care.

Study investigators will prioritize optimizing participant education and informed consent procedures for the study. Participant understanding of scientific information, research methods, and human subjects rights can be enhanced through the following: 1) full, standardized disclosure of study-related information in both written and verbal formats, 2) minimizing the reading level and enhancing the visual display of written documents, 3) providing adequate time and opportunity for participants to read/hear about, reflect upon, ask questions about, and discuss the research.

## Risks and Benefits

Risks associated with CAB-LA include the potential for INSTI resistance if seroconversion occurs while on the medication, and known side effects, which includes weight gain. Risks during breastfeeding are unknown. The potential benefit for women in this study is to avoid incident HIV infection. By selecting women at high risk of HIV, we expect that these benefits will outweigh the risks. There is also substantial scientific benefit from the knowledge gained, including benefit for other women and children in Botswana.

The risks of the study are mitigated by the close monitoring that will occur and the potential benefit for preventing incident HIV infection in this high risk group. There are also benefits associated with collecting data in lactating women that may lead to more widespread use of an effective HIV prevention medication in women in general and benefit others in the future.

* + 1. **Risks**
			1. **Stigma / Confidentiality / Coercion:** Some participants might be subject to stigma or the perception of stigma if they participate in the study, especially regarding being at high risk for HIV. However, the ability to take a medication only once every 2 months is likely to reduce this stigma, and avoiding HIV infection also reduces HIV-associated stigma. Measures to protect confidentiality of data are in place that include computer encryption and use of subject identifiers instead of personal identifiers, with limited/encrypted access to the linkage between subject and personal identifiers.

Coercion to enroll in the study will be avoided by the balanced consent process and the limited amount of travel and time reimbursement which is set at a level approved by the ethical review boards.

* + - 1. **Blood Draws:** Blood volumes are an important consideration for this protocol, and will be minimized to the greatest extent possible at every visit. A summary for multiple guidelines on this topic, published by WHO in the WHO Bulletin, is as follows: “Existing guidelines for blood sample volume limits (ranging from 1–5% of total blood volume within 24 hours and up to 10% of total blood volume over 8 weeks) are consistent with the limited evidence available on “minimal risk” to children. Lower limits for sick children are advisable, and a maximum of 3 ml/kg post-neonatally within 24 hours (3.8% of total blood volume), in line with United States policy, seems to be a reasonable guideline, although each study must be judged on its own merits and greater caution may be needed in children with illnesses that impair the replenishment of blood volume or hemoglobin [71].” The NIH Clinical Center also provides guidance for maximal allowed blood volumes allowed in research. The NIH recommends a limit of 5 mL/kg per single blood draw and a limit of 9.5 mL/kg in any 8-week period.

We will draw no more than 12 mL at any draw during the study for mothers and less at most visits. If additional clinical blood draws occur, study staff will account for these draws and make adjustments in the volume drawn for the study if needed. We will only collect infant dried blood spots from infants except for infants in the PK substudy. For infants in the PK substudy, we will draw 2mL at each study visit (occurring at month 1, 1 month 1 week, 5 months and 5 months 1 week) which is less than 9.5mL/kg in any 8 week period

* + - 1. **Cabotegravir-LA:** Cabotegravir has been approved for use for HIV treatment and PrEP with excellent safety profiles in clinical trials. However, less data exists on cabotegravir use in lactating women. Performing a PK study in lactating women and their infants before widespread availability will lead to safer and more rapid rollout of CAB-LA in women of reproductive potential**.** For antiretrovirals, there is an average 5-year lag between FDA approval of a medication and pregnancy/lactation PK data.76 Given the clear need and demand for CAB-LA PrEP in women, performing the PK studies now will allow will for more rapid—and safer—rollout of CAB-LA PrEP in settings with high HIV incidence in women of reproductive potential.This approach is in line with recent calls to action from multiple organizations (PHASES, ACOG, SMFM, WHO) to include pregnant and lactating people in research. We will also have the unique opportunity to provide the first PK data on CAB levels in infants. While infant toxicity is unlikely given low levels of infant exposure to other INSTIs via breastmilk, it is clinically important to understand whether infant CAB levels will be high enough to cause development of HIV resistance in an infant who becomes infected while exposed to CAB as this will impact empiric infant treatment decisions. Review of detailed safety and PK testing will occur (see section 8.13).

## Adherence to Human Subjects Requirements

Study participants will be recruited, enrolled, and followed in Botswana. Adherence to human subjects regulations will primarily be the responsibility of both Beth Israel Deaconess Medical Center Health and Botswana staff. The BHP team will adhere with all requirements by:

* Developing a study protocol that is able to meet its stated research objectives, and thus reflect adequate risk-benefit ratios for human subjects.
* Specifying study procedures in the protocols that protect the rights and safety of human subjects.
* Developing an informed consent form that includes all elements of informed consent required by Federal regulations and accurately represents study requirements, risks, and benefits in language that is understandable to study participants.
* Including human subjects considerations and requirements in study training sessions.
* Monitoring adherence to protocol specifications and human subjects requirements.

This proposal will be submitted to the Institutional Review Boards (IRB) of the Botswana Ministry of Health (the Health Research and Development Committee, or HRDC, and to Beth Israel Deaconess Medical Center.

## Procedures for Minimizing Potential Risks

1. Identification and disclosure: We have spent several years working to reduce the stigma concerns among participants in previous studies and have had success in these efforts. We have dedicated health educators at each site, and we provide extensive counseling during the screening process and at all subsequent visits, as needed.

Any information provided by the participant, including and especially their risk factors for HIV, will be subject to strict confidentiality. Special care will be exercised in all interactions with the participant to ensure that participation or non- participation in the study does not cause release of information.

The protection of information given in interviews is standard procedure. The identity of the participants will be only kept at the clinic sites in a locked facility, and in an encrypted database. Identifying information will be stored in separate locked file cabinets, and a numeric code will be assigned to the completed questionnaires.

1. Recruitment and Informed Consent: A participant will undergo an informed consent process and sign a written informed consent for their child’s participation in this study. The study informed consent form will contain all of the required elements as outlined by 45 CFR, 46.116. Informed consent forms for BHP studies are translated into Setswana, and back-translated into English. These translations are approved by both IRBs at Beth Israel Deaconess Medical Center and at the Botswana Ministry of Health (the HRDC). In general, study staff fluent in the participant’s preferred language, conducts the informed consent process with the potential participant in a private setting. If needed, a research nurse/physician may also review and discuss the process and answer any additional questions the potential participant might have. For more complicated studies, this process can take 1-2 hours, allowing sufficient time to ask questions. Sometimes, informed consent occurs over two separate visits. At the end of the discussion regarding the study (and full review of the informed consent form), the participant is given the opportunity to read the form, to ask questions, etc. Then, the nurse and/study physician usually review the understanding of study purpose, procedures, risks, benefits, etc.—the most important elements of the study from a human subjects perspective—to ensure that the parent/guardian understands these prior to the parent/guardian and the study staff member who conducted the informed consent process signing the form.
2. Safety and toxicity and PK monitoring plans outlined in the protocol, and approved by IRBs, SMC and study sponsor, will ensure the early identification of events and safe resolution of any possible toxicities. Post-injection monitoring for 30 minutes will occur following the first infusion. If initial doses are tolerated monitoring will not occur with subsequent infusions.
3. Blood Draws: The study will follow research criteria established by NIH for safe volumes of blood drawn.

## Participation of Children, Women and Minorities

Women are included in this research plan and will comprise 100% of those enrolled in the study. BHP study sites are accessible to individuals drawn from different ethnic groups, all of whom have access to government clinics and who are offered oral PrEP and HIV testing by the National Program to prevent MTCT and ART, if HIV-infected.

## Monitoring and Interim Analyses

An independent, external Safety Monitoring Committee (SMC) (functionally equivalent to a Data and Safety Monitoring Board/DSMB) will be established for this study, consisting of approximately 5 members, and approved by the study sponsor. Committee members will include those with expertise in PrEP treatment trials, research on pregnant and lactating women, and biostatistics. The primary role of the SMC is to help protect the safety of participants in the study. The reviews of interim data by the SMC will be triggered by criteria described below, but will occur at least every six months unless otherwise recommended by the SMC. The SMC will make recommendations to the study team and to the study sponsor. Following SMC reviews, the sponsor and study team will communicate and discuss the findings. A response plan will be developed by the study team, and sign off by the sponsor will be required to implement the response.

* + 1. **Reporting and SMC Triggers for Safety or Stopping Criteria.** Monthly safety and accrual monitoring by the protocol team will occur throughout the study. Regularly scheduled conference calls will occur between members of the study team (including the PI) and the Medical Officer at least twice yearly. Serious adverse events that are both related and unexpected will be reported on an expedited basis (EAE) and may trigger an early review by the SMC. The occurrence of any SAE will prompt immediate review by the PI and Medical Officer. The initial attribution of relationship of an SAE to study product will be discussed and the relationship will be confirmed by the study team, taking into account the site and the Medical Officer’s assessment of the event. Interpretation of CAB-LA relationship to adverse events will be based on the type of event, the relationship of the event to the time of injection, the known composition of the study product and the investigators’ medical judgment. Gradation of relationship will use the following terminology: “not related”, “possibly related,” and “related.” The occurrence of 2 EAEs determined to be related to study product will trigger an SMC review. This may lead to a recommendation for study discontinuation in consultation with the study team and sponsor. Due to the fact that Grade 3 neutropenia and anemia are common in children on ARVs, and the fact that hemolyzed specimens may falsely lead to Grade 3 hyperkalemia values, these toxicities will not be counted in the real-time safety review algorithm.

NOTE: If at any time during the study the triggers for SMC review are met, the study will be paused to new accruals and an SMC review performed within 7 days. The SMC will decide what additional data they would like to see and advise the study team on how to proceed.

* + 1. **Review of Data from PK Substudy**

We expect a median steady state trough of 0.71-6.7 mcg/mL (which is >10x the PA adjusted IC9) and 5% or fewer below 0.45 mgc/mL (which is >4x PA IC90). The SMC and the study team will review any cases of outlier PK concentrations and make individual recommendations about whether a participant should continue on CAB-LA. The SMC will evaluate the stead state PK levels (month 5) if >5% are below 0.45 mcg/mL or if the median steady state trough is not in the expected range. The recommendations by the study team or by the SMC may include no action, early termination of the study, if warranted by the safety/PK data, or a re-design of the study as approved by the study sponsors and regulatory agencies.

The SMC will review safety and HIV incidence data every six months, early review by the SMC will be triggered if there are serious unexpected adverse reactions.

## Biohazard Containment

As the transmission of HIV and other blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate blood and secretion precautions will be employed by all personnel in the drawing of blood, administration of injections, and shipping and handling of all specimens for this study, as currently recommended by the Centers for Disease Control and Prevention.

All infectious specimens will be sent using the ISS-1 SAF-T-PAK or equivalent package that is compliant to the International Air Transport Association Dangerous Goods Regulations Packing Instruction 602. Please refer to individual carrier guidelines (for example: Federal Express, Airborne, etc.) for additional specific instructions. Appropriate shipping permits will be obtained.

## Vertebrate Animals

This project does not involve vertebrate animals.

# ADMINISTRATIVE PROCEDURES

## Study Implementation

This study will be conducted in accordance with the protocol, international good clinical practice guidelines, and all applicable US, and non-US country and local regulations. Study implementation will also be guided by SOPs. These SOPs will be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study.

## ClinicalTrials.gov

This protocol will be subject to the United States Food and Drug Administration Amendments Act of 2007 (FDAAA), including registration in ClinicalTrials.gov.

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