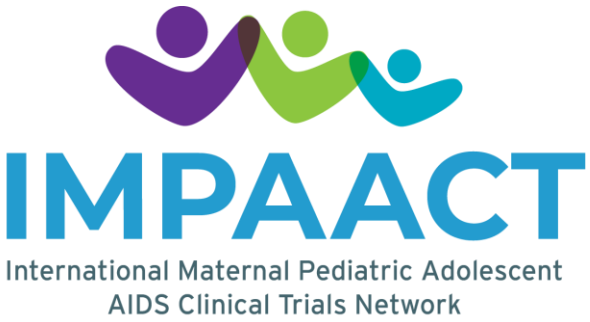

IMPAACT 2009

Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

IND # 136,735
DAIDS Study ID 30020

This file contains the current IMPAACT 2009 protocol, which is comprised of the following documents, presented in reverse chronological order:

- Clarification Memorandum #3, dated 31 May 2022
- Clarification Memorandum #2, dated 3 November 2021
- Clarification Memorandum #1, dated 9 April 2021
- Protocol Version 3.0, dated 19 November 2020

Clarification Memorandum # 3 for:

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

DAIDS Study ID # 30020
IND # 136735 Held By DAIDS

Clarification Memorandum Date: 31 May 2022

Summary of Clarifications and Rationale

The primary purpose of this Clarification Memorandum (CM) is to clarify that integrated next step counseling (iNSC) with drug level feedback will only be conducted if drug level results are available; otherwise, standard iNSC will be conducted. Updates to the Protocol Team and Study Site Rosters have also been made.

Implementation

This CM has been approved by the NIAID Medical Officer. Institutional Review Board/Ethics Committee (IRB/EC) approval of this CM is not required by the study sponsor prior to implementation. However, sites may submit this CM to the responsible IRBs/ECs for their information or, if required by the IRBs/ECs, for their approval prior to implementation.

The content of the CM does not impact the sample informed consent forms for the study or the benefit-to-risk ratio for study participants.

This CM should be maintained in each site's essential documents file for IMPAACT 2009. It is the responsibility of the Investigator of Record to ensure that all study staff are made aware of this CM.

The content of this CM will be incorporated into the next protocol amendment as specified below. Additions to the text are indicated in **bold**; deletions are indicated by ~~strike-through~~.

1) *Clarifications regarding timing of iNSC and iNSC with drug level feedback.*

Section 6.2.1.2, PrEP Comparison Component Maternal Antepartum Week 4, 8, and 12 (during pregnancy), Visit Procedural Table, Behavioral and Counseling row:

PrEP Comparison Component Maternal AP Weeks 4, 8 and 12 *(± 7 days)*	
Behavioral and Counseling	• CASI and COVID-19 questionnaire [see Section 6.16] *Week 12:* • Qualitative IDI Visit [see Section 6.2.1.8] *Cohort 1 and Cohort 2/Step 2* ***at Weeks 8 and 12*** *only:* • iNSC with drug level feedback [see Section 5.3.2] ***All Cohorts at Week 4 and*** *Cohort 2 only* ***at Weeks 8 and 12****:* • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]

Sections 6.2.1.2, 6.2.1.3, 6.2.1.4, 6.2.1.5, and 6.2.1.6, footnote added to Visit Procedural Tables corresponding to the bullet for iNSC with drug level feedback:

	• iNSC with drug level feedback [see Section 5.3.2]*****

***iNSC with drug level feedback will only be conducted if drug level results are available; otherwise, standard iNSC will be conducted.**

Appendix IIA (Schedule of Evaluations): Maternal PrEP Comparison Component: Cohort 1, Cohort 2/Step 2, rows for iNSC and iNSC with drug level feedback and footnote 8 (added):

MATERNAL PROCEDURES:	**SCR**	**Entry**	**Wk 4**	**Wk 8**	**Wk 12**	**Wk 24**	**Wk 36**	**L&D**	**PP Wk 6**	**PP Wk 14**	**PP Wk 26 / Exit and Early D/C**	**Interim Visit**
iNSC	X	X	**X**								X	As per Section 6.5
iNSC with drug level feedback[8]			~~X~~	X	X	X	X	X	X	X		

[8]iNSC with drug level feedback will only be conducted if drug level results are available; otherwise, standard iNSC will be conducted.

2) *Protocol Team Roster Updates*

As shown below, to reflect current protocol team membership, Frances Whalen was removed from the protocol team roster and Kathleen Shepherd, Ceora Beijer, and Helty Adisetiyo are added; the Protocol Pharmacist's name and email are also updated.

Protocol Pharmacist
Kelly **Colsh** ~~Parsons~~, PharmD, BCPS
Pharmaceutical Affairs Branch
NIH/NIAID/DAIDS/OCSO
5601 Fishers Lane, 9E15
Rockville, MD 20852
Office: 240-669-5721
Fax: 240-627-3112
Email: **kelly.colsh@nih.gov** ~~kelly.parsons@nih.gov~~

Protocol Laboratory Data Managers
Kathleen Shepherd, MPH
Frontier Science Foundation
4033 Maple Road
Amherst, NY 14226, USA
Phone: +1 (716) 834-0900, ext.7370
Email: kshepherd@frontierscience.org

Laboratory Center Representatives
~~Frances Whalen, MPH, MT(ASCP)~~
~~Clinical Project Manager~~
~~University of California Los Angeles~~
~~11075 Santa Monica Blvd., Suite 200~~
~~Los Angeles, CA 90025~~
~~Phone: 704-422-4055~~
~~Email: fwhalen@milabcentral.org~~

Helty Adisetiyo, PhD
Scientific Lead
IMPAACT Laboratory Center
University of California, Los Angeles
10990 Wilshire Blvd., Suite #260
Los Angeles, CA 90024
Phone: 424-946-5724
Email: hadisetiyo@milabcentral.org

Ceora Beijer, B.S.
Laboratory Support Manager
ACTG and IMPAACT Laboratory Center
University of California, Los Angeles
10990 Wilshire Blvd. Suite 260
Los Angeles, CA 90024
Phone: 424-946-5709
Email: cbeijer@milabcentral.org

A. Study Site Roster Updates

To reflect current site representatives, Vitumbiko Mandiwa is added, as shown below.

Site 30301, JHU-Blantyre

Vitumbiko Mandiwa, MBChB
Study Coordinator
Phone: +2651811885
Email: vmandiwa@jhp.mw

Clarification Memorandum # 2 for:

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

DAIDS Study ID # 30020
IND # 136735 Held By DAIDS

Clarification Memorandum Date: 03 November 2021

Table of Contents

Summary of Clarifications and Rationale

The primary purposes of this Clarification Memorandum (CM) are to clarify the prohibited medications list and associated eCRF reporting requirements, remove inconsistent language regarding gestational age at enrollment, correct discrepant language regarding timing of Qualitative IDIs, correct a discrepancy pertaining to study drug management for maternal reduced creatinine clearance, and clarify expectations regarding changes to study procedures for maternal participants who undergo a change to their use of study drug; updates to the Protocol Team and Study Site Rosters have also been made.

Implementation

This CM has been approved by the NIAID and NICHD Medical Officers. Institutional Review Board/Ethics Committee (IRB/EC) approval of this CM is not required by the study sponsor prior to implementation. However, sites may submit this CM to the responsible IRBs/ECs for their information or, if required by the IRBs/ECs, for their approval prior to implementation.

The content of the CM does not impact the sample informed consent forms for the study or the benefit-to-risk ratio for study participants.

This CM should be maintained in each site's essential documents file for IMPAACT 2009. It is the responsibility of the Investigator of Record to ensure that all study staff are made aware of this CM.

The content of this CM will be incorporated into the next protocol amendment as specified below. Additions to the text are indicated in **bold**; deletions are indicated by ~~strike-through~~.

1) *Clarify the prohibited medications list and associated eCRF reporting requirements.*

Section 5.4, Concomitant Medications, 3rd paragraph, first sentence (added)

All prohibited medications must be entered onto eCRFs. In addition, a~~A~~ listing of maternal and infant concomitant medications that must be entered into eCRFs is provided in Appendix III.

Section 5.5, Prohibited Medications, bulleted list under 1st paragraph, 1st bullet:

- ~~Investigational~~ ARV agents, interferon or interleukin therapy

2) *Remove inconsistent language regarding gestational age at enrollment to align with inclusion criteria 4.2.1.2.*

Section 3.2, PrEP Comparison Component Study Design, first paragraph:

The PrEP Comparison Component will assess feasibility, acceptability, adherence to, and safety of PrEP during pregnancy and postpartum in HIV-uninfected adolescents and young women. Women will be ~~enrolled~~ **screened** prior to 32 weeks' gestation **and enrolled** in two cohorts: those who agree to initiate PrEP (Cohort 1) and those who decline (Cohort 2). [*paragraph continues*]

Section 6.2.1, PrEP Comparison Component Maternal Screening, Enrollment and Follow-up Visits, first paragraph:

Refer to Section 4.5 for a description of the study recruitment, screening, and enrollment process. ~~Women will be evaluated for potential participation with a gestational age that will permit study enrollment before 32 completed weeks.~~ Infants will enroll into the PrEP Comparison Component, *in utero*, at the time their mothers enter the study. Infants will have study visits at birth, and 6, 14 and 26 weeks after birth.

Section 6.2.1.1, PrEP Comparison Component Maternal Enrollment Visit, first paragraph:
For eligible women, ~~enrollment must occur in time for study drug initiation prior to 32 completed weeks of gestation. T~~**t**he point of enrollment is taken to be the time when a participant has successfully been entered in the Subject Enrollment System (SES). [*paragraph continues*]

3) *Correct discrepant language in Section 3.2.1 and Section 6.2.1.8 regarding the timing of qualitative in-depth-interviews for pregnant participants. Revisions will ensure internal consistency within these sections, and between these sections and the Schedules of Evaluations.*

Section 3.2.1, Qualitative Evaluation Design, sixth paragraph, first sentence:

The interviews of participants identified for the "currently pregnant" strata will take place at or after ~~the~~ Week 12 ~~visit~~ and ~~prior~~ **through** to Labor and Delivery (Cohorts 1 and 2).

6.2.1.8, PrEP Comparison Component Maternal: Qualitative In-Depth Interview Visit, third paragraph, bulleted list, second bullet:

- For pregnant women in Cohort 2 (n=10) – at or after the Week 12 visit ~~and prior to delivery~~ **through to Labor and Delivery**.

4) *Correct a discrepancy between protocol Section 8.3 and Appendix V pertaining to study drug management for maternal reduced creatinine clearance. The creatinine clearance value in Section 8.3 will be corrected to match the management guidance provided in Appendix V.*

   Section 8.3, Criteria for Premature Discontinuation of Study Drug, bulleted list under first paragraph, bullet #5:

   - Confirmed maternal creatinine clearance **<60mL/minute** ~~<50mL/minute~~

5) *Clarify expectations regarding changes to study procedures for maternal participants in Cohort 1 and Cohort 2/Step 2 who undergo a change to their use of study drug. Guidance for discontinuing specific study procedures for maternal participants in Cohort 1 and Cohort 2/Step 2 who permanently discontinue study drug due to toxicity has been added. Guidance for discontinuing and restarting select study procedures following a change in study drug use due to participant choice has been clarified. The expectation that collection of vaginal secretion samples for PK testing will be stopped when collection of DBS samples for PK are stopped has also been clarified.*

   Section 6.2.3.1, Maternal Participants in Cohort 1 **and Cohort 2/Step 2** Who Subsequently Stop PrEP

   During follow-up, maternal participants in Cohort 1 and Cohort 2/Step 2 may choose to stop PrEP, or may permanently discontinue PrEP due to toxicity. To the extent possible, staff should try to identify the reasons for maternal participants choosing to stop PrEP. Once the maternal participant has stated that she is not willing to continue PrEP**,** ~~and~~ the decision to stop PrEP will be entered into an eCRF **and study drug dispensing will be discontinued immediately**. If not already required for the study visit at which this decision is made **or at which PrEP is permanently discontinued due to toxicity**, the following additional study procedures should be conducted:

   - iNSC
   - TFV-DP level (DBS)
   - HIV RNA test

   For participants in Cohort 1, t~~T~~This will not constitute a change from Cohort 1 to 2; **for participants in Cohort 2/Step 2, this will not constitute a step change back to Step 1. T**~~t~~he maternal participant will continue to be followed as per ~~her original assignment per~~ Appendix IIA with the exception of the following evaluations**, which will be discontinued at subsequent visits**:

   - iNSC with drug level feedback
   - **Study drug dispensing**
   - **Two-way SMS support (one-way SMS support should continue)**
   - **TFV-DP (DBS) level***
   - **Vaginal secretion sampling for drug level***

   ***Sample collection for drug level testing (blood & vaginal secretions) should still be performed at the Labor and Delivery visit.**

These study procedures should be resumed in the event a maternal participant in Cohort 1 or Cohort 2/Step 2 who chose to stop PrEP subsequently chooses to resume PrEP. As noted in Section 6.2.1.2, a negative rapid HIV test result must be available prior to prescribing and dispensing PrEP.

Section 6.3.3, Maternal HIV Seroconversion: Management at Maternal Participants at Every Visit (PrEP Comparison Component Only), Bulleted list under first paragraph, 7th bullet (added)

- **Vaginal secretion sampling for drug level**

6) *Protocol Team Roster Updates*

To reflect current protocol team membership, Kathryn Lypen has been removed from the protocol team roster and Ashley Mayo, Rebecca Dirschberger, and Megan Mueller Johnson have been added; contact details are shown below. Ashley Mayo is also added on the protocol cover page.

Clinical Trials Specialist
Ashley Mayo, MSPH
FHI 360
359 Blackwell Street, Suite 200
Durham, NC 27701, USA
Phone: (919) 321-3436
Email: amayo@fhi360.org

Protocol Data Managers
Rebecca Dirschberger, MPH
Office: (716) 834-0900 ext. 7268
4033 Maple Road, Amherst, NY 14226
dirsch@frontierscience.org

Investigators
Megan Mueller Johnson, MS
Department of Health Behavior and Health Education
1415 Washington Heights
Ann Arbor MI 48109
Phone: (734) 764-5425
Email: memuelle@umich.edu

A. Study Site Roster Updates

The Study Site Roster has been updated as shown below to reflect current site representatives and contact information.

Site 30301, JHU-Blantyre
P.O. Box 1131
Queen Elizabeth Central Hospital
Blantyre, Malawi

~~Frank Taulo, MBBS, FCOG~~
~~Investigator of Record~~
~~Phone: +2651875129~~
~~Email: ftaulo@yahoo.com~~

Sharon Mambiya, MBBS**, Registrar in Obstetrics/Gynecology**
Investigator of Record and Study Coordinator
Phone: +2651875129/ +**265999614533**

Email: **smambiya@jhp.mw**
~~skunkanga@jhu.medcol.mw~~

Rachel Chihana Kawalazira, MBBS, MSc, MPH candidate
Backup Study Coordinator
Phone: +2651875129/+265999894439
Email: rkawalazira@jhp.mw

~~Linly Seyama~~
~~Study Coordinator~~
~~Phone: +2651875129~~
~~Email: lseyama@jhu.medcol.mw~~

Site 30303, Saint Mary's
Suzen Maonera**, RGN, BSN, MSN**
Study Coordinator
Phone: +263772288160
Email: smaonera@uz-ctrc.org

Site 31890, Harare Family Care ~~Centre~~

Tichaona Vhembo MBChB, MPH
Investigator of Record
Phone: +**263 242 704 890** ~~2634704890~~
Email: tvhembo@uz-ctrc.org

Sukunena J. Maturure~~, RN, DCN~~
Study Coordinator
Phone: +263 **24**2 791946/ +263 712 437 682
Email: smaturure@uz-ctrc.org

Site 5126, Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration

Clemensia Nakabiito, MBChB, MMed. Obs & Gyn
Investigator of Record
Phone: ~~+256 414 540441/ +256 414 534 052~~
+256 200 404 100/ +256 772 405332
Email: cnakabiito@mujhu.org

Joel Maena MBChB, MPH
Study Coordinator
Phone: +256 200 404 100/ +256 779 692 426
Email: jmaena@mujhu.org

~~Brenda Gati, MBChB, MSc Epidemiology~~
~~Co-Investigator~~
~~Phone: +256 414 540441/ +256 772 881922~~
~~Email: bgati@mujhu.org~~

~~Phionah Kibalama, MBChB, MSc PH~~
~~Study Coordinator~~
~~Phone: 0414 541 044~~
~~Email: pkibalama@mujhu.org~~

Site 8051, Shandukani, South Africa
Wits Reproductive Health & HIV Research Institute (Wits RHI)
Shandukani Research Centre
~~Corner~~ **7** Esselen St**reet**~~. and Klein St.~~
Hillbrow, Johannesburg, Gauteng, South Africa

Elizea Horne, MBCHB
Investigator of Record
Phone: +27 82 334 7927/ +27 11 358 5500
Email: ehorne@wrhi.ac.za

Nakile Mabaso
Study Coordinator
Phone: +27 11 358 5500
Email: nmabaso@wrhi.ac.za

~~Maysseb (Masebole) Masenya, MD~~
~~Investigator of Record~~
~~Phone: +27 11 358 550 (ext. 5923)/~~
~~+27 82 303 0054~~
~~Email: MMasenya@wrhi.ac.za~~

~~Hedwig Kowo, RN~~
~~Study Coordinator~~
~~Phone: +27 82 849 5350~~
~~Email: hkowo@wrhi.ac.za~~

Clarification Memorandum # 1 for:

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

DAIDS Study ID # 30020
IND # 136735 Held By DAIDS

Clarification Memorandum Date: 9 April 2021

Summary of Clarifications and Rationale

This Clarification Memorandum (CM) updates protocol specifications to reflect current policies of the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Diseases (NIAID), and National Institutes of Health (NIH). It also includes updates to the protocol team and site representative rosters. These updates do not impact the study design or study-specific procedures.

Implementation

Institutional Review Board/Ethics Committee (IRB/EC) approval of this CM is not required by the study sponsor prior to implementation; however, sites may submit it to IRBs/ECs for their information or, if required by the IRBs/ECs, for their approval prior to implementation.

IRBs/ECs may have acknowledged and/or approved remote site monitoring strategies prior to the issuance of this CM. If so, documentation of the acknowledgement and/or approval should be filed in your essential document files for IMPAACT 2009. This CM and any applicable IRB/EC correspondence should also be filed in your essential document files for IMPAACT 2009.

The information included in this memorandum will be incorporated into the next protocol amendment.

A. DAIDS Policy Updates

1. Protocol Section 12 is updated to reflect current DAIDS policies for clinical site monitoring, which allow for on-site and remote monitoring. The prior contents of this section are replaced with the following:

   Under contract to DAIDS or NICHD, site monitors will inspect study site facilities and review participant study records — including informed consent and assent forms, paper-based CRFs (if used), eCRFs, medical records, laboratory records, and pharmacy records — to ensure protection of study participants, compliance with the IRB/EC approved protocol, and accuracy and completeness of records. Monitors also will review essential document files to ensure

compliance with all applicable regulatory requirements. Site investigators will make study facilities and documents available for inspection by monitors.

Monitoring visits may be conducted on-site or remotely. Remote visits may include remote source document verification using methods specified for this purpose by DAIDS or NICHD. Remote monitoring visits may be performed in place of, or in addition to, onsite visits to ensure the safety of study participants and data integrity (1). Site investigators will make study documents available for site monitors to review utilizing a secure platform that is 21 CFR Part 11 compliant. Potential platform options include: Veeva SiteVault, Medidata Rave Imaging Solution, Medidata Remote Source Review, site-controlled SharePoint or cloud-based portal, and direct access to electronic medical records. Other secure platforms that are 21 CFR Part 11 compliant may be utilized, as allowed by DAIDS or NICHD.

Reference:

1. **FDA Guidance on Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency: Guidance for Industry, Investigators, and Institutional Review Boards, March 2020, Updated on January 27, 2021. Accessed at: https://www.fda.gov/media/136238/download**

2. Protocol Section 14.5 refers to the DAIDS policy on identification and classification of critical events. This policy has been retired. Section 14.5 is removed from the protocol and Section 13.1 has been updated to refer to the reporting requirements that still apply for sites conducting this study. The prior contents of the first paragraph in Section 13.1 are replaced with the following:

   Prior to study initiation, site investigators must obtain IRB/EC review and approval of this protocol and site-specific informed consent and assent forms in accordance with 45 CFR 46; subsequent to initial review and approval, IRBs/ECs must review the study at least annually. Site investigators must promptly report to the IRBs/ECs any changes in the study and must comply with the requirements of 45 CFR 46.108(a)(4) and 21 CFR 56.108(b) for promptly reporting the following: unanticipated problems involving risks to participants or others; serious or continuing noncompliance with applicable regulations or the requirements or determinations of their IRBs/ECs; and any suspension or termination of IRB approval.

3. Protocol Section 14.6 (now re-numbered as Section 14.5) refers to requirements for entry of study results into ClinicalTrials.gov. To reflect current NIH and regulatory requirements, the prior contents of this section are replaced with the following:

   The NIH Policy on Dissemination of NIH-funded Clinical Trial Information establishes the expectation that clinical trials funded in whole or in part by the NIH will be registered and have summary results information submitted to ClinicalTrials.gov for public posting. The protocol team will comply with this policy as well as the requirements of 42 CFR 11.

4. Protocol Sections 11.1, 11.2, 11.3, 13.2, 13.3, 13.8, 14.3, and 14.4 refer to the following DAIDS policies:

   - Requirements for Essential Documents at Clinical Research Sites Conducting DAIDS Funded and/or Sponsored Clinical Trials
   - Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials
   - Requirements for Clinical Quality Management Plans
   - Requirements for Manual of Operational Procedures

- Enrolling Children (including Adolescents) in Clinical Research: Clinical Site Requirements

These policies have been retired and replaced with instructions for sites that are now contained in the DAIDS Site Clinical Operations and Research Essentials (SCORE) Manual. Throughout the protocol, references to the above-listed policies are replaced with requirements specified in the DAIDS SCORE Manual (edits not shown here). The SCORE Manual is available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

B. Protocol Team Roster Updates

To reflect current protocol team membership, Ellen Townley, Michael Whitton, and Chanell Wilkins have been added to the protocol team roster; contact details are shown below. Ellen Townley and Michael Whitton are also added on the protocol cover page.

NIAID Medical Officer
Ellen Townley, MSN, FNP
Maternal Adolescent Pediatric Branch
DAIDS, NIAID, NIH
5601 Fishers Lane, Rm 8B37
Rockville, MD 20852-9831
Phone: 240-292-4784
Email: ellen.townley@nih.gov

Westat Representative
Chanell Wilkins, MPH
Westat
1600 Research Boulevard
WB Building 215
Rockville, MD 20850
Phone: 301-610-5548
Email: ChanellWilkins@westat.com

Clinical Trials Specialist
Michael Whitton, BA
FHI 360
359 Blackwell Street, Suite 200
Durham, NC 27701, USA
Phone: (919) 544-7040
Email: mwhitton@fhi360.org

C. Study Site Roster Updates

To reflect recent administrative changes, the site number assigned to the Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration Clinical Research Site is changed from 30293 to 5126. In addition, to reflect recent institutional changes, the email addresses for representatives from CRS 30303 (Saint Mary's), CRS 30306 (Seke North), and CRS 31890 (Harare Family Care) are all updated as follows: ~~@uzchs-ctrc.org~~ **@uz-ctrc.org**. Finally, to reflect current site representatives, Tsungai Chipato (CRS 30303), Nicolette Nabukeera-Barungi, and Sarah Nanyunja (CRS 31798) are removed and the representatives shown below are added.

Site 30303, Saint Mary's
Patricia Mandima, MBChB, MPH
Investigator of Record
Phone: +263 4 704890
Email: pmandima@uz-ctrc.org

Site 31798, Baylor Uganda
Violet Korutaro, MBChB, MScPH, DPPM
Investigator of Record
Phone: +256 417-119200/100
Email: vkorutaro@baylor-uganda.org

Lilian Birungi, BSN, DPPM, MPH
Study Coordinator
Phone: +256 417-119200/100
Email: lbirungi@baylor-uganda.org

IMPAACT 2009

Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

A Study of the International Maternal Pediatric Adolescent AIDS Clinical Trials Network

Sponsored by:
National Institute of Allergy and Infectious Diseases
Eunice Kennedy Shriver
National Institute of Child Health and Human Development
National Institute of Mental Health

Pharmaceutical Support Provided by:
Gilead Sciences, Inc.

DAIDS ES # 30020
IND #136,735 Held By DAIDS

Protocol Co-Chairs:	Benjamin Chi, MD, MSc Lynda Stranix-Chibanda, MBChB, MMED
NIAID Medical Officer:	Hans Spiegel, MD, DrMedSc
NICHD Medical Officer:	Nahida Chakhtoura, MD, MsGH
Clinical Trials Specialists:	Kathryn Lypen, MPH Emily Brown, MA

FINAL Version 3.0
19 November 2020

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

DAIDS Study ID #30020

Version 3.0
19 November 2020
Protocol Signature Page

I will conduct this 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 (U.S.) Health and Human Service regulations (45 CFR 46); applicable U.S. Food and Drug Administration regulations; standards of the International Council 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., U.S. National Institutes of Health, Division of AIDS) and institutional policies.

Signature of Investigator of Record	Date

Name of Investigator of Record
(printed)

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

ABBREVIATIONS AND ACRONYMS

AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine transaminase
AP	Antepartum
AST	Aspartate aminotransferase
cART	Combination anti-retroviral therapy
CASI	Computer-assisted self-interviewing
CBC	Complete blood count
CFR	Code of Federal Regulations
CMC	Clinical Monitoring Committee
COVID-19	Coronavirus disease 2019
Css	Steady state concentration
DAIDS	Division of AIDS
DAERS	DAIDS Adverse Experience Reporting System
DBS	Dried blood spot
DMC	Data Management Center
DOT	Directly observed therapy
DXA	Dual-energy x-ray absorptiometry
EC	Ethics committee
eCRF	Electronic Case Report Form
EAE	Expedited adverse event
FDA	Food and Drug Administration
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (Truvada®)
GDPR	General Data Protection Regulation
HIV	Human Immunodeficiency Virus
IATA	International Air Transport Association
IDI	In-depth interview
IMPAACT	International Maternal Pediatric Adolescent AIDS Clinical Trials Network
iNSC	Integrated Next Step Counseling
IRB	Institutional review board
IND	Investigational new drug
IoR	Investigator of Record
IQR	Interquartile range
L&D	Labor and Delivery
LDMS	Laboratory data management system
LPC	Laboratory processing chart
MSM	Men who have sex with men
mHealth	Mobile health
MOP	Manual of procedures
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	National Institute of Child Health and Human Development
NIH	National Institutes of Health
NIMH	National Institute of Mental Health
OHRP	Office for Human Research Protections
PCR	Polymerase chain reaction

PID	Participant identification number
PK	Pharmacokinetic
PP	Postpartum
PrEP	Pre-exposure prophylaxis
RNA	Ribonucleic acid
SD	Standard deviation
SES	Subject Enrollment System
SMS	Short message service
SPDSMP	Study Progress, Data, and Safety Monitoring Plan
SOE	Schedule of Evaluations
SOP	Standard operating procedure
STI	Sexually transmitted infection
TDF	Tenofovir disoproxil fumarate
TFV-DP	Tenofovir disoproxil fumarate-diphosphate
U.S.	United States

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

PROTOCOL TEAM ROSTER

Protocol Co-Chairs
Benjamin Chi, MD, MSc
Department of Obstetrics and Gynecology
University of North Carolina at Chapel Hill
Chapel Hill, NC 27599
Phone: (919) 445-4174
Email: benjamin_chi@med.unc.edu

Lynda Stranix-Chibanda, MBChB, MMED
Department of Paediatrics and Child Health
College of Health Sciences
University of Zimbabwe,
PO. Box A178, Avondale
Harare, Zimbabwe
Phone: +263 4 704890
Email: lstranix@uzchs-ctu.org

Protocol Vice-Chair
K Rivet Amico, PhD
Associate Professor
University of Michigan
Department of Health Behavior and Health Education
3830 School of Public Health
1415 Washington Heights
Ann Arbor, Michigan 48109-2029
Phone: (734) 763-0051
Email: ramico@umich.edu

NIAID Medical Officer
Hans M.L. Spiegel, MD, DrMedSc, DTMH, FAAP, Contractor, Kelly Government Solutions
Sr. Medical Officer, PMPRB/Prevention Sciences Program,
DAIDS, NIAID, NIH
5601 Fishers Lane, 8B51A
Rockville, MD 20852Phone: (240) 292-4633
Email: hans.spiegel@nih.gov

NICHD Medical Officer
Nahida Chakhtoura, MD, MSGH
Eunice Kennedy Shriver National Institute of Child Health and Human Development
National Institutes of Health 6710B Rockledge Drive, Rm. 2140, MSC 7002
Bethesda, MD 20817
Phone: (301) 435-6872
Email: nahida.chakhtoura@nih.gov

NIMH Medical Officer
Susannah Allison, Ph.D.
Scientific Program Officer
Infant, Child, & Adolescent Research Programs
NIMH Division of AIDS Research
5601 Fishers Lane, 9G22, MSC 9830
Rockville, MD 20852-9830
Phone: (240) 627-3861
Email: Susannah.Allison@nih.gov

Clinical Trials Specialists
Kathryn Lypen, MPH
IMPAACT Operations Center
FHI 360
359 Blackwell Street, Suite 200
Durham, NC 27701, USA
Phone: (919) 544-7040, Ext. 11684
Email: klypen@fhi360.org

Emily Brown, MA
IMPAACT Operations Center
FHI 360
359 Blackwell Street, Suite 200
Durham, NC 27701, USA
Phone: (919) 544-7040, Ext. 11123
Email: embrown@fhi360.org

Protocol Investigators
Geri R. Donenberg, PhD
Associate Dean of Research,
School of Public Health
Professor, Department of Medicine
Director, Community Outreach Intervention Projects and Healthy Youth Program
1603 W. Taylor Street, 845 SPHPI
Chicago, IL 60612
Phone: (312) 996-8602
Email: gerid@uic.edu

Lisa Hightow-Weidman, MD, MPH
Associate Professor
Institute for Global Health and Infectious Diseases
University of North Carolina at Chapel Hill
Phone: (919) 843-0033
Email: lisa_hightow@med.unc.edu

John Shepherd, PhD
Population Sciences in the Pacific Program (Cancer Epidemiology)
University of Hawaii Cancer Center
701 Ilalo Street
Suite 431
Honolulu, HI 96813
Phone: 808-440-5230
Email: johnshep@hawaii.edu

Nicole Tobin, MD
David Geffen School of Medicine
Division of Infectious Diseases
University of California, Los Angeles
10833 Le Conte Ave.
Los Angeles, CA 90095
Phone: 310-825-5235
Email: NTobin@mednet.ucla.edu

Savita Pahwa, MD
University of Miami Miller School of Medicine
1580 NW 10th Avenue
BCRI 712, Miami, FL 33136
Phone: (305)-243-7732
Email: spahwa@med.miami.edu

Petina Musara, BSW
CTU Behavioral Sciences Coordinator
University of Zimbabwe College of Health Sciences
Clinical Trials Research Centre
10 Routledge Street, Milton Park
Harare, Zimbabwe
Phone: +263-42-915-421
Fax: + 263-4-704-897
Email: pmusara@uzchs-ctrc.org

Protocol Virologist
Lisa Frenkel, MD
Professor of Pediatrics, Laboratory Medicine
Adjunct Professor, Global Health and Medicine
University of Washington
1900 - 9th Ave
Box 359300/JMB-8
Seattle, WA 98101
Phone: (206) 987-5140
Fax: (206) 884-7311
Email: lfrenkel@uw.edu

Protocol Pharmacologists
Jennifer Kiser, PharmD
University of Colorado Denver
Skaggs School of Pharmacy and Pharmaceutical Sciences
12850 E Montview Blvd, V20-C238
Aurora, CO 80045
Phone: (303) 724-6131
Email: jennifer.kiser@ucdenver.edu

Peter Anderson, PharmD
University of Colorado Denver
Skaggs School of Pharmacy and Pharmaceutical Sciences
12850 E. Montview Blvd, V20-4101
Aurora, CO 80045
Phone: (303) 724-6128
Fax: (303) 724-7266
Email: Peter.Anderson@ucdenver.edu

Gilead Sciences Representatives
James Rooney, MD, PhD
Vice President, Medical Affairs
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404
Phone: (650) 522-5708
Email: jrooney@gilead.com

Protocol Pharmacist
Kelly Parsons, PharmD, BCPS
Pharmaceutical Affairs Branch
NIH/NIAID/DAIDS/OCSO
5601 Fishers Lane, 9E15
Rockville, MD 20852
Office: 240-669-5721
Fax: 240-627-3112
Email: kelly.parsons@nih.gov

Protocol Epidemiologist
Deborah Kacanek, ScD
Senior Research Scientist
Harvard School of Public Health
Center for Biostatistics in AIDS Research
Boston, MA 02115
Phone: (617) 432-2833

Email: dkacanek@sdac.harvard.edu

Protocol Statistician
Sharon Huang, MS
Harvard School of Public Health
Center for Biostatistics in AIDS Research
651 Huntington Avenue
Boston, MA 02115
Phone: (617) 432-2873
Email: sharon@sdac.harvard.edu

Protocol Data Managers
Benjamin Johnston, BS
Frontier Science Foundation, Inc.
4033 Maple Road
Amherst, NY 14226-1056
Phone: (716) 834-0900 x7407
Email: johnston@fstrf.org

Mattie Bartlett, MPH
Frontier Science
Foundation Inc.
4033 Maple Road
Amherst, NY 14226-1056
Phone: (716) 834-0900 x7270
Email: bartlett@fstrf.org

Protocol Laboratory Data Manager
Frederic Bone
Frontier Science Foundation, Inc.
4033 Maple Road
Amherst, NY 14226
Phone: (716) 834-0900 Ext. 7306
Email: bone@frontierscience.org

Laboratory Center Representative
Frances Whalen, MPH, MT(ASCP)
Clinical Project Manager
University of California Los Angeles
11075 Santa Monica Blvd., Suite 200
Los Angeles, CA 90025
Phone: 704-422-4055
Email: fwhalen@milabcentral.org

Protocol Laboratory Technologists
Cheryl Jennings
Laboratory Project Manager
Rush University Medical Center
1750 W. Harrison Street, 1121 Jelke
Chicago, IL 60612
Phone: (312) 942-5954
Email: cheryl_jennings@rush.edu

Dean Kamangadazi Soko
College of Medicine
Johns Hopkins Research Project
Queen Elizabeth Central Hospital
Blantyre, Malawi
Phone: 265 (0) 1 875 129
Emails: dsoko@jhu.medcol.mw /
sokodean@gmail.com

Community Program Manager
Marcus Bolton
IMPAACT Operations Center
FHI 360
359 Blackwell Street, Suite 200
Durham, NC 27701, USA
Phone: (919) 544-7040 x11678
Email: mbolton@fhi360.org

Community Advisory Board Members
Vincent Sserunjogi
Site 30293, MU-JHU Research Collaboration
Upper Mulago Hill Road
P.O. Box 23491 Uganda
Phone: +256 751674647 or +256 772 674647

Scovia Aseru
Baylor College of Medicine Children's
Foundation-Uganda
Block 5 Mulago Hospital
P.O. Box 72052, Kampala
Phone: +256 714-119200/100
Email: saseru@baylor-uganda.org

SITE ROSTER

Site 30301, JHU-Blantyre
P.O. Box 1131
Blantyre, Malawi

Frank Taulo, MBBS, FCOG
Investigator of Record
Phone: +2651875129
Email: ftaulo@yahoo.com

Sharon Mambiya, MBBS
Study Coordinator
Phone: +2651875129
Email: skunkanga@jhu.medcol.mw

Linly Seyama
Study Coordinator
Phone: +2651875129
Email: lseyama@jhu.medcol.mw

Site 30303, Saint Mary's
15 Phillips Avenue
Belgravia, Harare, Zimbabwe

Tsungai Chipato, MBChB, FRCOG, MSE
Investigator of Record
Phone: +263 4 704890
Email: tchipato@zol.co.zw

Suzen Maonera
Study Coordinator
Phone: +263772288160
Email: smaonera@uzchs-ctrc.org

Site 30306, Seke North
15 Phillips Avenue
Belgravia, Harare, Zimbabwe

Teacler Gamuchirai Nematadzira, MBChB, MSc Clin Epi
Investigator of Record and Study Coordinator
Phone: +263772288155
Email: tnematadzira@uzchs-ctrc.org

Site 31890, Harare Family Care Centre
15 Phillips Avenue
Belgravia, Harare, Zimbabwe

Tichaona Vhembo MBChB, MPH
Investigator of Record
Phone: + 2634704890
Email: tvhembo@uzchs-ctrc.org

Sukunena J. Maturure, RN, DCN
Study Coordinator
Phone: +263 4 791946/ +263 712 437 682
Email: smaturure@uzchs-ctrc.org

Site 30293, Makerere University-Johns Hopkins University (MU-JHU) Research Collaboration
MU-JHU Care LTD
Upper Mulago Hill Road
P.O. BOX 23491 Uganda

Clemensia Nakabiito, MBChB, MMed. Obs & Gyn
Investigator of Record
Phone: +256 414 540441/ +256 414 534 052/ +256 772 405332
Email: cnakabiito@mujhu.org

Brenda Gati, MBChB, MSc Epidemiology
Co-Investigator
Phone: +256 414 540441/ +256 772 881922
Email: bgati@mujhu.org

Phionah Kibalama, MBChB, MSc PH
Study Coordinator
Phone: 0414-541-044
Email: pkibalama@mujhu.org

Site 31798, Baylor Uganda
Baylor College of Medicine
Children's Foundation Uganda
Block 5 Mulago Hospital
P.O. Box 72052, Kampala Uganda

Nicolette Nabukeera-Barungi, MBChB, MMed, PhD
Investigator of Record
Phone: +256 417-119200/100
Email: nnabukeera@baylor-uganda.org

Sarah Nanyunja, BSc Nursing
Study Coordinator - Lead
Phone: +256 417-119200/100
Email: snanyunja@baylor-uganda.org

Site 8051, Shandukani, South Africa
Wits Reproductive Health & HIV Research Institute (Wits RHI)
Shandukani Research Centre
Corner Esselen St. and Klein St.
Hillbrow, Johannesburg, Gauteng, South Africa

Maysseb (Masebole) Masenya, MD
Investigator of Record
Phone: +27 11 358 550 (ext. 5923)/
+27 82 303 0054
Email: MMasenya@wrhi.ac.za

Hermien Gous, Pharm.D.
CRS Coordinator
Phone: +27-83-283-8360/
+27 11 358 5500 ext. 5502
Email: hgous@wrhi.ac.za

Hedwig Kowo, RN
Study Coordinator
Phone: +27-82-849-5350
Email: hkowo@wrhi.ac.za

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

SCHEMA – Pharmacokinetics Component

Purpose: To establish, among young HIV-uninfected women, the plasma drug concentrations associated with daily directly observed oral pre-exposure prophylaxis (PrEP) during pregnancy and postpartum. Findings from this component of the study will be used to guide the opening of the PrEP Comparison Component and interpretation of drug concentrations observed in that component.

Design: Pharmacokinetic (PK) study with oral PrEP drug concentrations determined under adequate adherence conditions.

Study Population: HIV-uninfected pregnant women 16-24 years of age and their infants

Group 1: Enrolled during singleton pregnancy at 14-24 weeks' gestation

Group 2: Enrolled postpartum within 6-12 weeks after delivery

Sample Size: Approximately 40 women (20 per group) to achieve at least 30 evaluable women (15 per group) and their infants.

Study Intervention: Fixed dose combination of 200 mg emtricitabine (FTC) and 300 mg tenofovir disoproxil fumarate (TDF) administered once daily under direct observation for 12 weeks.

Study Duration: Approximately nine months total for this component. Analysis of PrEP drug levels will be completed within approximately three months after the last participant follow-up visit.

Primary Objective

- To determine the concentration of tenofovir diphosphate (TFV-DP) associated with adequate adherence to FTC/TDF among women observed ingesting daily oral PrEP during pregnancy and postpartum.

Secondary Objective

- To compare TFV-DP concentrations observed in pregnant and postpartum women.

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SCHEMA – PrEP Comparison Component

Purpose: To determine among young HIV-uninfected women and their infants, the feasibility, acceptability, and safety of oral pre-exposure prophylaxis (PrEP) during pregnancy and postpartum.

Design: Parallel observational cohort study

Study Population: Pregnant HIV-uninfected women,16-24 years of age, with a confirmed singleton pregnancy of 32 weeks' gestation or less, and their infants.

Cohort 1: Woman accepts PrEP initiation during pregnancy at study entry
Cohort 2: Woman declines PrEP initiation during pregnancy at study entry

Sample Size: Approximately 350 women to achieve at least 300 evaluable (200 in Cohort 1 and 100 in Cohort 2) and their infants.

Study Intervention: *Both cohorts:*

1. Behavioral HIV risk reduction package, including cohort-appropriate SMS messages throughout follow-up.

Cohort 1 only:

1. Daily oral PrEP (200 mg FTC/ 300 mg TDF) throughout follow-up.
2. Enhanced adherence support, including SMS messaging and feedback of drug levels with tailored counseling.

Study Duration: Approximately 24 months total for this component. Women will be accrued over a 12-month period (from the date of first enrollment) and followed through 26 weeks postpartum. For each woman, the duration of follow-up is expected to range from seven to 12 months depending on gestational age at entry. For each infant, the expected duration of follow-up is approximately six months.

Primary Objectives

- To characterize PrEP adherence among HIV-uninfected young women during pregnancy and for twenty-six weeks postpartum, when provided with enhanced adherence support through mobile technology and counseling based on observed drug levels.
- To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety outcomes between cohorts.

Other Objectives

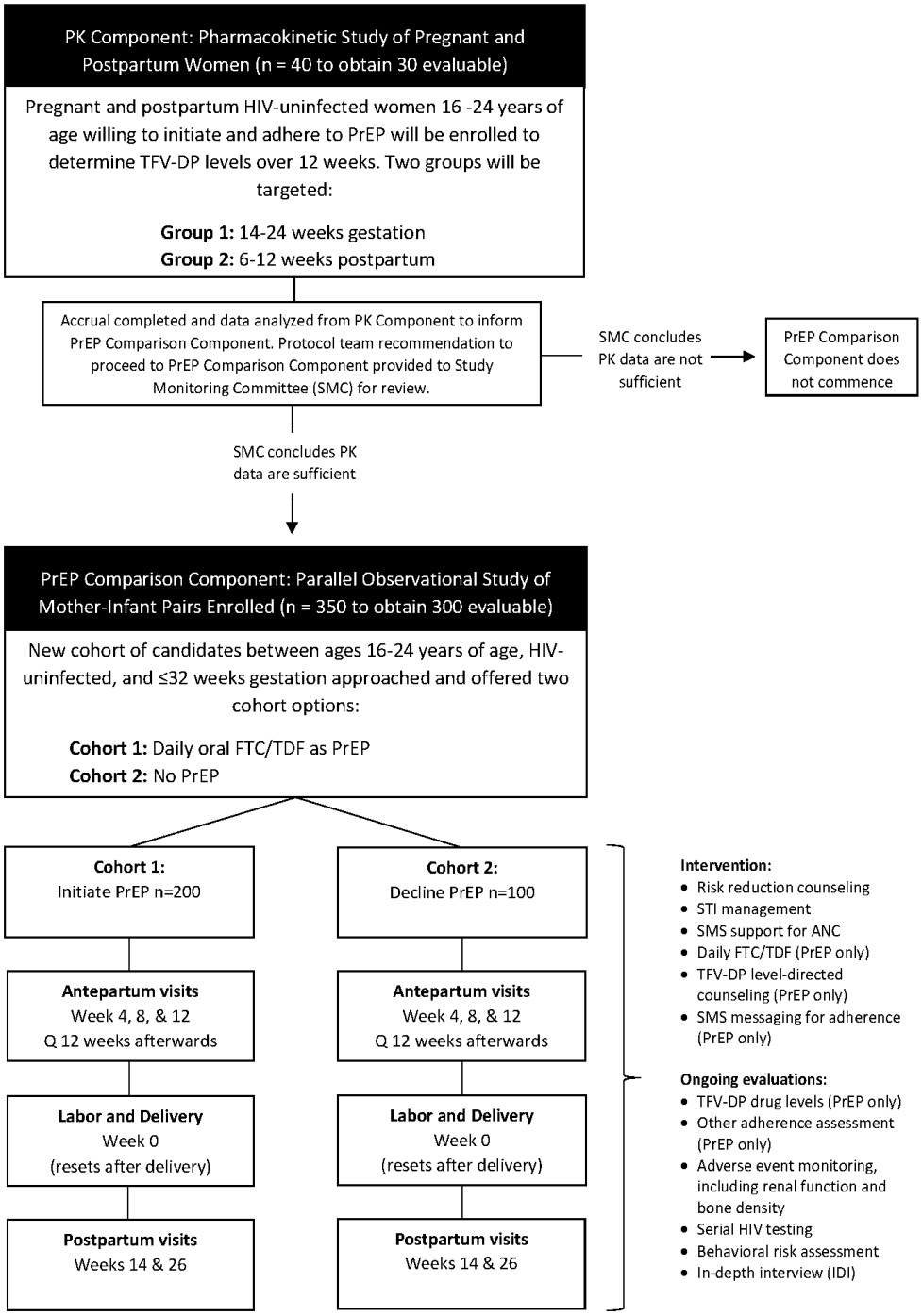
- To identify individual, social, and structural barriers and facilitators to PrEP uptake during pregnancy and to adherence during pregnancy and postpartum.
- To compare reported sexual risk behaviors and incidence of sexually transmitted infections, including HIV infection, among women who initiate PrEP during pregnancy versus women who decline PrEP.
- To compare antiretroviral drug resistance among women and infants who acquire HIV with and without exposure to FTC/TDF for PrEP, including whether resistance was transmitted or acquired at time of transmission.
- To compare bone density in women who initiated PrEP during pregnancy and women who decline PrEP.

Exploratory Objective

- To describe the composition of and changes in the maternal vaginal and gut microbiome and infant gut microbiome according to PrEP exposure.

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Figure 1: OVERVIEW OF STUDY DESIGN



1 INTRODUCTION

1.1 Background

Although oral PrEP with single- or dual-agent tenofovir-based regimens has been shown to be effective across a variety of settings, effectiveness is highly dependent on adherence to the regimen. For women, adherence may need to be near perfect because of lower cervical and vaginal drug concentrations as compared to the rectal mucosa. In randomized efficacy trials, disappointing findings among young women have traced back to poor adherence to the PrEP regimen. These findings have led to questions about the feasibility of PrEP as a public health intervention for young women. However, results from open label studies among women have demonstrated higher adherence levels (1). As such, PrEP has become an increasingly important component of combination HIV prevention for this group (2). Nonetheless, more work is needed to optimize PrEP uptake and adherence in populations at substantial risk for HIV acquisition, including young pregnant women (3). Although considerable evidence has been gathered about the safety of antiretroviral drug use in HIV-infected pregnant women, evidence supporting the safety, feasibility, and acceptability of PrEP use during the antenatal and postpartum periods among HIV-uninfected women is needed.

IMPAACT 2009 will evaluate the use of PrEP during pregnancy and postpartum among HIV-uninfected adolescent and young adult women. Demographically speaking, young women in Sub-Saharan Africa have the highest rate of new HIV infections globally (4, 5) and, because of a heightened risk for incident infection in pregnancy and postpartum (6), this population is in dire need of effective public health strategies for HIV prevention; however, it is unknown whether PrEP is feasible, acceptable, and safe among young women during pregnancy or in the early postpartum period. Women with HIV infection who become pregnant often display better health-seeking behavior than their non-pregnant counterparts. For example, 90% of women with improved adherence during pregnancy in the WITS study cited their baby's health as the primary reason for their adherence (7). A similar phenomenon has been observed among women without HIV, with adherence to daily medications such as multivitamins, ferrous sulfate, and folate being higher during pregnancy. The dual benefit of health interventions during pregnancy – for both mother and fetus – can be highly motivating and may represent a window in which PrEP, can be effectively introduced to young women without HIV infection.

1.2 Prior Research

Evidence Supporting PrEP Efficacy for HIV Prevention

Administration of antiretroviral agents to prevent sexual transmission of HIV has been studied in numerous settings. Single- and dual-agent tenofovir-based regimens have been shown to significantly reduce HIV acquisition among men who have sex with men (MSM), heterosexual men and women, injection drug users (8, 9) and HIV serodiscordant couples (9-13). The efficacy of oral daily PrEP has ranged from 0% to 86% in over ten clinical trials (1, 10-20). Interestingly, in at least two clinical trials of women, oral PrEP did not show a decreased risk of HIV transmission (14, 16); however, these results appeared to be related to poor adherence among study participants. In the FEMPrEP study, for example, only 12% of women had serum drug levels associated with good adherence throughout participation (16). In the Vaginal and Oral Interventions to Control the Epidemic (VOICE) study, less than 50% of women assigned to oral PrEP regimens had plasma tenofovir levels detected, despite participating in a rigorously conducted clinical trial (14).

Given the growing body of evidence about its effectiveness, including encouraging "real world" demonstration projects (21), oral daily PrEP is now recommended for HIV prevention by the United States Centers for Disease Control and Prevention and the World Health Organization (WHO) (22, 23). As with many medical interventions, its effectiveness is highly dependent on adherence to prescribed drugs. This appears to be particularly true for women, who require near perfect adherence (i.e. six to seven doses per week) to reach the required preventive drug concentrations in the female genital tract (24).

Understanding Adherence to PrEP among Pregnant and Postpartum Women

Measuring medication adherence in clinical trials is vitally important because inadequate adherence negatively affects clinical response (25). Rates of adherence among adolescents and young adults with chronic illnesses, such as HIV, diabetes, and cancer are concerning and are often estimated to be about 50% overall (26, 27). Data on adherence to antiretroviral therapy (ART) during pregnancy among HIV-infected women are mixed. Several studies have demonstrated that pregnant women have greater adherence to ART than non-pregnant women (7, 28-30); however, adherence during pregnancy, and even more so the postpartum period, can be improved. A meta-analysis conducted by Nachega and colleagues demonstrated adequate self-reported adherence in only 76% of pregnant women with HIV, which dropped to 53% following delivery (31). Phillips and colleagues (2014) found that missed visits and disengagement from care occurred frequently among HIV-infected pregnant and postpartum women in South Africa (32).

It is important to assess closely whether pregnant and postpartum women at risk for HIV infection would be willing and able to maintain consistent use of a daily medication regimen that is used solely for HIV prevention. Examination of the data on incident pregnancies during the Partners PrEP trial reveals that pill adherence was high overall and no differences were found between adherence among pregnant and non-pregnant women (33). Conversely, results from CAPRISA 004 showed that women who became pregnant during that trial were less likely to have adhered to pre-and post-coital study gel use (34). These discrepant results may be related to the lower median age of CAPRISA participants (22 years) compared to Partners PrEP participants (33 years), differences in oral versus vaginally applied products, differing perceived risk for acquiring HIV among study populations, and differences in eligibility criteria for enrollment. For example, Partners PrEP enrolled only couples with known discordancy in HIV status and used PrEP as a bridge until the infected partner had been on ART for six months, while CAPRISA 004 enrolled sexually active women.

Adherence behaviors can also be extrapolated from studies of non-pregnant adolescent and young adult women. The FACTS 001 licensure trial for tenofovir gel that followed CAPRISA 004 recently reported no effect on HIV acquisition among a large sample of young South African women, primarily because participants struggled to adhere to the prescribed regimen (15). Similarly, the VOICE trial (35) failed to show efficacy of either oral PrEP or daily vaginal gel, largely attributable to very low rates of adherence among a cohort of young women. Two recent randomized trials of the dapivirine vaginal ring showed modest efficacy overall, with an approximately 30% reduction in HIV incidence among those randomized to the intervention arm (36, 37). Interestingly, both studies demonstrated consistently low adherence among women under the age of 21, which attenuated the overall protective effect of the intervention.

Follow-up studies have further investigated PrEP adherence. In the FEM-PrEP study, only 12% of participants had drug levels consistent with four to five doses per week throughout participation (38). Characteristics associated with adherence were limited and included site of

recruitment (adjusted OR for the Bloemfontein site: 2.43, 95%CI: 1.32-4.48), liking the color of the pill (adjusted OR: 2.93, 05%CI: 1.18-7.27), and use of oral contraceptives at enrollment (adjusted OR: 0.37, 95%CI: 0.18-0.74) (38). In semi-structured interviews, five key facilitators for PrEP adherence emerged: a) participants supportive of research, b) HIV risk reduction counselling, c) adherence counseling, d) routine formation and use of reminder tools, and e) partner awareness and support (39). Interestingly, a significant proportion of women misrepresented their adherence behaviors during the study. In audio computer-assisted self-interviews of 224 participants, 31% admitted to reporting adherence to the study intervention when in fact they had not taken the drug. The main reason (69%) was the belief that admission of non-adherence would result in their termination from the trial (40). Women expressed concerns about the investigational nature of the drug and its potential side effects; they also reported discouragement from partners, community members, and other participants (41).

Similar adherence findings were observed in VOICE (MTN-003) (14), a randomized trial of oral and vaginal PrEP agents. Of particular relevance were the high levels of non-adherence determined by plasma drug levels (69%), a figure in stark contrast to the <10% non-adherence by participant self-report (42). A qualitative sub-study at the Johannesburg site (VOICE-C) revealed numerous potential barriers to study drug administration. These included perceptions that antiretroviral agents were associated with illness and concerns that they would be mistakenly identified as HIV-infected. The stigma associated with the PrEP agents occasionally led to relationship conflicts and early trial termination. While antiretroviral drugs were considered potent and beneficial for HIV-infected individuals, they were regarded as potentially harmful when taken by HIV-uninfected individuals (43). In a separate adherence disclosure study, where plasma drug level-based adherence assessments were provided to participants, women at all pharmacokinetic levels suggested that real-time drug monitoring and feedback could improve adherence to the PrEP agents (44).

While adherence data from these prevention trials are sobering, all PrEP trials with negative results to date have been randomized, placebo-controlled trials in which participants were not only blinded to product versus placebo arm, but also counseled that the efficacy of the product itself was unknown. Additionally, the issues around adherence to a vaginal product or coitally dependent dosing may differ substantially from those of oral daily PrEP use. The current study differs from those trials in that participants will be provided with information on the known efficacy of the study product. This knowledge alone may significantly improve adherence to levels seen in recent open label studies of oral PrEP among MSM and heterosexual couples (21, 45). Most encouraging are the recent results from the ADAPT trial (HPTN 067), which evaluated different oral PrEP dosing strategies in the context of open label use. At the Cape Town site, where half of the participants were women ≤25 years, adherence was highest in the daily dosing arm: 92.5% (week 10) and 79.3% (week 30) who had reported sex in the week prior had detectable serum tenofovir (1). In addition, semi-structured interviews and focus groups conducted in the ADAPT trial highlighted the ongoing importance of one's overall trust in PrEP as being safe and effective, and the potential for community distrust to erode confidence in study-provided information on these factors; however, this qualitative work also identified women who were strong advocates and champions for PrEP (46).

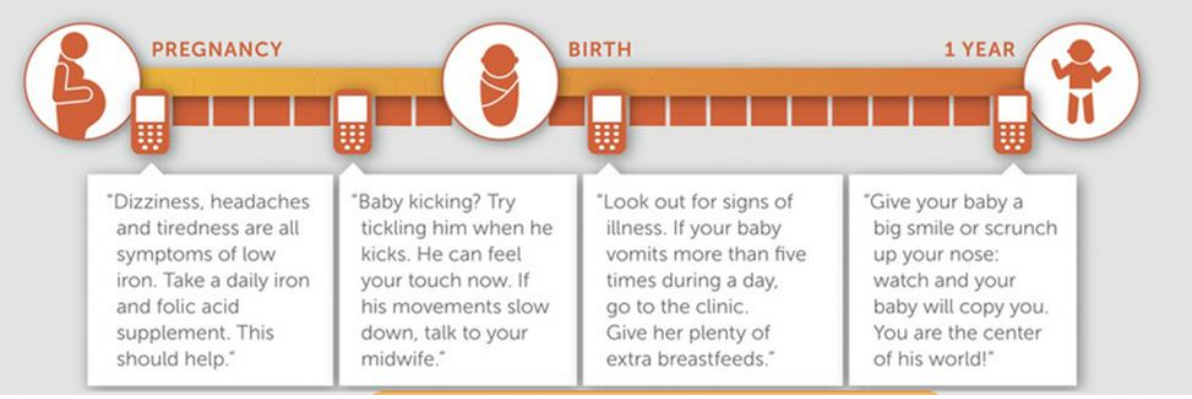
Interim findings from the Plus Pills open-label study of high risk South African adolescents aged 15-19 years (66% female) concluded that PrEP uptake and early adherence were reasonable (47). Good adherence to PrEP (>six pills/week) was reported by 72% of the sample with 76% having tenofovir plasma levels >40 ng/mL at 12 weeks. Ongoing evaluation, however, suggests that maintaining good adherence remains a challenge for these adolescents beyond the 12 week time point (48). The IMPAACT 2009 study specifically targets the distinctive period of pregnancy and

breastfeeding because this is a time when young women may be motivated to initiate PrEP and maintain adherence at uniquely high rates, to protect both themselves and their infants from acquiring HIV infection.

Among adopters of PrEP for HIV prevention, guidelines clearly promote the use of risk reduction and adherence counseling. Nonetheless, the exact kinds of support that will be most effective for youth and young adults is unknown. In the U.S., pilot projects have incorporated dried blood spot (DBS) monitoring with drug level feedback and integrated risk reduction counseling (49). DBS drug levels provide a vehicle for both monitoring as a proxy for adherence and an opportunity to target intervention efforts. Monitoring outcomes of preventative medications or behavioral strategies with biological measures is not uncommon (e.g., insulin monitoring for diabetics, oxygen saturation monitoring for asthmatics, and heart rate monitoring for relaxation training). Although the impact of drug level monitoring and feedback is unknown, early findings from several PrEP demonstration projects have been positive in terms of feasibility and acceptability (44, 50).

Similarly, the use of mobile technology (i.e., "mHealth") to support adherence has been shown to be effective across a broad range of settings (51, 52), and mobile phones are pervasive throughout Africa, especially among the youth population (53). Such technology-based strategies may have greater impact among adolescents and young adults, given their increased proficiency with such technologies even in resource-constrained settings (54-56), and SMS texting has been shown to be efficacious for encouraging HIV prevention, testing, care, and medication adherence (57, 58). Text messages developed by the Mobile Alliance for Maternal Action (MAMA) (59) have been shown to promote key outcomes such as prenatal care visit attendance and exclusive breastfeeding. MAMA has developed free, adaptable messages informed by experts in maternal, newborn and child health (Figure 2).

Figure 2. MAMA: personalized, stage-based messages



The PrEP adherence support package offered through the study is specifically designed to build on top of existing services at the study sites to accommodate the anticipated development of such capacity. Currently, public health maternity services make use of mobile technology in many countries across the region and cell phone penetration is already high. The major goal of the drug level analyses is scientific, to quantify PrEP uptake and use in this population. Nevertheless, there are efforts to develop low cost point of care assays for tenofovir testing, an ideal diagnostic modality for the study setting. This study will provide information on the acceptability and feasibility of drug-level guided adherence support in pregnant women. The intention is not to evaluate the efficacy of the adherence support package, although our work will offer important

information about women's experiences with it. A similar trial in non-pregnant adolescent and young women in sub-Saharan Africa has randomized participants to receive adherence counselling based on feedback from drug levels in addition to the standard of care support package (HPTN 082) and has assessed its effect on their motivation and ability to subsequently adhere to PrEP(60, 61). However, it remains vital for the experiences with drug level informed adherence support to be described in the context of pregnancy and breastfeeding, given that sexually active young women across the region targeted by PrEP implementation programs also have high rates of conception. The 2015 Zimbabwe Demographic Health Survey, for example, found that 17% of 15-19-year-old women have given birth and 27% have begun childbearing in rural areas (62). Findings are similar elsewhere in the region.

Mixed methods studies typically involve collecting and analyzing data derived from both quantitative and qualitative data sources in order to gain greater insight into a research problem than would be gained by using only one of the two methods in isolation (63). By combining methods, the limitations and biases inherent in one single methodology can be lessened by using multiple forms of data collection. Qualitative studies nested within previous trials of biomedical prevention (e.g. VOICE, FEM PrEP, HPTN067/ADAPT) have significantly contributed to our understanding of women's motivations to use PrEP (43, 64, 65) but it is not known yet how the context of pregnancy affects women's perceptions and use of PrEP. As such, this study will include qualitative evaluations which will consist of in-depth interviews (IDI) with maternal participants. These interviews will explore the barriers and facilitators to PrEP initiation and adherence among young women during pregnancy as well as postpartum. The use of qualitative methods will give young women the opportunity to discuss PrEP in their own words, which will be critical to understanding women's own perceptions of PrEP and adherence and to elucidating the mechanisms that underlie decisions about adherence among young women in the context of pregnancy. Refer to Section 3.2.1 for further detail.

Choice of Study Agent

Co-formulated TDF plus FTC was chosen for use in this study, based on efficacy data for both agents from pregnant animal studies, as well as the overall low rate of toxicity associated with their use in PrEP demonstration projects and implementation programs for women. At this time, FTC/TDF is the only PrEP product with sufficient safety and efficacy data during pregnancy to include in a clinical trial. Newer ARV agents are in development, with a gradual increase in available safety data from their use during pregnancy. For example, although tenofovir alafenamide (TAF) may appear promising, there are insufficient data about TAF use as treatment in HIV-infected pregnant women to consider its use for prophylaxis in uninfected pregnant women, where the risk-benefit ratio is quite different. Furthermore, there are no efficacy data yet available in humans on the efficacy of TAF as PrEP nor data to describe how TAF crosses the placenta in humans. While ongoing clinical trials plan to collect these data for TAF, the only PrEP agent available for use over the next several years is FTC/TDF. The primary aim of IMPAACT 2009 is to characterize adherence to and acceptability of oral PrEP, hence the study findings will apply to all forms of PrEP that utilize daily oral dosing. This protocol will fill critical gaps in knowledge related to the acceptability and feasibility of PrEP dosing during this time in a woman's life.

The Need for Safety Data for HIV-uninfected Pregnant and Postpartum Women

The safety data for TDF and FTC/TDF are taken predominantly from clinical trials involving HIV-infected adults undergoing treatment for HIV and non-pregnant adults receiving PrEP for HIV prevention. While the pool of opportunistic safety data on PrEP use in pregnancy is growing, prior studies of PrEP in women tested frequently for incident pregnancy and stopped dosing upon confirmation of pregnancy. Studies in men and non-pregnant women may not translate directly into the pregnant and postpartum context. This is most obvious with regard to fetal and/or infant development, but the physiological changes associated with the pregnant state (specifically renal and hepatic) also may alter toxicity risk and pharmacokinetics of PrEP. A better understanding of obstetrical outcomes with PrEP is also urgently needed. Thus far, few have investigated this important question. While clinical guidelines from the Center for Disease Control (CDC) (22), and more recently the WHO (23) do not consider pregnancy to be a contraindication to PrEP, they call for further research to be done in concern with the scale up of PrEP delivery to women at substantial risk of HIV who become pregnant or are lactating (66). A systematic review in 2016 concluded that there was no safety-related rationale to prohibit PrEP use in pregnant and breastfeeding women and that it should be continued in women who conceive on PrEP and remain at risk of acquiring HIV infection (67). Apart from characterizing important PrEP adherence patterns and drivers, the findings from our study will contribute to the body of evidence released to date by providing additional data collected under clinical trial conditions on safety outcomes experienced by HIV-uninfected women who take PrEP during pregnancy and lactation.

Evaluating risk-benefit ratio

Side effects associated with FTC/TDF (Truvada®) include diarrhea, nausea, fatigue, headache and rash which are seen in about 10% of participants during the initiation of therapy (68). While these early symptoms are generally well-tolerated and dissipate over a few weeks, such side effects may be less tolerable for pregnant or postpartum women. For HIV-infected pregnant and postpartum women, such risks – including the adverse bone and renal effects of TDF – are offset by the significant health benefits of taking the drug as part of ART. However, the risk of adverse drug effects in relation to the potential HIV prevention benefits for HIV-uninfected women at increased risk for infections requires further evaluation.

Tenofovir is a highly-charged anion that does not readily permeate across epithelial barriers. The water soluble pro-drug administered by mouth, TDF, is well absorbed in the gut and is rapidly converted to the active form - tenofovir. While tenofovir is present in amniotic fluid and cord blood, there is low penetration of active drug from maternal blood into breastmilk and subsequently across the infant gut. Low blood-milk penetration and infant drug levels were recently confirmed in a prospective study of 50 HIV uninfected lactating women receiving 10 days of directly observed oral dosing with FTC/TDF PrEP (69). While more ready excretion of FTC into breastmilk was evident, infant plasma concentrations were 0.5% of levels expected from therapeutic dosing in infants. This suggests the risk of postnatal exposure to PrEP in breastfed infants is low.

Renal damage

TDF use has been associated with a small and asymptomatic decrease in glomerular filtration rate in HIV-infected individuals. Reports from completed PrEP clinical trials reveal no clinically significant elevations in serum creatinine (70) but transient decrease in estimated glomerular filtration rates and a return to baseline levels within four weeks of PrEP discontinuation (71).

Glomerular filtration rates increase by up to 50% during pregnancy as part of normal physiologic changes. Reassuringly, in the women assigned to TDF-FTC-LPV/r (Arm C) in IMPAACT 1077BF (PROMISE), creatinine elevations were uncommon overall with <1% of women (n=2/424) having a confirmed Grade 2 or higher renal chemistry event up to 14 days postpartum (72). A recent randomized trial of TDF use in pregnant women who were positive for hepatitis B virus e-antigen found similar maternal and infant safety profiles between the intervention and control arms. Although TDF was associated with higher creatinine kinase and alanine aminotransferase levels, these differences were modest and not thought to be clinically meaningful (73).

Bone demineralization

Consistent with data on reduced bone mineral density (BMD) among HIV-infected adults on ARVs, PrEP use has been associated with an approximate 1% reduction in BMD among trial participants (11, 74). The effect on BMD was shown to be reversible in young African women adherent to PrEP in the VOICE trial with a follow-up dual-energy x-ray absorptiometry (DXA) scan 48 weeks after stopping study drug (75). Of special note is that the women studied in these trials were not lactating. There are no data at this time to evaluate the additive effect of lactation on bone loss due to PrEP. The bone mineral resorption required for breastmilk production results in a 3-5% reduction in BMD over six months of lactation that is generally restored within six months of cessation of breastfeeding. Preliminary data from IMPAACT P1084s – a sub study of PROMISE conducted at eight African sites – revealed a decline in BMD at 74 weeks postpartum in HIV-infected women receiving TDF-based cART who breastfed for a mean of 61 weeks when compared to women not receiving cART (76). The mean difference at the lumbar spine was -3.16% (95% CI -4.44, -1.87) and -2.33% (-3.23, -1.42) at the hip (both p-values<0.001). The IMPAACT 2009 study provides an important opportunity to study the effect of PrEP use and lactation on bone mass in young women who are yet to achieve peak bone mass.

For U.S. infant populations, Siberry and colleagues reported reduced bone mineral content of uncertain clinical significance among newborns who had been exposed *in utero* to TDF taken by their HIV-infected mothers (77). However, more recent work from Cape Town, South Africa found that fetal exposure to TDF did not appear to alter fetal long bone growth (78). IMPAACT P1084s found that *in utero* exposure to TDF-containing cART was not associated with lower bone mineral content among HIV-exposed newborns, when compared to other cART regimens. However, the data suggested a substantial and significant bone mineral content reduction in newborns exposed to maternal triple cART when compared to maternal zidovudine monotherapy (79). The effect of exposure to *in utero* and postnatal ARVs on long term infant bone health, growth and development remains so far still uncertain. Six-month longitudinal outcomes are currently being analyzed for P1084s.

DXA measurement of the hip and spine is the standard assessment to monitor BMD. BMD is expressed in absolute terms of grams of mineral per square centimeter scanned (g/cm2) and is compared to the expected BMD for the patient's age and sex (Z-score) or to young adults of the same sex (T-score). There are no normative data for DXA scan assessment of BMD in infants. Undergoing a DXA scan involves exposure to less radiation than experienced during a chest x-ray.

Adverse pregnancy outcome

A secondary analysis from the Partners PrEP study found no differences between women with incident pregnancies while taking PrEP in rates of preterm birth, congenital anomalies, or infant growth in the first year of life and women taking placebo; however, all women stopped the study medication at time of pregnancy detection, which occurred a median of 35 days (IQR: 29–45) after conception (69). In the ASPIRE trial (MTN 020) that evaluated the dapivirine ring for HIV prevention, 169 of 2551 women became pregnant and immediately stopped the study product upon diagnosis. The rates of adverse birth outcomes did not differ according to study arm (80). The Partners Demonstration Project, an open-label delivery study in Kenya and Uganda, HIV-uninfected women in serodiscordant relationships were given the option of continuing PrEP even after pregnancy was diagnosed. Of the 34 who became pregnant, 30 elected to continue PrEP antenatally. When compared to 96 women who became pregnant in the Partners PrEP clinical trial, there were no differences in pregnancy loss, preterm delivery, or congenital anomalies, though sample sizes were small (81). Finally, use of TDF in the last trimester of pregnancy to reduce hepatitis B vertical transmission is increasingly common, with no reports of an effect on pregnancy outcome to date (73, 82). Encouragingly, an ongoing randomized study in Thailand of tenofovir use among HIV-uninfected, hepatitis B-positive pregnant women (ClinicalTrials #NCT01745822) has not raised any safety concerns at the interim DSMB review in July 2015 (83, 84).

These findings are in contrast to results from the PROMISE trial, which demonstrated higher proportions of severe adverse pregnancy outcome in healthy HIV-infected pregnant women who received TDF-FTC-LPV/r (9%) versus ZDV-3TC-LPV/r (4%) to prevent *in utero* HIV transmission (72). The risk of very preterm delivery before 34 weeks of gestation was also significantly higher in women who received TDF-FTC-LPV/r versus ZDV-3TC-LPV/r (6.0% versus 2.6%, p=0.036), as was the risk of infant death by Day 14 (4.4% versus 0.6%, p<0.001). Further work is underway to better understand this finding, including whether drug interactions related to concomitant lopinavir-ritonavir may have contributed to these health outcomes. The gestational age at delivery for IMPAACT 2009 will be confirmed using obstetrical ultrasound, thus addressing an important limitation of studies thus far. The improved precision of gestational age dating will be an important contribution to the medical literature around antiretroviral drug exposure and adverse birth outcomes, without the potential confounding from HIV infection.

HIV drug resistance

Because PrEP comprises only two agents, concerns about ARV resistance have been raised for PrEP users who acquire HIV infection. Early study results provide some reassurance in this regard. Antiretroviral resistance has been consistently rare across PrEP trials, limited only to those with unidentified acute HIV infection at the time of PrEP initiation and decreasing rapidly in the absence of continued PrEP exposure (9, 85). For those individuals who acquire HIV after enrollment, there were no cases of resistance (10). The lack of resistance among seroconverters on PrEP was attributed in part to the low adherence among these participants. Despite these findings, vigilance is still required. One case of multiclass drug resistant HIV transmission has been documented in an individual who was likely adherent to PrEP (86). With high levels of TDF resistance reported among individuals failing first-line TDF-based ART – up to 57% in sub-Saharan Africa – (87) there are legitimate concerns about use of the same antiretroviral agent in both HIV prevention and treatment modalities. While early data remain encouraging, further investigation about adherence is needed in new populations such as pregnant and postpartum women.

Behavioral disinhibition

Alongside the potential for biological adverse events, there are behavioral risks that may be associated with the PrEP intervention as well. Risk compensation or risk homeostasis theory is based on the presumption that persons have an inherent set-point that determines their willingness to take risk (88, 89). According to this theory, any modification in the environment that reduces the external probability of risk could lead an individual to increase their risk-related behaviors (i.e., behavioral disinhibition) in order to maintain the homeostatic set point, thereby neutralizing the benefits of risk-reduction strategies. In this manner, risk compensation has the potential to threaten the benefits of emerging HIV prevention technologies. Despite considerable concerns for the potential for risk compensation (or safety offset), trials completed to date have not found supporting evidence for this. Rather, trials and projects consistently report "prevention synergies" where PrEP use has been associated with increased awareness of HIV prevention and commitment towards prevention (19, 90-94). In most trials to date, self-reported condom use increased, number of sexual partners decreased, and sexually transmitted infection diagnoses decreased in participants receiving PrEP (10, 13, 21, 95). While open label PrEP demonstration projects have shown high rates of STIs while on PrEP, these rates appear consistent with baseline diagnoses of STIs and thus aren't rising in the context of PrEP (19, 90, 96).

Other safety concerns

Discontinuation of FTC/TDF in HIV-infected individuals with hepatitis B co-infection has been associated with subsequent liver injury. These "hepatic flares" have been documented in several settings when an agent with anti-hepatitis B activity (e.g., TDF, FTC, lamivudine [3TC]) is stopped (97-99). However, less is known about the incidence of such events in the absence of HIV co-infection. In the iPrEx study, HIV-uninfected participants with chronic hepatitis B infection on FTC/TDF did not encounter hepatic flares after PrEP was stopped, but the sample size was small (n=5) (100). Investigators reported similar findings in a multicenter study of TDF for PrEP in West Africa. Among 23 women with active hepatitis B in the intervention arm, none experienced acute exacerbation following discontinuation of the study agent (101). Nevertheless, participants will be screened for hepatitis B surface antigen prior to enrollment. Because of this potential risk of hepatitis B exacerbation and its potential impact during pregnancy, those with a positive result will be excluded from study participation.

Potential changes in the microbiome with PrEP use in pregnancy

Recent studies suggest that the vaginal microbiome in pregnancy could play an important role in maternal as well as neonatal health outcomes. It is well established that the vaginal bacterial community undergoes a shift in composition in pregnancy (102). Changes primarily involve dominance of *Lactobacillus* species that are believed to secrete antibacterial products and produce metabolites such as lactic acid. The resulting low pH represents an effort by the host to inhibit pathogen growth (103). Consequently, circumstances that lead to dysbiosis of the vaginal microbiome could result in complications of pregnancy including risk of preterm birth (104-106).

Intravaginal practices are common in sub-Saharan African women, specifically vaginal drying, washing and insertion of herbs, which are associated with altered vaginal pH and bacterial vaginosis (107, 108). These practices continue during pregnancy and post-delivery (109), with specific cultural practices linked to preparing the vaginal tract for childbirth. It is known that the vaginal microbiome is more stable during pregnancy (110, 111); however, intravaginal practices that promote dysbiosis could affect wound healing, (112) which may be especially important following delivery in high HIV prevalence settings.

Additionally, the microbiome appears to be important for local drug level concentrations and HIV acquisition risk. In a sub-study of a PrEP trial conducted by the Centre for the AIDS Programme of Research in South Africa (CAPRISA) of TFV vaginal gel to prevent HIV-infection in young South African women, efficacy varied by the composition of the vaginal microbiome. Among women whose microbiome contained >50% *Lactobacilli*, TFV vaginal PrEP use protected 61% of women from HIV acquisition. In contrast, only 18% of women whose microbiome was composed of <50% *Lactobacilli* were protected *(113)*. *In vitro* studies demonstrated that TFV levels decreased by approximately half in the presence of *Gardnerella vaginalis* (114). Furthermore, in the FRESH study, women with bacterial communities dominated by *Gardnerella vaginalis* or diverse bacterial communities had up to a four-fold greater risk of acquiring HIV (115). These results are supported by more recent work by Hillier S. et al, showing that trough levels of TFV were lower in cervicovaginal fluid and plasma among women with higher levels of *G. vaginalis*, when intravaginal PrEP agents were used (116). This is in contrast to a secondary analysis from the Partners PrEP Study, which studied oral (vs. intravaginal) PrEP formulations. Heffron and colleagues found that bacterial vaginosis did not alter the efficacy of the oral PrEP intervention, though vaginal drug concentrations were not measured (117). Clearly, more work in this area is needed, particularly in the context of pregnancy.

Most microbiome studies available assess the gut microbiome. While a healthy vaginal microbiome is often dominated by only a few bacteria, a healthy gut microbiome is characterized by greater diversity. While it is known that antibiotics and proton-pump inhibitors alter the gut microbiome, there are only a few studies about antiretroviral drugs which report a decrease of alpha diversity in treated individuals (118-120) . In the existing studies of the gut microbiome in HIV-infection, antiretroviral therapy does not restore the microbial dysbiosis of HIV-infection and HIV-associated dysbiosis correlates with immune activation and disease progression (121-124). Furthermore, in a study of the HIV-associated microbiome in Uganda, the HIV-associated microbiome changes documented in previous studies were not seen in this East African cohort, likely due to differences in the baseline microbiota in the developed versus developing world (125). Given that as many as 20% of women report engaging in heterosexual anal intercourse in sub-Saharan Africa (126), the underlying changes to the maternal gut microbiome with oral PrEP may be an important factor in HIV acquisition

Therefore, it is scientifically important to assess changes to both the vaginal and rectal microbiota of women on oral PrEP. Although the mechanisms of action appear different, changes in both the vaginal and rectal microbiome could affect the efficacy of PrEP interventions. At this time, however, data remains sparse, particularly among adolescent and young women who are pregnant. IMPAACT 2009 provides an ideal opportunity to characterise the microbiome in this sub-population and begin to study its relationship with FTC/TDF use.

Maternal PrEP use and the infant gut flora

The seeding of the neonatal gut by the maternal vaginal microbiome during birth and then by the microbiome present in breast milk constitute major mechanisms for protection of the infant from infections, atopy, and autoimmunity by influencing infant metabolism and immune system development (127). The host–microbial symbiosis that is established in early life contributes to host development and influences disease progression later in life. The immune system is intimately linked to the microbiome and dependent on the presence of commensal microbiota, particularly in the gastrointestinal tract and the infant immune system is modulated by the nature of colonizing bacterial species. In germ-free mice the immune system is altered, with deficits in both innate and adaptive immune components of the gut mucosa (128). Interestingly, emerging evidence indicates that the infant microbiota may be populated *in utero*, is seeded much earlier

than was previously thought, and can be altered by gestational exposures (129). In pre-term and healthy term pregnancies, there is now evidence for maternal transmission of bacteria to the fetal gut during gestation, with bacteria associated with the placenta and amniotic fluid (130, 131), attributed to swallowing of amniotic fluid by the infant continuously from mid to late gestation. The evidence for transmission of microbes from the mother to the fetus during gestation is further corroborated by the observed similarity of the neonatal microbiota at the time of delivery with maternal skin and vaginal microbiota.

A recent review by S Tamburini (132) discussed factors such as host genetics, prenatal environment and delivery mode that shape the newborn microbiome at birth. After birth, other factors such as antibiotic treatment, diet or environmental exposure further modulate the development of the infant's microbiome and immune system, underscoring the relationship between composition of gut microbiome in infancy and risk for several diseases.

In studies by Aldrovandi et al in Haiti (133), HIV infected women were found to manifest an altered microbiome with the result that the microbiome of their HIV exposed infants was disrupted. The studies of Madan JC et. al (132) have clearly demonstrated that the infant intestinal microbiome at approximately six weeks of age is significantly associated with both delivery mode and feeding method.

The increasing evidence of the impact of the maternal microbiome on the infant gut microbiome highlights the importance of understanding the microbiome composition in mothers and infants. In the context of maternal oral FTC/TDF PrEP use, preliminary data generated in IMPAACT 2009 will add to the pool of knowledge relating to the origin of the infant gut microbiome and explore the potential effect of maternal PrEP use on the infant microbiome.

1.3 Rationale

1.3.1 Feasibility and Acceptability of Pre-Exposure Prophylaxis during Pregnancy and Breastfeeding

Adolescent and young adult women in sub-Saharan Africa have the highest HIV incidence rates globally (35). Three recent HIV prevention trials in this population (FEMPrEP, VOICE, and ASPIRE) reported HIV incidence rates of 5-6% despite monthly HIV testing, individualized risk-reduction counseling, provision of condoms, and treatment of sexually transmitted infections (16, 134, 135). In resource-limited settings with HIV incidence of this magnitude, pregnant and postpartum women are at high risk of acquiring HIV. In a meta-analysis and systematic review, Drake and colleagues found an overall incidence of 3.8 per 100 person-years, a figure that was comparable to (or even higher than) those observed in studies of "high risk" non-pregnant populations: female sex workers (2.7 per 100 person-years), HIV-discordant couples from South and East Africa (2.0–3.6 per 100 person-years), and men who have sex with men (MSM) in North and South America (6.1 per 100 person-years) (6). A more recent meta-analysis, conducted by Graybill and colleagues, found similarly high HIV incidence during pregnancy and breastfeeding (3.6 per 100 person years), even with numerous studies from the era of combination HIV prevention(136). A variety of biomedical and behavioral factors are thought to contribute towards this risk. First, observational data suggest that the hormonal changes women experience during pregnancy may increase HIV acquisition risk (137). As a result, HIV acquisition may be as much as four times higher among pregnant women than among non-pregnant women, with young, single women being at particularly high risk (6, 138-140). Second, reports from South Africa revealed that less than half (40%) of women who had been pregnant in the past year were aware

of their sexual partner's HIV status (141, 142). Third, cultural norms may increase a woman's risk for HIV exposure, including decreased condom use during pregnancy or the sanctioning of external sexual partners for the husband while his wife or primary partner is pregnant or postpartum (70, 143). A recent cohort study of pregnant young women in Kenya (n=1304; median age 22 years), HIV incidence was 2.31/100 person years, condom use was reported by only 8% of participants, and none of the women who seroconverted identified that they had an HIV-infected partner (144).

PrEP for HIV prevention has been shown to be highly efficacious among MSM, heterosexual men and women, serodiscordant couples and injection drug users (10-12, 45). While pregnant women have been excluded from enrollment into PrEP trials, very high rates of pregnancy (range 3.9 to 52 per 100 person years) have occurred among participants in HIV prevention trials despite ready access to effective contraception (141, 145, 146). In the case of incident pregnancy, PrEP trial participants were instructed to stop study drug immediately, at an average gestational age of 6 weeks (147). Data from these incident pregnancies as well as the registry data show that risk for toxicity appears to be low and neither pregnancy nor breastfeeding is considered a contraindication for PrEP initiation per the CDC and WHO clinical guidelines (148). Exposure to PrEP in early pregnancy, for example, was not associated with detectable differences in later pregnancy outcomes (147). The PrIYA PrEP implementation program in Kenya recorded no observed difference in pregnancy outcomes among women with prenatal PrEP use(149). In addition, the recommended regimen for PrEP, FTC/TDF, has been used safely for treatment of pregnant women with chronic hepatitis B infection as well as pregnant women with HIV for over a decade and forms the backbone of the first line treatment regimen recommended by the World Health Organization (WHO) (82, 150-153).

Although there are numerous female-initiated antiretroviral prophylaxis strategies currently under study, daily oral PrEP is one of the few proven modalities supported by scientific evidence at this time. As PrEP trials move from efficacy to implementation, and licensing is being sought in low-resource countries, several PrEP implementation projects are enrolling young women at high risk of acquiring HIV infection through unprotected sexual activity. Despite PrEP becoming available to young women in sub-Saharan Africa, critical scientific gaps remain concerning the safety and efficacy of PrEP use in young pregnant and postpartum women (10, 33, 154-156) and access remains restricted (157). While published reports suggest that young women may find the concept of oral PrEP acceptable during pregnancy and breastfeeding and are able to engage with PrEP, PrEP adherence may be particularly challenging during pregnancy(158-160). Importantly, the IMPAACT 2009 study will document context-specific barriers and facilitators of engaging with PrEP during pregnancy and postpartum, contributing much needed evidence to inform the PrEP implementation approach for adolescent and young women in high-burden settings(161).

Pregnant and postpartum women are at increased high risk for HIV acquisition and acute infection during these periods is associated with higher risk of transmission to the fetus or infant. As highly efficacious ART regimens are implemented widely, including "Option B+" and Treat All strategies that do provide lifelong ART to all HIV-infected pregnant and postpartum women, primary prevention must also be implemented widely, delivering proven interventions to those at risk. Given the concentration of the HIV epidemic among young women in sub-Saharan Africa – and the lack of specific pregnancy and postpartum data from current PrEP studies – there is urgent need for targeted study in this critical area.

IMPAACT 2009 will be conducted at sites in Sub-Saharan Africa, the region with the highest HIV incidence among adolescent and young adult women, including during pregnancy. It is in this population at substantial risk of acquiring HIV infection that the potential benefits of PrEP

could outweigh risks and associated financial cost. Similarly, this population stands to benefit the most from participation in such a study.

1.3.2 Safety of Pre-Exposure Prophylaxis during Pregnancy and Breastfeeding

Based on the existing evidence on safety and effectiveness, the World Health Organization does not list pregnancy as a contraindication to PrEP use. In settings of high HIV prevalence and serodiscordancy, the benefits of PrEP in pregnancy likely outweigh the associated potential risks (23). Despite reassuring data to date, in their comprehensive systematic review of TDF safety in pregnancy, Mofenson and colleagues acknowledged the limited number of studies examining safety among HIV-uninfected pregnant women (67). Most randomized studies of oral and vaginal PrEP, for example, discontinued study product once pregnancy was diagnosed (80, 147). Continued surveillance is needed to confirm the safety of PrEP during pregnancy and breastfeeding (67). Safety data from PrEP demonstration projects will contribute to this body of evidence. Early reports from the Partners Demonstration Project have been reassuring – with no increases in pregnancy loss, preterm birth, or congenital anomalies – but the sample of exposed pregnancies remain small (n=30) (81). There may be inherent limitations to this approach as well. PrEP adherence can be difficult to measure in clinical settings making it challenging to differentiate between a favorable safety profile vs. poor medication adherence to PrEP. A focus on sustainability has been a hallmark of many demonstration projects in sub-Saharan Africa; however, the integration of services into existing health systems can impose constraints on available health outcomes such as bone mineral density and vaginal microbiome. Further research is needed to enhance ongoing surveillance activities, to better understand the overall safety of PrEP during pregnancy and breastfeeding.

Finally, the WHO recommends that PrEP be considered for HIV prevention during pregnancy and breastfeeding after carefully weighing the potential risks and benefits (23). To date, there have been no clinical studies directly evaluating safety among HIV-uninfected pregnant and breastfeeding women, a gap that – despite WHO guidance thus far – may limit implementation. Such a delay has already been observed in South Africa, where the Medicines Control Council (MCC) awaits additional safety data from this specific population before removing the current restriction on PrEP use for pregnant and breastfeeding women (personal communication, L-G Bekker). Without better information to support policy, the full implementation of PrEP in in pregnant and breastfeeding women appears unlikely. Given the high HIV incidence rates observed in this population, this could have important consequences for HIV prevention efforts globally.

1.3.3 Pharmacokinetic Thresholds of Adequate PrEP Adherence in Pregnancy and Breastfeeding

Adherence to PrEP is crucial to overall effectiveness, but it has been traditionally difficult to quantify. Intracellular TFV-DP in red blood cells (measured with dried blood spots) is a relatively new modality that, given the agent's long half-life of 17 days, can provide an objective measure for cumulative FTC/TDF PrEP adherence. The long half-life translates into drug accumulation such that increasing numbers of ingested doses produces higher TFV-DP that can be interpreted as cumulative adherence. Approximately 12 weeks of dosing (5 half-lives) is needed to achieve steady-state in red blood cells. For instance, the following TFV-DP concentration thresholds have been used in MSM studies to estimate average adherence: below the limit of quantitation (BLQ to <350 fmol/punch (< 2 doses per week), 350-699 fmol/punch (two to three doses per week), ≥700 fmol/punch (≥4 doses per week). These interpretations were used in MSM in the iPrEx Open-Label Extension and PrEP demonstration projects, and were correlated with PrEP efficacy (20,

162). The relatively simple requirements for specimen collection and storage – using dried blood spots on filter paper – makes it adaptable to both programmatic and clinical settings.

Among MSM, specific TFV-DP thresholds such as those above have been correlated with reduced HIV acquisition in the setting of FTC/TDF PrEP usage (20, 163). At present, however, there exists no analogous data among women in general including pregnant women. There are numerous physiologic changes associated with pregnancy (e.g., cardiovascular, hematologic, increased renal clearance of TDF (164), that may affect the drug levels observed with adequate adherence to FTC/TDF. For instance, pregnant women exhibited 39% higher renal clearance of tenofovir compared with non-pregnant women, which could translate to lower than expected TFV-DP in DBS during pregnancy (164). Additionally, pregnant women undergo plasma volume expansion and increased erythropoiesis during pregnancy, which could also translate into differences in expected TFV-DP in red blood cells (165). Generally, these changes are not marked in the first trimester of pregnancy but become more significant in the second and third trimesters.

Given that adherence to PrEP is the primary outcome of this study, a Pharmacokinetic (PK) Component was included to establish expected TFV-DP thresholds in DBS for adequate adherence over time in this important population, when FTC/TDF is administered daily. Prior research in non-pregnant women has already established TFV-DP drug levels at various levels of adherence following initiation of daily oral PrEP (166); these levels are likely similar in the first trimester of pregnancy. The focus was instead on TFV-DP levels in the second and third trimesters of pregnancy in this study. Determination of these levels provides important general knowledge about PrEP in pregnancy. More importantly for this study, these results will directly inform the "biofeedback" counseling in the PrEP Comparison Component.

1.3.3.1 PK Component Results

The PK Component evaluated PK characteristics of daily oral PrEP (FTC 200mg/TDF 300mg) among 20 pregnant (median gestational age: 18 weeks) and 20 postpartum (median time after delivery: 7 weeks) women at a median age of 20 years. Daily FTC/TDF was administered under direct observation for 12 weeks; of 3360 doses, 3352 [>99%] were directly observed). Weekly TFV-DP was measured from DBS using a validated liquid chromatography-tandem mass spectrometry assay. TFV-DP distributions were determined at 12 weeks and groups compared with the Wilcoxon test. Population PK models were fit to estimate half-life and steady state concentrations.

TFV-DP accumulated with a half-life of 15.3 days (95%CI: 12.8,17.8) in pregnancy and 18.0 days (95%CI: 15.3,20.7) postpartum, with steady state achieved by 8-10 weeks in both groups. Median TFV-DP was 965 fmol/punch (IQR: 691,1166) in pregnancy vs 1406 fmol/punch (IQR: 1053,1859) postpartum (p=0.006). Predicted median TFV-DP was 890 fmol/punch (IQR: 704,1143) in pregnancy vs 1418 fmol/punch (IQR: 1179,2139) postpartum. Under conditions of near perfect adherence, TFV-DP concentrations in DBS were approximately one-third lower in pregnancy compared to postpartum. TFV-DP levels in DBS observed in pregnancy also appeared lower than those reported in analogous studies of men and non-pregnant, non-lactating women in the US(167, 168).

These results confirm previous findings (see Section 1.3.2) that suggested drug levels in pregnant women may be lower than in a non-pregnant population. The PK Component results do not, however, provide information about PrEP efficacy among pregnant women, and data about the adherence-efficacy relationship during pregnancy remain limited.

1.3.4 Microbiome composition among mothers and infants with PrEP exposure

There is an emerging literature demonstrating the association between intravaginal PrEP formulations and the vaginal microbiome. Work from the CAPRISA 004 study has shown decreased efficacious of intravaginal PrEP when vaginal dysbiosis is present (113); other studies have shown similar outcomes, either in elevated rates of HIV acquisition (115) or local decreases of drug concentrations (114, 116). To date, there has only been one analysis of *oral* PrEP and vaginal dysbiosis, which showed unchanged efficacy in the presence of bacterial vaginosis (117).

While there are studies of the vaginal microbiome and HIV-risk, there are no published studies on the rectal microbiome and risk of HIV-acquisition. However, there is clear evidence that certain microbes are associated with gut inflammation (169) and inflammation and the presence of inflammatory cells are associated with elevated risk for HIV acquisition (115). If oral PrEP altered the rectal microbiome of pregnant women to a more inflammatory state, this could have important implications for HIV prevention measures. A recent meta-analysis found that as many as 20% of women reported heterosexual anal intercourse within the past three months (126).

The scientific question remains as to whether use of oral FTC/TDF may alter microbial communities in the mother and infant, and how this might impact efficacy and/or downstream health outcomes. This is particularly important in the context of pregnancy and the early postpartum period, given the associated physiological changes that occur for both mother and newborn. In an exploratory aim, preliminary data on how FTC/TDF may alter the maternal (vaginal/gut) and infant gut microbiome will be obtained Repeat specimens will be collected over time to see how the bacterial communities change over time based on FTC/TDF exposure. Comparisons by PrEP allocation arm and investigation of associations with vaginal drug concentrations of TFV will be made. IMPAACT 2009 provides a unique platform to collect these important data and inform the ongoing scientific debate about how antiretroviral interventions may affect the local microbiome for both mother and infant.

1.4 Hypothesis for the PK Component

In the context of monitored daily administration of PrEP, a TFV-DP threshold can be determined to track adequate adherence during pregnancy and breastfeeding.

1.5 Hypotheses for the PrEP Comparison Component

- At least 75% of women who opt to initiate PrEP will achieve a high level of adherence throughout pregnancy, as measured by adequate TFV-DP concentrations in DBS specimens.

- The rate of maternal and infant adverse events (including adverse pregnancy outcomes) will be higher among mother-infant pairs in which the mother initiates PrEP during pregnancy than in pairs in which the mother declines PrEP.

- The mean bone mineral density for the lumbar spine and hip at baseline and at Week 26 postpartum will be lower among women who initiated PrEP during pregnancy than among women who decline PrEP.

2 OBJECTIVES

2.1 Primary Objective for PK Component

The primary objective of this component is:

2.1.1 To determine the concentration of tenofovir diphosphate (TFV-DP) associated with adequate adherence to FTC/TDF among women observed ingesting daily oral PrEP during pregnancy and postpartum.

2.2 Secondary Objective for PK Component

The secondary objective of this component is:

2.2.1 To compare TFV-DP concentrations observed in pregnant and postpartum women.

2.3 Primary Objectives for PrEP Comparison Component

The primary objectives of this component are:

2.3.1 To characterize PrEP adherence among HIV-uninfected young women during pregnancy and for twenty-six weeks postpartum, when provided with enhanced adherence support through mobile technology and counseling based on observed drug levels.

2.3.2 To assess the safety of FTC/TDF for PrEP during pregnancy and postpartum by comparing pregnancy outcomes and maternal and infant safety between cohorts.

2.4 Other Objectives for PrEP Comparison Component

The other objectives of this component are:

2.4.1 To identify individual, social, and structural barriers and facilitators to PrEP uptake during pregnancy, and to adherence during pregnancy and postpartum.

2.4.2 To compare reported sexual risk behaviors and incidence of sexually transmitted infections, including HIV infection, among women who initiate PrEP during pregnancy versus women who decline PrEP.

2.4.3 To compare antiretroviral drug resistance among women and infants who acquire HIV with and without exposure to FTC/TDF for PrEP, including whether resistance was transmitted or acquired at time of transmission.

2.4.4 To compare bone density in women who initiated PrEP during pregnancy and women who decline PrEP.

2.5 Exploratory Objective for PrEP Comparison Component

The exploratory objective for this protocol is:

2.5.1 To describe the composition of and changes in the maternal vaginal and gut microbiome and infant gut microbiome according to PrEP exposure.

3 STUDY DESIGN

This study is comprised of two components: a) PK Component to establish the TFV-DP concentrations associated with adequate adherence to a daily FTC/TDF for PrEP regimen during pregnancy and postpartum period, and b) a PrEP Comparison Component to assess the feasibility, acceptability, and safety of PrEP provided to young women during pregnancy and postpartum. Refer to Figure 1 for an overview of the study design.

3.1 PK Component Study Design

The PK Component will establish PK parameters for TFV-DP under adequate adherence during pregnancy and postpartum. This initial component is necessary because TFV-DP concentrations will be used in the PrEP Comparison Component both to measure adherence (as the primary outcome) and to guide counseling provided as part of the adherence support intervention. The PK Component will be open to all sites participating in the PrEP Comparison Component.

Participants will be enrolled in two groups: pregnant women at 14-24 weeks' gestation and postpartum women who delivered 6-12 weeks prior to enrollment. The rationale for combining participants in the 2nd and 3rd trimesters is that physiological changes such as plasma volume increase and RBC mass increase start early (Weeks 6-8) and peak late (~Week 32) (170). Similarly, renal drug clearances change gradually during pregnancy and are similar in trimesters two and three (171), removing the need to assess each trimester separately. These gradual changes, coupled with the 12 week TFV-DP buildup to steady-state, creates a challenge to study PK in isolated trimesters. Therefore, the PK component will combine the 2nd and 3rd trimesters to capture the "averaged" physiological changes over these two trimesters. In the postpartum period, enrollment will begin approximately six weeks following delivery, when most women first attend their routine postpartum visit. The enrollment window is extended to 12 weeks following delivery, to ensure that the follow-up period will occur in the first six months when most women continue to breastfeed. The PK Component is not powered to detect small differences in TFV-DP concentrations between the pregnancy and postpartum periods.

Participants will be screened for HIV infection; only those who test negative will be eligible to initiate once-daily FTC/TDF for PrEP. Participants must be willing to initiate and adhere to PrEP and to be observed daily for adherence for 12-weeks. Refer to Section 4 for the study eligibility criteria.

Immediately prior to PrEP initiation – and at weekly intervals during the PK Component – a maternal blood specimen will be collected via venipuncture. This specimen will be used to create a dried blood spot (DBS) specimen at the local designated laboratory, prior to storage and shipment to the reference pharmacology laboratory for testing. All maternal participants will have study drug administered under direct observation daily for a total of 12 weeks. Enrolled maternal participants who have not delivered by their Week 12 visit may elect to continue PrEP thereafter through their labor and delivery visit. This is offered to provide additional HIV prevention during

the period of highest risk for vertical transmission of HIV and with the knowledge that unprotected intercourse, thus potential HIV exposure, may continue in the last trimester of pregnancy.

At participating sites, pregnancy and postpartum periods are associated with elevated risk for HIV acquisition. As such, it would not be ethically possible to vary the dosing levels of PrEP during observation. Instead, the study aims to determine the drug levels associated with adequate adherence to the once-daily FTC/TDF regimen. Study staff will monitor and document maternal participants' daily administration of FTC/TDF, as described in Section 5.1.1.1. Once the PK Component is complete, the distribution of TFV-DP concentrations measured in evaluable maternal participants will be analyzed. Refer to Section 9.1.1 for the definition of an evaluable participant.

PrEP doses taken will be categorized into one of the following:

1) Directly observed in-person by study staff
2) Directly observed in real-time video (live streaming or time/date stamped recording)
3) Self-reported, including still photos of dose ingestion
4) Missed dose (missing data or reported as missed)

Only doses directly observed in-person by study staff and doses observed in real-time video recording will be considered adequate documentation of the PrEP doses taken for the purposes of defining an evaluable participant, as described in Section 9.1.1.

These data will be used to determine the drug thresholds to be used in the PrEP Comparison Component to categorize drug adherence. The drug threshold corresponding to adequate adherence will be defined from the distribution of steady-state TFV-DP concentrations at Week 12 of the PK Component (refer to Section 9.1.6). The PK testing laboratory will provide sites with a drug level result and adherence status as it relates to the categorized level of adherence to use during adherence counselling sessions.

Upon completion and analysis of the PK Component, the Clinical Management Committee (CMC) will make recommendations to the Study Monitoring Committee (SMC) to proceed to the PrEP Comparison Component with established TFV-DP thresholds. With approval from the SMC, the PrEP Comparison Component will commence with notification to sites being issued from the Protocol Team and operational guidance on managing the transition between protocol components. This notification will include the drug threshold established from review of the PK Component data. Refer to Section 9 for a complete description of statistical considerations, data monitoring, and data analysis plans.

3.2 PrEP Comparison Component Study Design

The PrEP Comparison Component will assess feasibility, acceptability, adherence to, and safety of PrEP during pregnancy and postpartum in HIV-uninfected adolescents and young women. Women will be enrolled prior to 32 weeks' gestation in two cohorts: those who agree to initiate PrEP (Cohort 1) and those who decline (Cohort 2). Infants will be enrolled *in utero* at the same time the woman is enrolled. A randomized trial in which one arm would not receive PrEP would be unethical at this time due to the overwhelming evidence supporting the efficacy of PrEP to prevent HIV infection (172). All women enrolled in the study will receive a standard of care package of HIV prevention services and a study-specific behavioral risk reduction intervention. Additionally, all women will receive support for antenatal, delivery and early infant care through

one-way mHealth messaging services. Women who choose to initiate PrEP will be provided PrEP as well as a study-specific adherence support intervention.

Regardless of cohort, all maternal participants will follow similar schedules of evaluations through the course of pregnancy and in the 26 weeks following delivery. Following Cohort 2 provides an opportunity to document the process of decision-making around HIV prevention during this time of substantial risk; as these women may choose to initiate PrEP at any stage following study entry, triggers for change in PrEP perceptions and acceptability over time will be recorded to better understand women's motivation to seek additional HIV prevention (see Section 3.2.1) services. As Cohort 2 is not exposed to antiretroviral agents during pregnancy or postpartum, the study will also obtain preliminary safety data for TDF use in HIV-uninfected women during this period. Cohort 2 will serve as a comparison group for estimates of incidence and prevalence of maternal and infant safety outcomes including a) pregnancy outcomes, b) maternal adverse events, and c) infant growth and development between the two cohorts (see Section 9.2.2). The primary analysis will be based on original Cohort designation at baseline. Additional secondary analyses that consider the population of women who may initially decline PrEP and then later decide to initiate it will be considered, refer to Section 9.

The primary objectives focus on women who initiate PrEP during pregnancy and continue through the first 26 weeks postpartum (Cohort 1). Adherence to PrEP will be assessed in this population by measuring TFV-DP drug levels in dried blood spots. Participants in this arm will receive an intervention designed to promote PrEP adherence, comprised of a) two-way SMS messaging related to PrEP adherence, and b) targeted adherence counseling guided by TFV-DP drug levels from past visits, refer to Section 6. Participants will be contacted and provided with their DBS TFV-DP concentration results, as described in Section 6.5. Less than adequate concentration results are defined during the PK Component by pharmacokinetic parameters established when drug adherence is measured using TFV-DP levels from dried blood spots.

Maternal participants who change their intent regarding PrEP will be allowed to do so. Participants in Cohort 1 who choose to stop PrEP will continue in follow-up undergoing the same procedures. If these participants later elect to resume PrEP, HIV-infection status must be confirmed before resuming PrEP, refer to Sections 4.2.1 and 6.2.3. Maternal participants in Cohort 2 who subsequently choose to initiate PrEP will undergo a Step Change, during which eligibility for starting PrEP will be confirmed, refer to Sections 4.2.3 and 6.2.3. These participants are referred to as Cohort 2/Step 2 and will follow the procedures for Cohort 1. These switches onto or off PrEP will be considered in the feasibility/acceptability outcomes, refer to Section 9.2. While a formal step change will only be required when those originally declining PrEP later chose to initiate it, there is no upper limit set on the number of switches allowed. Switching will be closely monitored as part of feasibility and adherence outcomes.

3.2.1 Qualitative Evaluation Design

Qualitative evaluations will complement the quantitative data collected through the measures of TFV-DP drug levels in dried blood spots allowing for a mixed methods approach to evaluating adherence. These evaluations will consist of in-depth interviews (IDI) with maternal participants who chose to take PrEP as well as those who decline. These data will contribute to secondary objective 2.4.1 in identifying individual, social, and structural barriers and facilitators to PrEP uptake during pregnancy, and adherence and continued use during pregnancy and postpartum, as well as factors influencing decisions not to initiate PrEP or to stop once started. Additionally, given the circumstances pertaining to the circulating SARS-CoV-2 and associated coronavirus disease 2019 (COVID-19), data may be collected related to the potential influence of the COVID-

19 pandemic on participants' life and study participation, including study product adherence and acceptability.

The sampling method will be designed to include a range of possible perspectives on the phenomenon under study, thus ideally qualitative samples are purposive in nature. A stratified purposive sample will be used, which will allow for consideration of the concepts of range, saturation/redundancy, and stratification in the sampling frame (173). The sample will be stratified based on PrEP initiation (Cohort 1 vs. Cohort 2) and PrEP adherence (Cohort 1 only). Stratification on these two factors will allow for a diverse purposive sample that will include a range of women who may have various experiences with PrEP use. Interviews will be conducted with three distinct groups of antepartum and postpartum adolescent and young women as shown in Table 1: Those a) who initiate and adhere to PrEP (Cohort 1), b) who initiate but do not adhere to PrEP, including early discontinuation (Cohort 1) and c) who decline PrEP at enrollment (Cohort 2). In addition, the site teams will aim to have equal representation in each group by pregnancy status (i.e., pregnant vs. postpartum). Interview groups may be enriched or expanded as needed given experiences gathered during the trial.

Table 1: Expected number of interviews conducted with three distinct groups of antepartum and postpartum women

Group	Antepartum	Postpartum
Adherent PrEP maternal participants	10	10
Non-adherent PrEP maternal participants	10	10
Maternal Participants who decline PrEP	10	10

Study staff members at each site will conduct IDIs with up to 60 young women. These interviews will be audio-recorded, transcribed and translated to English for analysis. With as many as ten respondents in each of the six strata, the protocol team expects saturation/redundancy to be reached. To monitor the sampling, each site will be allotted two to three slots per cell at a time, with additional slots opened as needed. If challenges arise with recruiting the targeted number of women from each site, adjustments may be made to the sampling frame by allowing other sites to enroll additional participants. Such adjustments will be done in a manner that allows for the overall target sample size to be reached within each stratum, and provides the desired representation or experiences across PrEP use and pregnancy status.

All maternal participants enrolled in the PrEP Comparison Component of this study are eligible to complete the qualitative evaluations. However, determination of adherence will need to be assessed for maternal participants of Cohort 1 who are included. For enrollment purposes, adherence/non-adherence will be defined as either achieving or not achieving target TFV-DP levels measured by DBS (which will be defined following the PK phase):

- Currently pregnant: At two consecutive antepartum visits
- Postpartum: At both the Week 6 and Week 14 visits

Maternal participants will be identified and solicited for participation by site study team members until each cell (Table 1) is complete. The interview will be conducted after written informed consent is provided.

The interviews of participants identified for the "currently pregnant" strata will take place at or after the Week 12 visit and prior to Labor and Delivery (Cohorts 1 and 2). For the postpartum groups, because selection of Cohort 1 maternal participants will depend on the DBS results from

the Week 14 visit, the interview may take place following the Week 14 visit through to and including the Week 26 visit. Women selected for the postpartum group from Cohort 2 may have their interviews anytime at or after the Week 14 visit through to and including the Week 26 visit. Interviews may be scheduled to take place at a regular study visit or an alternative date.

The interviews will be conducted by study staff members at each site who will be trained in conducting the qualitative interview. Following the training, interviewers will practice conducting digitally recorded in-depth interviews with mock participants. Through the application of the interview guide, these interviews will identify the individual, social, and structural barriers and facilitators to PrEP uptake during pregnancy, and experiences with PrEP adherence (adequate and below adequate) during pregnancy and postpartum. The interview guide will be developed with input from the qualitative interviewers at the sites to ensure that the guide is both geographically and culturally appropriate.

3.2.2 Exploratory Objective for PrEP Comparison Design

The microbiome exploratory objective will assess whether PrEP alters the maternal and subsequently the infant microbiome. The samples for this objective will be collected during the regularly scheduled study visits - Entry, late pregnancy, and Weeks 6 and 26 postpartum - during the PrEP Comparison Component and will include maternal vaginal and optional rectal swabs. The Entry sample will serve as a baseline microbiome for the maternal participant during pregnancy. The re-sampling at 12 weeks will determine if the microbiome has changed significantly on FTC/TDF PrEP; pregnant women who decline PrEP will serve as the control group. The six-week postpartum time point will allow assessment of the microbiome composition after childbirth in women receiving or not receiving FTC/TDF PrEP. Finally, the 26-week postpartum time point will determine if the microbiome changes once pregnancy is over.

Infant rectal swabs or fresh stool will be collected at each timepoint to characterize the changes over time of the infant gut microbiome and potential changes resulting from exposure to maternal FTC/TDF PrEP. Changes in the microbiome will be assessed as described in Section 9.

3.3 Standard of Care

Sites will ensure that all participants access a basic standard of care (SOC) package which will generally include: a) HIV testing and risk reduction counseling at all visits; b) counseling and provision of contraception options; c) STI testing at screening, and STI testing and presumptive syndromic STI treatment, as needed; d) information on proper use of male and female condoms, including condom demonstrations, and provision of condoms; e) pregnancy testing; and f) discussion of HIV testing and prevention for their sexual partner(s) and referrals to local testing options for their partners. Refer to Section 5.3.2 for additional details on counseling procedures.

At all visits, participants presenting with physical signs or symptoms, consistent with an STI will be referred for or provided with treatment as indicated. Participants with a laboratory diagnosis consistent with an STI will be referred for or provided with treatment as indicated. Due to the timing of specific lab testing, this may occur as an interim visit.

4 STUDY POPULATION

This study will be conducted among approximately 390 women and their infants (40 in the PK Component, and 350 in the PrEP Comparison Component) who will be selected for the study according to the criteria in Sections 4.1 - 4.4. The study-specific approach to recruitment, screening, and enrollment is described in Section 4.5. Considerations related to participant retention and withdrawal from the study are provided in Sections 4.6 and 4.7, respectively.

4.1 PK Component (Groups 1 and 2)

4.1.1 Inclusion Criteria

Mothers must meet all the following criteria prior to study entry to be included in this component.

4.1.1.1 At study entry, mother is 16-24 years of age.

- *For mothers who are of legal age to provide independent informed consent as determined by site SOPs and consistent with site IRB/EC policies and procedures:* The mother is willing and able to provide written informed consent for her and her infant's study participation.

- *For mothers who are not of legal age to provide independent informed consent.* The parent/guardian or other legally authorized representative of the mother and her infant is willing and able to provide written informed consent for the mother and her infant's study participation; in addition, when applicable the mother is willing and able to provide written assent for her and her infant's study participation.

4.1.1.2 At screening, evidence of a viable singleton pregnancy (Group 1 only) with sonographic confirmation*

**Note: If adequate sonographic results are not available from medical records at screening, an ultrasound must be performed so that the result is available at study entry.*

4.1.1.3 At study entry, pregnant or recently delivered, in one of the following two enrollment windows:

- Group 1: Gestational age of 14 to 24 weeks, defined as greater than 13 weeks plus six days and less than 24 completed weeks of gestation with sonographic confirmation*, or
- Group 2: 6 to 12 weeks postpartum, defined as between 42 and 84 days after the date of delivery.

**Note: If adequate sonographic results are not available from medical records at screening, an ultrasound must be performed so that the result is available at study entry.*

4.1.1.4 At study entry, willing to initiate once-daily oral PrEP and continue use for at least 12 weeks under directly observed therapy and support for adherence.

4.1.1.5 Within 14 days prior to study entry, HIV negative by HIV RNA test.

4.1.1.6 At study entry, rapid test negative and absence of symptoms of acute HIV infection (i.e. acute viral illness).

4.1.1.7 At screening, Hepatitis B negative by Hepatitis B surface antigen test.

4.1.1.8 At screening, has the following laboratory test results:

- Grade 1 or normal (<2.5 x ULN) ALT
- Grade 1 or normal (≥9.5g/dL) hemoglobin
- Grade 1 or normal (≥800cells/mm3) absolute neutrophil count (ANC)
- Normal (≥90mL/min) estimated creatinine clearance (CrCl; Cockcroft-Gault formula)

4.1.1.9 At screening, mother has negative or trace proteinuria (less than Grade 1).

4.1.1.10 At screening, mother has normal dipstick urine for glucose (less than Grade 1).

4.1.1.11 At study entry, mother weighs > 35 kg.

4.1.1.12 Intention to stay within the study site's catchment area for at least 12 weeks (or through delivery).

4.1.2 Exclusion Criteria (PK Component and PrEP Comparison Component)

Mothers must be excluded from the study if any of the following are identified at any time prior to study entry:

4.1.2.1 Mother has any current significant uncontrolled, active or chronic disease process that, in the judgment of the site investigator, would make participation in the study inappropriate.

4.1.2.2 Mother has a known history of any of the following, as determined by the site investigator or designee based on maternal report and available medical records:

- Sickle cell anemia (excluding sickle cell trait), chronic bleeding, blood transfusion within the past 120 days (excluding for chronic illness) or other blood dyscrasias
- Bone fracture not explained by trauma
- Allergy/sensitivity to FTC/TDF or its components

4.1.2.3 Fetus has a known or suspected major congenital anomaly, from chart review of prior data, defined as a structural malformation with surgical, medical, or cosmetic importance

4.1.2.4 Mother has confirmed renal insufficiency, a history of known renal parenchymal disease, or known single kidney at screening

4.1.2.5 Current use of prohibited medications listed in Section 5.5

4.1.2.6 Concurrent participation in a study of any biomedical HIV prevention intervention or investigational drug in an HIV vaccine study or microbicide study

4.1.2.7 Past participation in an HIV vaccine study

4.1.2.8 Currently taking a PrEP regimen from non-study sources

4.1.2.9 Any other condition or adverse social situation that, in the opinion of the site investigator, would preclude informed consent, make study participation unsafe, complicate interpretation of study outcome data, or otherwise interfere with achieving the study objectives

4.1.2.10 Past participation in IMPAACT 2009

4.2 PrEP Comparison Component (Cohorts 1 and 2)

4.2.1 Inclusion Criteria

Mothers must meet all the following criteria prior to study entry to be included in this component.

4.2.1.1 At study entry, mother is 16-24 years of age.

- *For mothers who are of legal age to provide independent informed consent as determined by site SOPs and consistent with site IRB/EC policies and procedures:* The mother is willing and able to provide written informed consent for her and her infant's study participation.

- *For mothers who are not of legal age to provide independent informed consent.* The parent/guardian or other legally authorized representative of the mother and her infant is willing and able to provide written informed consent for the mother and her infant's study participation; in addition, when applicable the mother is willing and able to provide written assent for her and her infant's study participation.

4.2.1.2 At screening, evidence of a viable singleton pregnancy with gestational age of 32 weeks or less, defined as 224 days or less after the date of conception with sonographic confirmation *

**Note: if adequate sonographic results are not available from medical records at screening, an ultrasound must be performed in the interim so that the result is available at study entry.*

4.2.1.3 Within 14 days prior to study entry, negative by HIV RNA test.

4.2.1.4 At study entry, HIV rapid test negative and absence of symptoms of acute HIV infection (i.e. acute viral illness).

4.2.1.5 At screening, Hepatitis B negative by Hepatitis B surface antigen test performed.

4.2.1.6 At screening, has the following laboratory test results:

- Grade 1 or normal (<2.5 x ULN) ALT
- Grade 1 or normal (≥9.5g/dL) HB
- Grade 1 or normal (≥800cells/mm^3) ANC
- Normal (≥90mL/min) for estimated creatinine clearance (CrCl; Cockcroft-Gault formula)

4.2.1.7 At screening, mother has negative or trace proteinuria (less than Grade 1).

4.2.1.8 At screening, mother has normal dipstick urine for glucose (less than Grade 1).

4.2.1.9 Intention to stay within the study site's catchment area through 26 weeks postpartum

4.2.1.10 Regular access to a cellular phone that is able to receive SMS messages, and for Cohort 1 only, is also able to send SMS messages.

4.2.1.11 *Cohort 1 only*: At study entry, expresses willingness to take PrEP from pregnancy up to 26 weeks postpartum

Cohort 2 only: At study entry, expresses unwillingness to take PrEP from pregnancy up to 26 weeks postpartum

4.2.1.12 At study entry, mother weighs > 35 kg

4.2.1.13 Based on site investigator assessment at screening, mother is literate in one or more of the study languages

4.2.2 Exclusion Criteria

Mothers who meet any of the criteria specified in Section 4.1.2 will be excluded from this component.

4.2.3 Step 2 Inclusion Criteria: Cohort 2 only

Cohort 2 maternal participants who subsequently choose to initiate PrEP complete Step Change Visits 1 and 2, refer to Section 6.2.3.2 and Appendix IIB and must meet all of the following criteria in order to enter Cohort 2/Step 2 to initiate PrEP:

4.2.3.1 HIV negative by HIV RNA testing at Step Change Visit 1 and HIV rapid test negative and absence of symptoms of acute HIV infection (i.e. acute viral illness). at Step Change Visit 2.

4.2.3.2 Prior to entering Step 2, Hepatitis B negative by Hepatitis B surface antigen test performed at Step Change Visit 1.

4.2.3.3 Normal (≥90mL/min) estimated creatinine clearance (CrCl; Cockcroft-Gault formula) at previous assessment and negative or trace proteinuria (less than Grade 1) at Step Change Visit 1.

4.2.3.4 Willing to take PrEP up to 26 weeks postpartum.

4.2.3.5 Regular access to a cellular phone that is able to receive and send SMS messages.

4.3 Infant Enrollment

Infants enter the study at the same time their mother is enrolled. Infants in the PK Component - Group 1 and the PrEP Comparison Component enter the study, *in utero,* and infants in the PK Component - Group 2 enter the study six to 12 weeks after birth. There are no set inclusion or exclusion criteria for infants. If an infant is deemed too ill to undergo study procedures, the Site Investigator may opt to omit specific study procedures and prioritize those necessary for clinical management.

4.4 Co-Enrollment Considerations

Maternal and infant participants should not take part in any concurrent research studies that use drugs or medical devices while in follow up. Co-enrollment in observational or other studies may be allowable with approval in advance by the Protocol Teams of both studies. Requests for such approval should be emailed to the CMC.

4.5 Recruitment, Screening, and Enrollment Process

Sites will be responsible for developing appropriate recruitment processes that are geared toward their respective local communities. Recruitment materials should undergo approval, if applicable, per institutional review board (IRB)/Ethics Committee (EC) policies and procedures.

Women at heightened risk of HIV acquisition will be recruited for the PK Component and PrEP Comparison Component from public health centers where standard of care HIV pre- and post-test counseling procedures include a standardized HIV risk assessment. Study staff will approach women who, based on this risk assessment, perceive themselves to be at heightened risk and express interest in learning more about methods to protect themselves. After introducing the study, study staff will invite women who express potential interest in the study to initiate the informed consent process; those who provide informed consent will then initiate the study screening process. Women who do not express potential interest in the study will be referred to standard of care HIV prevention services.

All sites are eligible to participate in the PK Component; however, some may elect to opt out. It is also possible that not all sites will be needed to reach full accrual for this component. It is anticipated that all sites will participate in the PrEP Comparison Component.

The IMPAACT Data Management Center (DMC) Subject Enrollment System (SES) will be used to assist with tracking the screening and enrollment process for both the PK Component and PrEP

Comparison Component. When informed consent is obtained, participant identification numbers (PIDs) will be assigned to the mother and infant and a study-specific screening number will be obtained for the mother-infant pair through the SES. For pairs found to be eligible, enrollment will occur upon successful entry of required eligibility data into the SES. For pairs who are found to be ineligible for the study, or who do not enroll in the study for any reason, an eCRF will be entered to record the screening outcome. Refer to Section 9.3 for more information on monitoring participant accrual in this study.

4.6 Participant Retention

Once a participant is enrolled in this study, the study staff will make every effort to retain them for the expected duration of follow-up in order to maximize statistical power and minimize potential bias associated with loss-to-follow-up. Study staff are responsible for developing and implementing local procedures to reach this goal. Refer to Section 9.3.1 for more information on monitoring participant retention in this study.

4.7 Participant Withdrawal or Termination from the Study

Regardless of the participant retention methods used, maternal participants may voluntarily withdraw from the study for any reason at any time. The site IoR or designee also may withdraw participants from the study to protect their safety and/or if they are unwilling or unable to comply with required study procedures.

Participants may also be withdrawn/discontinued from the study by the site investigator or designee under the following circumstances:

- Participant re-locates away from access to any study site or is otherwise determined by the study staff to be lost-to-follow-up and unlikely to return to the study.
- Participant judged by the investigator or designee that continued participation in the study would be unsafe or otherwise not in the best interest of the participant, after consultation with the Protocol Team.
- Participant fails to comply with the study requirements so as to cause harm to him/herself or to seriously interfere with the validity of the study results.
- At the discretion of the IMPAACT leadership, NIAID, NICHD, the Office for Human Research Protections (OHRP), the U.S. FDA, the pharmaceutical supplier(s), an in-country national health or regulatory agency, or the IRB/EC.

Every reasonable effort will be made to complete final evaluations for participants who terminate from the study prior to the last study assessment or visit; study staff will record the reason(s) for all withdrawals from the study in the participants' study records.

For any participant who is withdrawn from the study prior to scheduled completion of follow-up, study staff will document the reason for the withdrawal in detail and will make every effort to complete final evaluations as described in Section 6.4. If the circumstances that led to a participant's withdrawal change (e.g., she returns to the study site area after having re-located previously), the site investigator or designee should contact the Protocol Team to discuss options for resumption of follow-up.

5 STUDY DRUG CONSIDERATIONS

Site pharmacists should consult the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* for standard pharmacy operations.

Study drug is defined for this study as FTC/TDF 200 mg/300 mg (Truvada®). Truvada® is approved by the U.S. Food and Drug Administration (FDA) for the treatment of HIV-1 infection and for PrEP to reduce the risk of sexually acquired HIV in adults at substantial risk. Further information on Truvada is available in the current package insert available at: https://rsc.niaid.nih.gov/clinical-research-sites/pi-list

Maternal participants in this study may be exposed to study drug through direct ingestion; infants may be exposed to study drug *in utero* and through breastfeeding.

5.1 Study Drug Regimen

Maternal participants in Group 1 and Group 2 of the PK Component, in Cohort 1 of the PrEP Comparison Component, and Cohort 2/Step 2 of the PrEP Comparison Component will receive a fixed dose combination of FTC/TDF 200 mg/300 mg to be taken by mouth once daily for the duration specified in Section 5.1.2.

5.1.1 Study Drug Administration

FTC/TDF will be administered orally as one fixed dose oral combination tablet (FTC 200 mg/TDF 300 mg) once daily, with or without food. In the PK Component, administration will be directly observed. Instructions for maternal participants in the event of vomiting relevant for both components are below. Further guidelines for study drug administration in the PK Component and PrEP Comparison Component are specified in Sections 5.1.1.1 and 5.1.1.2, respectively.

In the event of vomiting, maternal participants in both the PK and PrEP Comparison Components, will be instructed by study staff as follows:

- If the maternal participant vomits within 15 minutes after taking a tablet, and suspects that the tablet has been thrown up (i.e., the tablet is visible in the vomit), she should wait approximately 30 minutes and then take another FTC/TDF tablet.

  Note: Participants in the PK Component should notify staff immediately upon vomiting and the same DOT procedures described below should be followed for the additional tablet.

- If this maternal participant vomits again after taking the second tablet, she should not take any more tablets that day, but try again to take a tablet at her daily dose time the next day.

- If the maternal participant is not able to determine that the tablet has been thrown up (i.e., the tablet is not visible in the vomit), she should not take any more tablets that day, but try again to take a tablet at her daily dose time the next day.

5.1.1.1 PK Component

In the PK Component, study staff must monitor maternal participants' daily administration of FTC/TDF. Each study site must establish standard operating procedures (SOPs) describing how this will be done; depending on site-specific feasibility, options may include:

- Directly observed therapy (DOT) at the study site
- DOT at the participant's home or other community-based location (see also Section 6.1.1)
- Real-time video-based DOT (e.g., live streaming via FaceTime, Tango, Skype, or other similar method)

The first two options listed above, which involve in-person direct observation by study staff, are preferred and should be used whenever possible. When use of these options is not possible, the third option of observation via live streaming video should be used. If live streaming is not possible, a video file may be saved by the participant and sent to study staff for non-live viewing, provided the file is dated and time-stamped.

In the expected rare event that one of the three options listed above cannot be used, participants will be contacted to self-report their daily dose administration via telephone call or audio message, text message, and/or still photo. Whether directly observed or self-reported, all types of documented dose administrations will be adequately source documented and entered into electronic case report forms (eCRFs); however, dose administrations that are not directly observed will not be considered in primary analyses.

5.1.1.2 PrEP Comparison Component

In the PrEP Comparison Component, maternal participants will be encouraged to select a consistent daily "dose-time;" however, if a maternal participant forgets to take a dose at that time, the dose may be taken later in the day, as long as only one dose is taken on any calendar day.

5.1.2 Study Drug Duration

5.1.2.1 PK Component

Maternal participants in Group 1 and Group 2 of the PK Component will take FTC/TDF for 12 weeks, beginning on the day of enrollment and continuing through the day of the Week 12 visit. Participants who have not delivered by their Week 12 visit may elect to continue PrEP thereafter through their labor and delivery visit.

5.1.2.2 PrEP Comparison Component

Maternal participants in Cohort 1 of the PrEP Comparison Component will take FTC/TDF through pregnancy and for 26 weeks postpartum, beginning on the day of enrollment and continuing through the day of the Week 26 postpartum visit.

Maternal participants in Cohort 2 of the PrEP Comparison Component who declined PrEP initiation during pregnancy will not take FTC/TDF for the duration of the study unless they undergo a step change and enter Cohort 2/Step 2.

Maternal participants in Cohort 2/Step 2 of the PrEP Comparison Component will take FTC/TDF for a variable duration, depending on timing of Step 2 entry. The duration will begin on the day of enrollment in Step 2 and continuing through the day of the Week 26 postpartum visit.

5.2 Study Drug Formulation

FTC/TDF (supplied for this study as Truvada®) is a fixed dose combination tablet containing 200 mg of emtricitabine (FTC) and 300 mg of tenofovir disoproxil fumarate (TDF) in each tablet.

Tablets must be stored in their original containers at 25°C (77°F); with excursions permitted to 15-30°C (59°-86°F) (see USP Controlled Room Temperature). Each container is packaged with a child-resistant screw cap and contains a silica gel to protect the product from humidity. The silica gel should not be removed from the container.

5.2.1 Study Drug Supply

FTC/TDF will be supplied by Gilead Sciences, Inc. and made available to study sites through the National Institute of Allergy and Infectious Disease (NIAID) Clinical Research Products Management Center (CRPMC). Upon successful completion of protocol registration procedures, study drug may be obtained by the site pharmacist following instructions provided in the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trial Networks*.

5.2.2 Study Drug Accountability

Site pharmacists must maintain complete records of all study drugs received from the CRPMC and subsequently dispensed.

The site pharmacist must follow the instructions in the Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks for the destructions of unused study products.

5.3 Behavioral Counseling and Adherence Support

5.3.1 PK Component

Adequate study drug adherence is required from maternal participants in the PK Component to accurately establish the target TFV-DP levels in DBS for use in the PrEP Comparison Component. To support near perfect dosing in the PK Component, study teams will have daily contact with maternal participants and directly observe each dose, providing counseling as directed by the circumstances. The mechanism for achieving this may differ by site and over time, being laid out in a standard operating procedures document. At in-clinic planned study visits, adherence and sexual health protection counseling will be offered as detailed below.

Adherence and Sexual Health Protection Counseling:

Integrated Next Step Counseling (iNSC, described in Section 5.3.2) will be adapted for context of PK Component. All maternal participants in both groups are provided education, support and problem solving at time of observed dose by the team member observing the dose (in person or virtually). Added efforts to engage participants failing to come-in, be present at home when a home visit has been scheduled, or call-in for observation of dose taking will include exploration of factors that could facilitate near-perfect/perfect coverage of observation. At study visits,

women will be asked to engage in brief, discussions about their current strategies for sexual health protection and experiences with DOT. For women engaging well with DOT, support is provided to maintain this success. For women engaging intermittently or insufficiently with DOT, discussions will focus on problem solving and targeting increased DOT engagement and high adherence regardless of DOT. Thus, the iNSC approach will be used to promote non-PrEP related sexual health protection and support participants in their engagement with DOT and adherence.

5.3.2 PrEP Comparison Component

Adherence and Sexual Health Protection Counseling:

Integrated Next Step Counseling (iNSC). At study visits, all maternal participants in both cohorts receive iNSC, a participant-focused discussion of sexual health and well-being during the delivery of HIV-negative test results. iNSC includes exploration, problem solving and skills building around non-biomedical strategies to prevent HIV and other STIs and general well-being for all participants. For those receiving PrEP (Cohort 1 or Cohort 2/Step 2), counselors assess maternal participants' desire to remain on PrEP. With confirmation of desire to continue with PrEP it subsequently focuses on a discussion of PrEP adherence. For those not on PrEP, the discussion ends with a discussion on whether she is interested in starting PrEP and providing appropriate follow-up, accordingly. For those who have been prescribed PrEP but have yet to start taking it, the discussion focuses on her decision to start PrEP andon regimen adherence education and planning. iNSC is participant-focused, relies on participant experiences rather than self-reported performance (e.g., does not ask participants to report numbers of missed doses or number of occurrences of condomless sex, but rather asks about experiences [positive and negative] with sexual health protection, areas where the participant would like to feel more confident, skilled or comfortable), uses Motivational Interviewing and problem solving strategies, and is based on a situated information, motivation and behavioral skills model of sexual health protection (which contextualizes individual behavior within its social-cultural and structural context).

Drug level monitoring with feedback. DBS samples to measure TFV-DP levels will be obtained from participants in Cohort 1 and Cohort 2/Step 2 as per Appendix IIA. DBS levels strongly correlate with PrEP efficacy, so users and clinicians can interpret the drug level in the context of HIV prevention. TFV-DP levels in red blood cells (measured with DBS), by virtue of a 17-day half-life, represent cumulative adherence rather than only recent adherence. Monitoring outcomes of preventive medications with biological measurements is common in the management of chronic diseases. Per drug thresholds, established in the PK Component, specific ranges will be defined for a minimum three adherence categories, aligned with other similar studies and will be used to provide guidance for adherence counseling. (e.g. below adequate, adequate and above adequate). All participants in Cohort 1 and Cohort 2/Step 2 will receive iNSC with drug level feedback based on this categorization.

Participants will be contacted when their DBS TFV-DP concentration results become available to site staff. Targeted iNSC, an adapted version of the standard PrEP check-in used at each visit (iNSC), will be used in delivering results of drug testing to participants. The approach allows staff to share results with participants in a systematic manner and differs based on result being delivered. Discussions around adherence and sexual health protection are offered (as an option for those with optimal levels) or requested as part of study procedures (for sub-optimal levels) to participants. Based on current evidence, any concentration below excellent will be considered below adequate. This threshold will be updated as needed should evidence emerge suggesting that women can achieve high protection with lower concentrations. Detailed steps for these

procedures are provided in the study-specific iNSC manual/workbook and associated training material.

During the PrEP Comparison Component, completion of iNSC sessions will be documented via eCRF. Counselors will complete a tracking form at the end of each session documenting the specific elements of iNSC included or deferred and related details.

SMS Support:

Beginning with enrollment and continuing through follow-up, all maternal participants, regardless of cohort, will receive periodic informational one-way messages about pregnancy and infant care. Message content and frequency, as well as standardized procedures for delivering messages via an automated computer system will be described in the study-specific MOP.

Maternal participants in Cohort 1 and Cohort 2/Step 2 will also receive weekly two-way text messages that provide general adherence support. Beginning the week following enrollment (participants in Cohort 1), or the week following Step Change Visit 2 (participants in Cohort 2/Step 2), and continuing through follow-up, each week, participants will receive a text message reminding them about the study support and inquiring how they are doing (e.g. 'are you ok?'). Participants will be instructed to respond within 48 hours either that they are doing well or that they have a problem and request assistance. A study staff member will call to follow-up with any participant whose response indicates that there is a problem, and participants who fail to respond within two business days. Site staff will be expected to respond to text messages only during regular clinic hours, but are not restricted to do so. Participants will be instructed that all emergencies should be handled through their usual means such as contacting the nearest hospital emergency department. Message content and standardized procedures for delivering messages and tracking responses for two-way messaging via an automated computer system will be described in the study-specific MOP.

Delivery of the SMS support package will be documented via usage statistics and automated system reporting of message delivery data. Telephone and in-person follow-up will be tracked manually and source documented.

5.4 Concomitant Medications

The term concomitant medications is used in this study to refer to medications other than FTC/TDF.

All concomitant medications received by maternal participants and their infants throughout the duration of study participation must be source documented as part of the medical and medication histories obtained at each study visit (see Section 6.8). This includes prescription and non-prescription (over-the-counter) medications; vaccines and other preventive medications; contraceptives; antenatal vitamins, therapeutic foods, and other nutritional supplements; and alternative, complementary, and traditional medications and preparations.

A listing of maternal and infant concomitant medications that must be entered into eCRFs is provided in Appendix III.

eCRFs will also capture whether maternal participants received any traditional medications during follow-up.

5.5 Prohibited Medications

Any mother who requires a medication considered prohibited while on study drug must have the study drug held. The CMC must be notified in advance (or as soon as possible thereafter) of any instances in which prohibited medications are administered. The following medications are prohibited during administration of FTC/TDF:

- Investigational ARV agents, interferon or interleukin therapy
- Agents with substantial nephrotoxic potential
- Other agents that may inhibit or compete for elimination via active renal tubular secretion (e.g., probenecid), and/or other investigational agents

6 STUDY VISITS AND PROCEDURES

The study visit and evaluation schedules for maternal participants and infants are provided in Appendices I - II; blood draw volumes for each visit are also detailed in these appendices. Presented in this section is additional information on visit-specific study procedures. Information related to scheduled visits is presented in Sections 6.1 – 6.4. Additional details related to study-specific procedures, such as medical history, physical exams, ultrasound scans, DXA scans, and Computer-Assisted Self-Interview (CASI) evaluations are provided in Sections 6.8 – 6.16.

All visits and procedures must be performed at the clinical research site or approved associated facilities except for certain visits in the PK component which may be done in the home, refer to Section 6.1.1. Unless otherwise specified, visits may be split, with required procedures performed on more than one day within the allowable visit window if necessary. Whenever possible, maternal and infant visits should be scheduled on the same date. All visits and procedures must be documented in accordance with the NIAID Division of AIDS (DAIDS) policies for source documentation; refer to Section 11 for more information on documentation requirements and completion of eCRFs. Refer to Section 7.3 for information on expedited adverse event (EAE) reporting, which may be required at any time during follow-up.

For sites experiencing disruptions or limitations of usual operations due to COVID-19, operational guidance is provided in Appendix X. All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.

In addition to the protocol-specified procedures listed in this section, study staff may complete other tasks consistent with site SOPs or local standard of care, including but not limited to collecting, reviewing, and updating demographic and locator information; reviewing elements of informed consent; scheduling telephone contacts and visits; providing instructions for contacting study staff between visits; providing visit reminders; and following up on missed visits. All such tasks should be documented consistent with site SOPs. Study staff should inform maternal participants of clinically meaningful physical exam findings and laboratory test results when available.

6.1 PK Component

6.1.1 Maternal Screening, Enrollment and Follow-up (Group 1 and Group 2)

Refer to Section 4.5 for a description of the study recruitment, screening, and enrollment process. For eligible women, enrollment must occur in time for study drug initiation within the following timeframes:

- Group 1: 14 to 24 weeks gestation
- Group 2: 6 to 12 weeks post delivery

Generally, the visit requirements are the same regardless of whether the maternal participant is in Group 1 or Group 2: there will be a screening visit and an enrollment visit followed by 12 weekly visits. Other requirements at these visits that are specific to one group or the other are noted in the text and tables that follow. Participants in Group 1 will have a Labor & Delivery Visit (see Section 6.1.1.4). For participants who are still in weekly follow-up at the time of delivery, the Labor & Delivery visit may be combined with a weekly study visit. Participants who complete weekly follow-up prior to delivery will be permitted to continue the study drug through delivery with the labor and delivery visit considered their final visit.

Further operational guidance for required procedures is as follows:

- Maternal visits are described in Sections 6.1.1.1 – 6.1.1.4

- Infant visits are described in Sections 6.1.2 – 6.1.3

- Maternal creatinine testing is required in relation to the eligibility criteria in Section 4.1.2.4 and to select follow up visits; as soon as the creatinine test result is obtained for a given visit, the estimated CrCl rate should be calculated using the Cockcroft-Gault formula, and all results should be graded for severity as specified in Section 7.2.1 and assessed for clinical significance concurrent with all other laboratory test results.

- For Group 1, a fetal ultrasound report is required prior to entry to estimate gestational age and to assess for multiple gestation. Biometry from the ultrasound will be used to confirm (or change) the gestational age at study entry and gestational age at delivery. If ultrasound is performed during the screening period, results must be considered for purposes of eligibility determination. For example, if ultrasound indicates multiple gestation during the screening period, the woman (and her fetuses) should not be enrolled. Because ultrasound-based gestational dating is more accurate the earlier a woman is in her pregnancy, prior ultrasound results can be used to meet this requirement, if a report of biometric measurements is available as documentation.

- Infants in Group 1 will have two study visits: at birth and at six weeks postpartum. Infants in Group 2 will have three study visits; at study entry and at six and 12 weeks after study entry.

- Should a woman in Group 1 or Group 2 have any ongoing adverse events at the time of her last visit, the event will be followed as described in Section 8.1.

- If allowed per local law and regulations and/or institutional policies, women in the PK Component may have study visits conducted in their home or in other off-site locations. Specifically, the visits scheduled at Weeks 1-3, 5-7 and 9-11 may be conducted off-site. At sites where off-site visits are permitted, study staff will discuss this option with participants in advance and agree on where and when such visits may take place, with adequate protections for participant privacy and confidentiality. Prior to each off-site visit, study staff will again

confirm the date, location, and time of each visit with the participant. At off-site visits when specimen collection is required, the procedures specified in Section 6.17 must be followed. Off-site visits may only be conducted by designated study staff who are qualified to perform all protocol-specified procedures and have undergone study-specific and all other applicable training relevant to the procedures they will perform off site (e.g., HSP, GCP, IATA). These staff should also be adequately trained and qualified to immediately manage any adverse events and/or social impacts that may occur during off-site visits (e.g., fainting during phlebotomy, family dispute in the home). If adverse events requiring further evaluation or management are identified during an off-site visit, study staff conducting the visit will arrange for the participant to return to the study site as soon as possible.

6.1.1.1 PK Component Maternal Screening (Group 1 and Group 2)

Refer to Section 4.5 for a description of the study recruitment, screening, and enrollment process.

Screening may be initiated after informed consent is obtained. All screening procedures except for sample collection for HIV RNA are expected to be performed within 30 days prior to study entry. Screening evaluations may be repeated during the 30-day period, with the latest outcomes used for eligibility determination. An HIV negative NAT test result, from a specimen collected within 14 days prior to enrollment, is required prior to entry. Multiple screening visits may be conducted within the 30-day time frame to complete all required procedures. If the 30-day screening period is exceeded, the screening process may be repeated. For potential participants who do not meet the eligibility criteria, screening may be discontinued once ineligibility is determined.

PK Component Maternal Screening Visit Procedures		
Administrative and Regulatory		• Obtain written informed consent • Assign participant identification number (PID) • Obtain screening number from SES
Behavioral and Counseling		• Integrated Next Step Counseling (iNSC) [see Section 5.3.1]
Clinical		• Obtain available medical records and medical/medication history • Perform complete maternal physical exam
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test (within 14 days prior to enrollment) • Rapid HIV test • Complete blood count (CBC) with differential and platelets • ALT, Creatinine, and CrCl • Hepatitis B surface antigen testing • Glucose (random)
	Urine	Collect urine for: • Urinalysis (protein and glucose)
Other	**Ultrasound**	*For Group 1 only*: Review medical records for results of any fetal ultrasound performed earlier in this pregnancy to confirm/determine gestational age. If no results are available, or if documented results are not adequate for study purposes, schedule ultrasound so that results are available prior to enrollment.

6.1.1.2 PK Component Maternal Enrollment Visit (Group 1 and Group 2)

All Entry Visit procedures are expected to be performed on the day of enrollment; procedures that may provide information relevant to eligibility for the study (e.g., medical history, physical examination, HIV rapid test), should be performed first, prior to final eligibility determination and enrollment. If a participant is found to be ineligible on the day of enrollment, enrollment should not occur.

Additional requirements for sequencing of procedures at the Entry Visit are as follows:

- Rapid HIV test, and final eligibility determination and confirmation must precede enrollment
- Blood collection for baseline TFV-DP level must precede ingestion of the first dose of study drug
- Prescribing must precede dispensing and administering of study drug

PK Component Maternal Enrollment Visit Procedures *(Day 0)*		
Administrative and Regulatory		• Complete final eligibility determination and confirmation • Complete eligibility checklist[1], enter checklist data into SES to enroll the participant, print and file a copy of the confirmation • Collect comprehensive locator information
Behavioral and Counseling		• iNSC [see Section 5.3.1] • Demographic/Behavioral Questionnaires [see Section 6.16]
Clinical		• Update medical and medication history since last visit • Perform complete maternal physical exam
Laboratory	**Blood**	*Prior to first dose of study drug, collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test[1] [see Section 6.3 in the event the test is (+)] • Baseline TFV-DP level (DBS)[2]
	Other	• Collect vaginal swab for chlamydia and gonorrhea
Other	**Ultrasound**	*For Group 1 only*: Review results of ultrasound performed prior to this visit and assign gestational age.
Study Drug		• Prescribe and dispense study drug • Conduct training on observed study dose administration based on site's preferred method

[1] A negative test result must be available prior to enrollment.
[2] Baseline sample must be collected prior to ingestion of study drug.

6.1.1.3 PK Component Maternal Follow Up (Group 1 and Group 2)

Maternal participants will have once weekly follow-up visits for 12 weeks following the enrollment visit. Target dates for each visit are counted from the date of enrollment as Day 0, with an allowable visit window of ± 1 day. At all visits when study drug is dispensed (Weeks 4 and 8), a negative rapid HIV test result must be available prior to prescribing PrEP. Weekly study visits scheduled at Weeks 4, 8 and 12 must be conducted in the study clinic. Weekly study visits at other time points (Weeks 1-3, 5-7 and 9-11) may be conducted in the study clinic or at an approved offsite location, for example the participant's home, refer to Section 6.1.1. For off-site weekly study visits, a home visit plan will be developed with the participant prior to the visit to indicate her permission for this activity and record any special instructions for the study staff.

In addition, daily adherence will be assessed with directly observed dosing. The participant and study staff will develop an individualized plan for accomplishing this, determined by the maternal participant's preferred method for observing doses as stated at study entry and reviewed during follow-up to accommodate any changes, refer to Section 5.1.1.1.

For participants in Group 1 who deliver prior to completing all 12 weekly visits, the weekly follow-up schedule should be maintained to the extent possible after delivery The Labor and Delivery Visit may be combined with a weekly visit (refer to Section 6.1.1.4).

PK Component Maternal Weekly Follow-up Procedures *(every 7 days ± 1 day)* x 12		
Behavioral and Counseling		• iNSC [see Section 5.3.1] • Demographic/Behavioral Questionnaires [see Section 6.16] (*Week 12 only)*
Clinical		• Obtain interim medical/medications history • *Weeks 4, 8, and 12 only*: perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC as necessary)
Laboratory	**Blood**	*Collect blood for:* • *Week 12 only:* • CBC with differential • Hepatitis B surface antigen testing • Pregnancy testing *(Group 2 only):* blood or urine test may be performed • *Weeks 4 and 12, only:* • ALT, Creatinine, and CrCl • *Weeks 4, 8, and 12, only:* • Rapid HIV test [see Section 6.3 in the event the test is (+)] • HIV RNA test • Store plasma for HIV drug resistance testing • *All visits; Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12:* • TFV-DP level (DBS)
	Urine	• Pregnancy testing *(Group 2, Week 12 only):* blood or urine test may be performed
Study Drug		• *Weeks 4 and 8, only:* Prescribe and dispense study drug • *Week 12 only*: collect all remaining study drug Note (*Group 1 only*): If a participant is still pregnant at Week 12, and wants to continue to receive study drug, study drug may be dispensed through delivery. No PK evaluations will be required. The final visit for maternal participants who choose to continue study drug will be at Labor & Delivery (L&D).

6.1.1.4 PK Component Maternal Labor and Delivery (Group 1)

Regardless of whether a maternal participant has completed the 12 weeks of PK follow up, a visit will be conducted within 14 days after delivery to document the pregnancy outcome and evaluate the infant (refer to Section 6.1.2 for details regarding the infant). If the participant is unable to return to the research clinic within the allowable visit window, follow-up and safety testing may be conducted at approved off-site facilities. If the Labor and Delivery Visit is within the window of one of the weekly visits, those visits may be combined. If the participant has completed the 12 weeks of follow-up prior to labor and delivery, then this visit will be the final maternal study visit. If the participant had elected to remain on study drug beyond the 12 weeks of PK assessment, then study drug should be retrieved at the Labor and Delivery Visit.

PK Component (Group 1): Maternal Labor and Delivery Visit Procedures *(within +14 days after delivery)*		
Administrative and Regulatory		• Review locator information
Behavioral and Counseling		• iNSC [see Section 5.3.1]
Clinical		• Obtain interim medical/medications history and pregnancy outcome • Perform targeted physical exam • Identify/review/update adverse events
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)] • TFV-DP level (DBS)
Study Drug		• *If the participant has completed the 12 weeks of follow-up prior to this visit:* collect all remaining study drug • *If the participant is still having weekly study visits for PK assessment:* see Section 6.1.1.3

6.1.2 PK Component Infant Birth and PP Week 6 (Group 1)

Infants in Group 1 will be evaluated for safety at birth and postpartum Week 6. Infant creatinine testing is required at all infant visits; the infant creatinine test result for a given visit will be recorded on the appropriate eCRF.

6.1.2.1 PK Component Infant Birth (Group 1)

The Group 1 infant birth visit will be conducted within 14 days after birth. Whenever possible, this visit should occur on the same day as the Labor & Delivery visit.

PK Component (Group 1) Infant Birth Visit Procedures *(within 14 days after birth)*		
Clinical		• Perform complete infant physical exam • Obtain birth history, interim medical and medications history since birth, and feeding history since birth
Laboratory	**Blood**	*Collect blood for:* • Creatinine • TFV-DP level (DBS)

6.1.2.2 PK Component Infant Postpartum Week 6 Visit (Group 1)

This visit should be conducted six weeks (42 days) after infant birth (± 14 days). There is no corresponding maternal visit at this time.

PK Component (Group 1) Infant Postpartum Week 6 Visit Procedures (Day 42 ± *14 days*)		
Clinical		• Obtain interim medical, medications, and feeding history • Perform targeted physical exam
Laboratory	**Blood**	*Collect blood for:* • Creatinine

6.1.3 PK Component Infant Entry, Week 6 and Week 12 after study entry (Group 2)

Infants in Group 2 will be evaluated for safety at three time points: Entry, Week 6 and Week 12 after study entry. These time points correspond to the maternal enrollment visit and two of the maternal follow up visits. Whenever possible, infant visits should be scheduled on the same date of the corresponding maternal visits. Because Group 2 maternal participants are enrolled at 6-12 weeks' post-partum, infants in this group enter the study at 6-12 weeks of age. Follow-up visit scheduling is then based on the date of enrollment and is unrelated to infant date of birth.

6.1.3.1 PK Component Infant Entry (Group 2)

PK Component (Group 2) Infant Entry Visit Procedures *(Day 0 ± 14 days*)		
Clinical		• Obtain birth history, interim medical and medications history since birth, and feeding history since birth • Perform complete infant physical exam
Laboratory	**Blood**	*Collect blood for:* • Creatinine • TFV-DP level (DBS)

6.1.3.2 PK Component Infant Week 6 and 12 (Group 2)

The Group 2 infant follow up visits will be conducted at Week 6 and 12 (± 14 days). Whenever possible, infant visits should be scheduled on the same date of the associated maternal visits.

PK Component (Group 2) Infant Week 6 and 12 Visit Procedures *(Days 42 and 84 ± 14 days*)		
Clinical		• Obtain interim medical, medications, and feeding history • Perform targeted infant physical exam
Laboratory	**Blood**	*Collect blood for:* • Creatinine • TFV-DP level (DBS) (Week 6 visit only)

6.2 PrEP Comparison Component

6.2.1 PrEP Comparison Component Maternal Screening, Enrollment and Follow-up Visits

Refer to Section 4.5 for a description of the study recruitment, screening, and enrollment process. Women will be evaluated for potential participation with a gestational age that will permit study enrollment before 32 completed weeks. Infants will enroll into the PrEP Comparison Component, *in utero*, at the time their mothers enter the study. Infants will have study visits at birth, and 6, 14 and 26 weeks after birth.

Screening may be initiated after informed consent is obtained. All screening procedures except for sample collection for HIV RNA are expected to be performed within 30 days prior to study entry. Screening evaluations may be repeated during the 30-day period, with the latest outcomes used for eligibility determination. A HIV negative NAT test result, from a specimen collected within 14 days prior to enrollment, is required prior to entry. Multiple screening visits may be conducted within the 30-day time frame to complete all required procedures. If the 30-day screening period is exceeded, the screening process may be repeated. For potential participants who do not meet the eligibility criteria, screening may be discontinued once ineligibility is determined.

- Maternal creatinine testing is required in relation to the eligibility criteria in Section 4.1.2.4 and to select follow up and step change visits; as soon as the creatinine test result is obtained for a given visit, the estimated CrCl rate should be calculated using the Cockcroft-Gault formula, and all results should be graded for severity as specified in Section 7.2.1. and assessed for clinical significance concurrent with all other laboratory test results.

- Fetal ultrasound should be performed during the study screening period to estimate gestational age and to assess for multiple gestation. Specific biometric measurements (e.g., crown-rump length, femur length, biparietal diameter, head circumference, abdominal circumference) to ensure that gestational age estimate is consistent across sites will be documented. Biometry from the ultrasound will subsequently be used to confirm (or change) the gestational age at study entry and gestational age at delivery. If ultrasound is performed during the screening period, results must be considered for purposes of eligibility determination. For example, if ultrasound indicates multiple gestation during the screening period, the mother (and her fetuses) should not be enrolled. Because ultrasound-based gestational dating is more accurate the earlier a woman is in her pregnancy, the prior ultrasound results will be used to meet this requirement, provided that a report of biometric measurements is available as documentation.

PrEP Comparison Component Maternal Screening Visit Procedures		
Administrative and Regulatory		• Obtain written informed consent for PrEP Comparison Component Screening and Enrollment • Assign participant identification number (PID) • Obtain screening number from SES • Assess eligibility for screening
Behavioral and Counseling		• iNSC [see Section 5.3.2]
Clinical		• Obtain available medical records and medical/medication history • Perform complete maternal physical exam
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Rapid HIV test • CBC with differential • ALT, Creatinine, and CrCl • Hepatitis B surface antigen • Glucose (random)
	Urine	*Collect urine for:* • Urinalysis (protein and glucose)
Other	**Ultrasound**	Review medical records for results of any fetal ultrasound performed during this pregnancy to determine gestational age. If none are available, or if documented results are not adequate for study purposes, schedule ultrasound so that results are available prior to enrollment.
Study Drug		Not applicable

6.2.1.1 PrEP Comparison Component Maternal Enrollment Visit

For eligible women, enrollment must occur in time for study drug initiation prior to 32 completed weeks of gestation. The point of enrollment is taken to be the time when a participant has successfully been entered in the Subject Enrollment System (SES). All Entry Visit procedures are expected to be performed on the day of enrollment; procedures that may provide information relevant to eligibility for the study (e.g., rapid HIV test, medical history, physical examination), should be performed first prior to final eligibility determination and assignment to a study cohort. If a participant is found to ineligible on the day of enrollment, enrollment should not occur.

Additional requirements for sequencing of procedures at the Entry Visit are as follows:

- Rapid HIV testing, and final eligibility determination and confirmation must precede enrollment.
- For women in Cohort 1:
  - Enrollment must precede prescribing of study drug.
  - Study drug prescribing must precede dispensing.
  - Blood collection for baseline TFV-DP drug level evaluation must precede ingestion of study drug.

PrEP Comparison Component Maternal Enrollment Visit Procedures *(Day 0)*		
Administrative and Regulatory		• Complete final eligibility determination and confirmation • Complete eligibility checklist, enter checklist data into SES to enroll the participant, print and file a copy of the confirmation • Collect comprehensive locator information
Behavioral and Counseling		• iNSC [see Section 5.3.2 for specific counseling for each cohort] • CASI and COVID-19 questionnaire [see Section 6.16]
Clinical		• Update medication and medical history since last visit • Perform complete maternal physical exam
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)][1] • Baseline TFV-DP level (DBS)[2] • Syphilis testing • HSV2
	Other	*Collect vaginal swab or urine for:* • Chlamydia and gonorrhea *Collect vaginal swab for:* • Microbiome *Collect rectal swab (if consented) for:* • Microbiome
Study Drug		*Cohort 1 only:* • Prescribe and dispense study drug

[1] A negative test result must be available prior to enrollment.
[2] Baseline sample must be collected prior to ingestion of study drug for Cohort 1 participants; the sample for Cohort 2 participants can be collected at any time and is required to confirm the participant is not receiving PrEP as standard of care.

6.2.1.2 PrEP Comparison Component Maternal Antepartum Week 4, 8, 12 (during pregnancy)

The Antepartum (AP) Week 4, 8 and 12 visits are targeted to take place on Study Days 28, 56 and 84 respectively, counted from the date of study entry as Day 0, with an allowable window of ± 7 days. A negative rapid HIV test result at each visit must be available, for participants in Cohort 1 and Cohort 2/Step 2, prior to prescribing study drug.

In addition to the standard of care HIV prevention package provided and routine safety evaluations for all study participants at these visits, adherence and acceptability assessments are performed in Cohort 1.

Participants in Cohort 1 and Cohort 2 may deliver prior to completion of the AP Week 8 or Week 12 visit. If so, evaluations indicated for the AP Week 8 and Week 12 visit should be conducted in addition to the evaluations required for the Maternal Labor and Delivery Visit (Week 0) (Section 6.2.1.4). In those cases, the visit window of the L&D visit (+14 days) will apply.

Note that women who are selected for and consent to participation in the Qualitative In-Depth Interview during pregnancy may be scheduled for their interview at or after the Week 12 visit. See Section 6.2.1.8 for details about this visit.

PrEP Comparison Component Maternal AP Weeks 4, 8 and 12 *(± 7 days)*		
Behavioral and Counseling		• CASI and COVID-19 questionnaire [see Section 6.16] *Week 12:* • Qualitative IDI Visit [see Section 6.2.1.8] *Cohort 1 and Cohort 2/Step 2 only:* • iNSC with drug level feedback [see Section 5.3.2] *Cohort 2 only:* • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)] *Weeks 4, 12:* • ALT, Creatinine, and CrCl *Cohort 1 and Cohort 2/Step 2 only:* • TFV-DP level (DBS)
	Other	*Collect vaginal swab once at EGA 34-38/40 weeks for:* • Microbiome C*ollect rectal swabs once at EGA 34-38/40 weeks (if consented)* for: • Microbiome *Cohort 1 and Cohort 2/Step 2 only; collect at one visit at EGA 34-38/40 weeks:* • Collect vaginal secretions for drug level
Study Drug		*Cohort 1 and Cohort 2/Step 2 only:* • Prescribe and dispense study drug

6.2.1.3 PrEP Comparison Component Maternal Antepartum Week 24 and 36

Participants in both Cohort 1 and Cohort 2 who enroll early in gestation (i.e., prior to 20 weeks' gestation) may return for visits at AP Study Weeks 24 and 36. These visits are targeted to take place 168 and 252 days from the date of study entry (Day 0), respectively, with an allowable window of ± 14 days.

In addition to the standard of care HIV prevention package provided and routine safety evaluations for all study participants at these visits, assessments are conducted in the PrEP study cohort of PrEP acceptability and adherence.

Note that women who are selected for and consent to participation in the Qualitative In-Depth Interview during pregnancy may be scheduled for their interview at the time of their regular study visit at Week 24 or 36 or at an alternative date. See Section 6.2.1.8 for details about this visit.

PrEP Comparison Component Maternal AP Week 24 and 36 Visit Procedures *(± 14 days)*		
Administrative and Regulatory		• Review locator information
Behavioral and Counseling		• CASI and COVID-19 questionnaire [see Section 6.16] • Qualitative IDI Visit: [see Section 6.2.1.8] *Cohort 1 and Cohort 2/Step 2 only:* • iNSC with drug level feedback [see Section 5.3.2] *Cohort 2 only:* • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC team if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)] • ALT, Creatinine, and CrCl *Cohort 1 and Cohort 2/Step 2 only:* • TFV-DP level (DBS)
	Other	*Collect vaginal swab once at EGA 34-38/40 weeks for:* • Microbiome *Collect rectal swabs once at EGA 34-38/40 weeks (if consented) for:* • Microbiome *Cohort 1 and Cohort 2/Step 2 only; collect once at EGA 34-38/40 weeks:* • Collect vaginal secretions for drug level
Study Product		*Cohort 1 and Cohort 2/Step 2 only:* • Prescribe and dispense study drug

6.2.1.4 PrEP Comparison Component Maternal Labor and Delivery Visit

Note: The calendar will reset after delivery for postpartum visits, with the delivery date considered as Day 0, and subsequent visits calculated from this date.

The maternal labor and delivery visit will be conducted within 14 days of delivery. If the delivery visit is conducted within 30 days of the last visit, procedures do not need to repeated except for the rapid HIV test, HIV RNA test and plasma storage for future resistance testing. If the mother does not deliver a live born infant the mother should still complete the Delivery Visit and all subsequent postpartum visits if she is willing to do so.

PrEP Comparison Component Maternal Labor and Delivery Visit Procedures *(L/D +14 days)*		
Administrative and Regulatory		• Review locator information
Behavioral and Counseling		• Qualitative Interview IDI Visit: to take place at the L/D visit or at an alternative date [see Section 6.2.1.8] *Cohort 1 and Cohort 2/Step 2 only*: • iNSC with drug level feedback [see Section 5.3.2] *Cohort 2 only:* • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical/medications history, including pregnancy outcome • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)] • TFV- DP level (DBS)[1] • ALT, Creatinine, and CrCl
Other	**Radiology**	*DXA scan of:* • Hip • Lumbar Spine
Study Product		*Cohort 1 and Cohort 2/Step 2 only:* • Prescribe and dispense study drug

[1] DBS sampling is required for Cohort 1 and Cohort 2/Step 2 and Cohort 2; this requirement for Cohort 2 is to confirm and document the absence of PrEP exposure.

6.2.1.5 PrEP Comparison Component Maternal Postpartum Week 6

The PP Week 6 visit will be conducted six weeks (± 14 days) following the delivery date.

PrEP Comparison Component Maternal PP Week 6 Visit Procedures *(Week 6 ± 14 days)*	
Behavioral and Counseling	*Cohort 1 and Cohort 2/Step 2 only:* • iNSC with drug level feedback [see Section 5.3.2] *Cohort 2 only:* • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]

PrEP Comparison Component Maternal PP Week 6 Visit Procedures *(Week 6 ± 14 days)*		
Clinical		• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)] *Cohort 1 and Cohort 2/Step 2 only:* • TFV-DP level (DBS)
	Other	*Collect vaginal swab for:* • Microbiome *Collect rectal swabs (if consented) for:* • Microbiome *Cohort 1 and Cohort 2/Step 2 only:* • Collect vaginal secretions for drug level
Study Product		*Cohort 1 only and Cohort 2/Step 2 only:* • Prescribe and dispense study drug

6.2.1.6 PrEP Comparison Component Maternal Postpartum Week 14

The PP Week 14 visit will be conducted 14 weeks (±14 days) following the date of delivery.

Women in Cohort 2 who have been selected for and consented to participate in the Qualitative Evaluations postpartum may undergo their in-depth interview at this visit or any time up to and including the PP Week 26 visit.

Because the DBS results from this visit will be used to evaluate adherence in Cohort 1, women from Cohort 1 may not be formally selected for participation in the Qualitative Evaluations until those results are available; however, if slots are still available study staff could approach potential participants to discuss the Qualitative Evaluations and to arrange for potential follow up for the in-depth interview, refer to Section 6.2.1.8.

PrEP Comparison Component Maternal PP Week 14 Visit Procedures *(± 14 days)*	
Behavioral and Counseling	• CASI and COVID-19 questionnaire [see Section 6.16] • Qualitative IDI Visit [see Section 6.2.1.8] *Cohort 1 and Cohort 2/Step 2 only:* • iNSC with drug level feedback [see Section 5.3.2] *Cohort 2 only:* • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical	• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)

PrEP Comparison Component Maternal PP Week 14 Visit Procedures *(± 14 days)*		
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid HIV test[1][see Section 6.3 in the event the test is (+)] • ALT, Creatinine, and CrCl • CBC with differential • Pregnancy testing (blood or urine test may be performed) *Cohort 1 and Cohort 2/Step 2 only:* • TFV-DP level (DBS)
	Urine	• Pregnancy testing (blood or urine test may be performed)
Study Product		*Cohort 1 only and Cohort 2/Step 2 only:* • Prescribe and dispense study drug

6.2.1.7 PrEP Comparison Component Maternal Postpartum Week 26: Final Study Visit

The PP Week 26 visit will be conducted 26 weeks (± 14 days) following the date of delivery.

If slots are still available for the Qualitative In-Depth Interview and/or if a mother had previously consented to participate at the time of this visit, the in-depth interview may take place during this visit. It is acceptable for the interview to take place on a different date within the Week 26 visit window.

PrEP Comparison Component Maternal PP Week 26 Visit Procedures *(Week 26 ± 14 days)*		
Behavioral and Counseling		• iNSC [see Section 5.3.2] • CASI and COVID-19 questionnaire [see Section 6.16] • Qualitative IDI Visit [see Section 6.2.1.8] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • Rapid test [see Section 6.3 in the event the test is (+)] • ALT, Creatinine, and CrCl • Hepatitis B surface antigen • Syphilis testing • HSV2 • Pregnancy testing (blood or urine test may be performed) *Cohort 1 and Cohort 2/Step 2 only:* • TFV-DP level (DBS)

PrEP Comparison Component Maternal PP Week 26 Visit Procedures *(Week 26 ± 14 days)*		
	Other	*Collect vaginal swab or urine for:* • Chlamydia and gonorrhea *Collect vaginal swab for:* • Microbiome *Collect rectal swabs (if consented) for:* • Microbiome *Cohort 1 and Cohort 2/Step 2 only:* • Collect vaginal secretions for drug level
	Urine	• Pregnancy testing (blood or urine test may be performed)
Other	**Radiology**	*DXA scan of:* • Hip • Lumbar Spine *Note: Pregnancy test required within 14 days prior to DXA scan.*
Study Drug		*Cohort 1 and Cohort 2/Step 2 only:* • Collect study drug

6.2.1.8 PrEP Comparison Component Maternal: Qualitative In-Depth Interview Visit

A subset of women from both cohorts of the PrEP Component will be invited to participate in the Qualitative In-Depth Interviews. The primary activity for this evaluation will be a single in-depth interview which will be audio-recorded and transcribed. All women in this subset must provide written informed consent prior to participating.

Up to 30 antepartum women and up to 30 postpartum women will be selected and interviewed. For Cohort 1, DBS results from two consecutive visits will be reviewed to categorize maternal participants as adherent or not prior to selection; up to ten antepartum women and up to ten postpartum women in each adherence category will be selected. Cohort 2 is comprised of women who declined PrEP.

The timing of the interviews will differ according to pregnancy status and Cohort, as below:

- For pregnant women in Cohort 1 (n=20) – after review of DBS results obtained between Week 12 and delivery.
- For pregnant women in Cohort 2 (n=10) – at or after the Week 12 visit and prior to delivery.
- For postpartum women in Cohort 1 (n=20) – after review of Week 14 DBS results and prior to study exit.
- For postpartum women in Cohort 2 (n=10) – at or after Week 14 and prior to study exit.

All interviews explore experiences around decision making around use of PrEP, which include PrEP start and experiences with taking PrEP over time (Cohort 1) and reasons PrEP was declined (Cohort 2). Experiences with PrEP from a socio-ecological perspective will be discussed and factors related to PrEP drug, self-perceptions of HIV exposure risk and need for PrEP, self-administration/dosing, community influence, experiences at site and structural supports or challenges will be explored.

Interview topics may include how women incorporated daily pill-taking into their daily routines, where they stored PrEP, and whether they disclosed their PrEP use with family members, peers, and their partner(s), and experiences with side-effects for Cohort 1. Women may also be asked

questions regarding the impact of COVID-19 on their study participation, including PrEP adherence and acceptability.

Among women who declined PrEP (Cohort 2), interview topics include factors that were associated with not initiating PrEP, including risk perception, personal and community beliefs about PrEP, and challenges related to COVID-19 that may have influenced PrEP decision making, and opinions about appropriateness of PrEP for other expecting or nursing women in the community.

Interview guides used during the interviews will be developed using reviews of local literature and community advisory board (CAB) feedback. Local researchers/ethnographers will conduct all qualitative interviews with oversight from the study staff. All interviews will be audio-recorded and transcribed and translated into English by trained study staff. Coding will identify main themes emerging from each of the areas explored in the interview.

6.2.2 PrEP Comparison Component Infant Birth and PP Week 6, 14 and 26 Visit

Infants born to women in the PrEP Comparison Component (both cohorts) will be evaluated for safety at four time points: birth and Weeks 6, 14 and 26 after birth. The date of birth is considered Day 0 and subsequent visits will be calculated from this date. These time points correspond to the maternal labor and delivery visit and maternal postpartum follow up visits at Weeks 6, 14 and 26. Whenever possible, infant visits should be scheduled on the same date of the associated maternal visits.

6.2.2.1 PrEP Comparison Component Infant Birth Visit

The allowable window for all evaluations expected at birth is within 14 days after birth; however, it is understood that sites may not be able to conduct the DXA scan on the same date as the other evaluations and may not be able to arrange a scan within that window. Sites should make every effort to schedule the DXA scan to take place within the 14-day window, but up to 21 days is allowed. See Section 6.14 for further details.

PrEP Comparison Component Infant Birth Visit Procedures *(Birth +14 days)*		
Clinical		• Obtain birth history, interim medical and medications history since birth, and feeding history since birth • Perform complete infant physical exam
Laboratory	**Blood**	*Collect blood for:* • Creatinine • TFV-DP level (DBS) (for storage)
	Other	• Rectal swabs or fresh stool for microbiome
Other	**Radiology**	*DXA scan of:* • Whole body • Lumbar Spine

6.2.2.2 PrEP Comparison Component Infant PP Week 6 and PP Week 14 Visits

Study visits will be conducted six and fourteen weeks following birth for the infant (± 14 days). Whenever possible, infant visits should be scheduled on the same date of their associated maternal visits.

PrEP Comparison Component Infant PP Week 6 and PP Week 14 Visit Procedures (± *14 days*)	
Clinical	• Perform targeted physical exam • Obtain interim medical/medications history and feeding history
Laboratory	• Rectal swabs or fresh stool for microbiome

6.2.2.3 PrEP Comparison Component Infant PP Week 26

The final study visit will be conducted 26 weeks following birth for the infant (± 14 days). Whenever possible, infant visits should be scheduled on the same date of their associated maternal visits.

PrEP Comparison Component Infant PP Week 26 Visit Procedures *(Week 14 ± 14 days)*		
Clinical		• Perform complete infant physical exam • Obtain interim medical/medications history including feeding history
Laboratory	**Blood**	*Collect blood for:* • Creatinine
	Other	• Rectal swabs or fresh stool for microbiome
Other	**Radiology**	*DXA scan of* • Lumbar spine

6.2.3 Management of Maternal Participants Who Subsequently Switch from Choice Made at Enrollment During the PrEP Comparison Component

All participants enter the study on Step 1; however, the study will accommodate maternal participants who change their minds after enrollment about whether to receive PrEP. Management of these participants will be different depending on the cohort to which they were originally enrolled.

6.2.3.1 Maternal Participants in Cohort 1 Who Subsequently Stop PrEP

To the extent possible, staff should try to identify the reasons for maternal participants choosing to stop PrEP. Once the maternal participant has stated that she is not willing to continue PrEP and the decision to stop PrEP will be entered into an eCRF. If not already required for the study visit at which this decision is made, the following additional study procedures should be conducted:

- iNSC
- TFV-DP level (DBS)
- HIV RNA test

This will not constitute a change from Cohort 1 to 2; the maternal participant will continue to be followed as per her original assignment per Appendix IIA with the exception of the following evaluations:

- iNSC with drug level feedback

6.2.3.2 Maternal Participants in Cohort 2 Who Elect to Begin PrEP during Follow-up (Cohort 2/Step2)

Maternal participants who initially declined PrEP may subsequently inform study staff of an interest in starting PrEP. In preparation of PrEP initiation, evaluations should be performed as per

the Step Change column in Appendix IIB. The Step Change will occur across two visits, Visit 1 and Visit 2. Note that the Step Change Visit 1 may be combined with a regular study visit and procedures combined. The participant may not initiate PrEP until she has been determined to be HIV-uninfected via a rapid test, and has otherwise satisfied the eligibility criteria for a Step Change per Section 4.2.3. Blood collection for baseline TFV-DP drug level evaluation must precede ingestion of the first dose of study drug for Cohort 2/Step 2.

PrEP Comparison Component Step Change from non-PrEP to PrEP Visit 1		
Administrative and Regulatory		• Review PrEP Comparison Component follow up and risks
Behavioral and Counseling		• CASI and COVID-19 questionnaire • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical records and medical/medication history • Perform targeted physical exam
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Store plasma for HIV drug resistance testing • CBC with differential • ALT, creatinine, and CrCl • Hepatitis B surface antigen • Glucose (random)
	Urine	*Collect urine for:* • Urinalysis (protein and glucose)

PrEP Comparison Component Step Change from non-PrEP to PrEP Visit 2		
Administrative and Regulatory		• Complete final eligibility determination and confirmation[1] • Complete eligibility checklist[1], enter checklist data into SES to enter the maternal participant into Step 2, print and file a copy of the confirmation
Behavioral and Counseling		• CASI and COVID-19 questionnaire • iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical records and medical/medication history • Perform targeted physical exam
Laboratory	**Blood**	*Collect blood for:* • Rapid HIV test[2] [see Section 6.3 in the event the test is (+)] • Baseline TFV-DP level (DBS)[3]
	Other	*Collect vaginal swab for:* • Microbiome *Collect rectal swabs (if consented) for:* • Microbiome
Study Drug		• Prescribe and dispense study drug

[1] Perform prior to step change (entering Step 2)
[2] Review results prior to prescribing study drug.
[3] Baseline sample must be collected prior to ingestion of study drug.

The maternal participant should continue with her regular follow up appointments. However, at subsequent visits, the study procedures applicable to Cohort 1 throughout this section and as per Appendix IIA will be conducted through study exit, and participants will be referred to as Cohort 2/Step 2.

6.3 Management of Participants with Suspected or Confirmed HIV Seroconversion (PK and PrEP Comparison Components)

It is imperative that pregnant and breastfeeding women newly diagnosed with HIV begin ART in a timely manner. To ensure that study participants who acquire HIV infection during follow-up initiate ART as soon as possible, each site will develop a site-specific SOP to outline their operational plan for ensuring prompt linkage to care and treatment. This will include details for rapid referral to local ART providers and mechanisms for frequent follow-up to ensure that women are initiated on ART within days of being diagnosed. Sites will also be required to include information about prophylaxis, screening, and possible treatment options for the infant. Women with newly diagnosed HIV infection (and their infants) may also co-enroll in other protocols focused on acute HIV, with prior agreement from both Protocol Teams.

6.3.1 Maternal Participants Who Test HIV-Positive (Confirmatory Visit)

Appendices IIA and IIB specify frequent HIV testing for maternal participants by using both rapid HIV tests and an HIV RNA tests. In addition to the testing specified in these appendices, testing will also be performed if a participant has signs or symptoms consistent with acute HIV infection, or expresses a concern about recent HIV acquisition.

Rapid test results are expected to be available at the visit when blood is collected; HIV RNA results are expected to be available within one to two weeks after blood collection. For any participant with a test result indicative of HIV infection, PrEP will be immediately stopped and additional Confirmatory Visit procedures will be performed as indicated in the table below and in Appendix IID. Confirmatory HIV testing should be performed according to the site's HIV testing algorithm, which will be reviewed and approved by the IMPAACT Laboratory Center prior to study initiation. The CMC should be notified of initial positive test results, as well as confirmatory test results. PrEP should continue to be held for participants with discordant test results or not confirmed as infected, pending consultation with the CMC. PrEP can only be resumed with approval from the CMC.

In general, the guidelines below should be followed for confirmatory testing.

- If the initial test indicating infection is a rapid HIV test, additional Confirmatory Visit procedures should be performed on the same day as the initial test.

- If the initial test indicating infection is an HIV-1 RNA test, the participant should be contacted immediately upon receipt of the test result to advise her to stop PrEP and request that she return to the clinic for confirmatory HIV testing and other required Confirmatory Visit procedures as soon as possible (within two business days).

The Confirmatory Visit can be combined with other scheduled study visits if the visits are conducted within the allowable window of another scheduled visit. For maternal participants who have given birth when HIV infection is first indicated, infant HIV testing should also be performed at the Confirmatory Visit, as specified in Section 6.3.2.

In all cases, maternal HIV-1-infection will be based on documented positive test results from two separate blood samples collected at different time points and following the site-specific approved HIV testing algorithm.

Sample collection and processing must follow procedures as indicated in Section 6.17.2. Participants with confirmed HIV infection will be immediately referred to non-study sources of appropriate HIV care and treatment, consistent with local standards of care, and scheduled for additional study follow-up four weeks from the date of specimen collection for the initial test result indicating HIV infection (see Section 6.3.2 for more information about this visit).

Results from HIV drug resistance testing performed after transmission has been confirmed will be provided to participants and their HIV care providers when these results become available.

Confirmatory Visit Procedures (PK Component and PrEP Comparison Component)		
Behavioral and Counseling		• iNSC [see Section 5.3.2]
Clinical		• Refer to HIV care and treatment
Laboratory	**Blood**	*Collect blood for:* • Confirmatory HIV (specimen collection for this testing must occur at a different timepoint from the initial positive test result) • CD4 count • Plasma and non-viable PBMC storage for HIV drug resistance testing
Study Drug		• Collect any remaining study drug (if applicable)
Follow-up		• Schedule visit four weeks from date of the initial HIV positive blood draw; this may be combined with scheduled study visit if windows overlap.

6.3.2 Maternal HIV Seroconversion: Management of Maternal Participants Four Weeks Post Date of Initial Positive HIV Test

Maternal participants in the PK and PrEP Comparison Components should be scheduled for a visit four weeks after the date of the blood draw for the initial positive HIV test, per the table below and Appendix IID. This visit is in addition to scheduled visits and can be combined with a scheduled visit if conducted within the allowable window of the scheduled visit. At this visit, it will be important to confirm that the mother has received appropriate HIV care and treatment and – if still pregnant or breastfeeding – appropriate PMTCT interventions. Study staff should review any available laboratory results from the prior visit. This will be the final study visit for the women participating in the PK Component. Participants in the Prep Comparison Component will continue in the study, refer to Section 0.

Maternal Procedures 4 Weeks (± 7 days) Following Diagnosis of HIV		
Clinical		• Obtain interim history, and confirm receipt of appropriate HIV care and ARV medications started as treatment (and PMTCT, if still pregnant or breastfeeding)
Laboratory	**Blood**	*Collect blood for:* • Plasma and non-viable PBMC storage for HIV drug resistance testing • CD4 count
Follow-up		*PK Component* • None. This will be the final study visit *PrEP Component* • Confirm time/date of next scheduled visit

6.3.3 Maternal HIV Seroconversion: Management of Maternal Participants at Every Visit (PrEP Comparison Cohort only)

Maternal participants in the PrEP Comparison Component with confirmed HIV infection will remain on-study and continue to be followed through scheduled study exit per the applicable SoE (Appendix IIA or IIB). However, because they will no longer be on PrEP, the following procedures will no longer be performed among these participants:

- Rapid HIV test
- Study drug dispensing
- iNSC with drug level feedback
- Two-way SMS support (one-way SMS support should continue)
- PrEP acceptability assessment
- TFV-DP (DBS) level

In addition, ALT, creatinine, CrCl and HIV RNA testing will be performed at a decreased frequency−approximately every six months during scheduled study visits−to monitor chemistries and HIV viral load among these participants.

6.3.4 Maternal HIV Seroconversion: Management of Infants

Regardless of whether the mother is diagnosed as HIV-infected during participation in the PK or PrEP Comparison Component, her infant should remain on study through his or her scheduled study exit for safety monitoring.

Infants of women diagnosed as HIV-infected during pregnancy should undergo HIV nucleic acid testing (NAT) as soon as possible after birth. Infants of women diagnosed after delivery should undergo HIV NAT on the day that the mother undergoes confirmatory HIV testing. If uninfected, the infant should be tested for HIV at each subsequent visit through study exit (see Appendix IIE). For all infants or infected mothers, site staff should provide referrals and otherwise facilitate to non-study standard of care services to prevent vertical HIV transmission.

If an infant's HIV NAT result is positive, the mother should be contacted immediately to return to the clinic with the infant, as soon as possible, to receive the results and undergo confirmatory testing. The CMC should be notified upon site awareness of a positive infant HIV result. Infant blood should be collected for confirmatory HIV testing, according to the site's HIV testing algorithm reviewed and approved by the IMPAACT Laboratory prior to study initiation, and for HIV drug resistance testing per Appendix IIE. The CMC should be updated of the confirmatory test result.

A confirmed diagnosis of infant HIV infection will be based on positive test results from two separate blood samples collected on different days following the site-specific approved infant HIV testing algorithm, which may include the following assays:

- HIV DNA PCR
- Plasma HIV RNA (quantitative or qualitative)
- HIV total nucleic acid tests

Sample collection and processing must follow procedures as indicated in Section 6.17.2.

Infants with confirmed HIV infection will remain on study through their scheduled study exit per the applicable SoE (Appendix IB, IC or IIC) and have additional procedures related to their HIV infection as per Appendix IIE. Site staff are responsible for ensuring that infants with confirmed HIV infection have access to non-study sources of appropriate HIV care and treatment, consistent with local standards of care.

6.4 Early Discontinuation Visit

Refer to Section 4.7 for criteria for withdrawal from the study. Study staff will record the reason(s) for all withdrawals in participants' study records. For any maternal or infant participant who is withdrawn or terminated from the study prior to scheduled completion of follow-up, every effort should be made to perform a final series of study evaluations possible, per the "early D/C" column of the Schedule of Evaluations; however, any evaluations for maternal and infant participants completed within 30 days prior to Early Discontinuation Visit need not be repeated at the visit except for HIV rapid testing which should be completed at the early discontinuation visit.

6.4.1 Early Discontinuation Visit During the PK Component

6.4.1.1 Maternal Procedures at Early Study Discontinuation

PK Component Maternal Early Study Discontinuation Procedures		
Behavioral and Counseling		• iNSC [see Section 5.3.2]
Clinical		• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Plasma storage for HIV resistance testing • Rapid HIV test [see Section 6.3 in the event the test is (+)] • TFV-DP level (DBS) • CBC with differential • ALT, creatinine and CrCl • Hepatitis B surface antigen
Study Drug		• Collect study drug

6.4.1.2 Infant Procedures at Early Study Discontinuation

If early discontinuation occurs after delivery, study procedures should be conducted for the infant where possible.

PK Component Infant Early Study Discontinuation Procedures		
Clinical		• Perform complete infant physical exam • Obtain interim medical/medications history including feeding history
Laboratory	**Blood**	*Collect blood for:* • Creatinine

6.4.2 Early Study Discontinuation During the PrEP Comparison Component

6.4.2.1 Maternal Procedures at Early Study Discontinuation

PrEP Comparison Component Maternal Early Study Discontinuation Procedures		
Behavioral and Counseling		• iNSC [see Section 5.3.2] • Assess for social harms [see Section 13.6]
Clinical		• Obtain interim medical/medications history • Perform targeted physical exam • Identify/review/update adverse events • Perform additional evaluations per Section 8 and/or if clinically indicated (consult CMC if indicated)
Laboratory	**Blood**	*Collect blood for:* • HIV RNA test • Plasma storage for resistance testing • ALT, creatinine, and CrCl • Rapid HIV test [see Section 6.3 in the event the test is (+)] • Hepatitis B surface antigen • Syphilis testing • HSV2 • Pregnancy testing, as needed (blood or urine test may be performed) *Cohort 1 and Cohort 2/Step 2 only:* • TFV-DP level (DBS) •
	Other	*Collect vaginal swab or urine for:* • Chlamydia and gonorrhea *Collect vaginal swab for:* • Microbiome *Collect rectal swabs (if consented) for:* • Microbiome *Cohort 1 and Cohort 2/Step 2 only:* • Collect vaginal secretions for drug level
	Urine	• Pregnancy testing, as needed (blood or urine test may be performed)
Other	**Radiology**	*DXA scan of:* • Hip • Lumbar Spine • *Note: Pregnancy test required within 14 days prior to DXA scan.*
Study Drug		*Cohort 1 or Cohort 2/Step 2 only:* • Collect study drug

6.4.2.2 Infant Procedures at Early Study Discontinuation

PrEP Comparison Component Infant Early Study Discontinuation Procedures		
Clinical		• Perform complete infant physical exam • Obtain interim medical/medications history including feeding history
Laboratory	**Blood**	*Collect blood for:* • Creatinine
	Other	• Rectal swabs or fresh stool for microbiome

6.5 PrEP Comparison Component – Interim Visit for Drug Level Feedback

Study staff will contact maternal participants within three business days of site receipt of their DBS TFV-DP concentration results. Delivery of test results will follow the approach detailed in Section 5.3.2 and the study-specific iNSC manual/workbook and associated training material. Drug level feedback should be scheduled to take place as soon as possible after receipt of the PK result. If an interim visit cannot be scheduled in-person, the visit may take place over the phone.

6.6 Management of Infants Whose Mother Dies

For infants of maternal participants who die during the PK or PrEP Comparison Component, the infant will remain enrolled through scheduled exit to complete safety evaluations, although study procedures will be held until informed consent for the infant's continued participation is obtained from the surviving parent or legal guardian.

6.7 Management of Women Whose Infant Dies or Experience Fetal Demise

For maternal participants whose infants die or who experience fetal demise during the PK or PrEP Comparison Component, maternal study procedures will not change, with the following exception:

- One-way SMS support messages should be discontinued upon site awareness of infant death or fetal demise. For maternal participants in Cohort 1 or Cohort 2/Step 2, two-way SMS support messages should continue.

6.8 Maternal Medical and Medication History

Collection of medical and medication history is required at each scheduled maternal visit during the PK and PrEP Comparison Components. A baseline history is established at the screening and enrollment visits, and interim (since the last visit) histories are obtained at subsequent follow-up visits. All history may be based on maternal self-report but available medical records should be obtained when possible to supplement self-reported information.

Documented medical conditions will be assessed for severity as described in Section 7.3.3, and new conditions occurring during follow-up will also be assessed for relationship to study drug as described in Section 8.1. Relevant dates will be recorded for all conditions and medications; see Section 5.4 for more information on concomitant medications.

The following should be source documented as part of the baseline medical and medication history:

- Date of birth
- Reproductive and obstetrical history (including date of last menstrual period prior to the current pregnancy and dates and outcomes of all prior pregnancies)
- History of allergy and/or hypersensitivity
- History of bone fracture (traumatic and non-traumatic)
- History of substance use (e.g. tobacco smoking and/or alcohol use)
- History of STI
- Medical conditions (signs, symptoms, illnesses, and other diagnoses) occurring during the 30 days prior to enrollment and/or ongoing at the time of enrollment

- Medications, including alternative therapies, taken within the 30 days prior to enrollment and/or ongoing at the time of enrollment
- Any other information needed to determine eligibility for the study

The following should be source documented as part of interval medical and medication histories:

- Current status of conditions that were ongoing at the previous visit
- Current status of medications that were ongoing at the previous visit
- Occurrence of any new conditions (signs, symptoms, illnesses, and other diagnoses) since the last visit (specifically including incident HIV and other STIs)
- Use of any new medications, including alternative therapies, since the last visit

In addition, at the Delivery Visit, all delivery-related history, including pregnancy outcome, should be documented.

Refer to Sections 7.2 and 5.4, respectively, for information on entering medical and medications history information into eCRFs.

6.9 Maternal Physical Examinations

A physical examination is required at each scheduled maternal visit during the PrEP Comparison Components. Complete exams are required at the Screening and Entry Visits; targeted exams are required at all other visits. For maternal participants in the PK Component, complete examinations are required at the screening and entry visits, and targeted examinations are required at Weeks 4, 8, 12, and at the Labor and Delivery visit (Group 1 only).

Complete exams should include the following:

- Height measurement (at screening only)
- Weight measurement
- Blood pressure measurement
- Obstetric exam at AP visits (including fundal height measurement and assessment of fetal heart rate)
- Auscultation of chest (heart and lung exam)
- Examination of:
  - Skin
  - Head
  - Mouth
  - Neck
  - Abdomen
  - Extremities
  - Visual inspection of the genitalia

Targeted exams should include the following:

- Weight measurement
- Blood pressure measurement
- Obstetric exam at AP visits (including fundal height measurement and assessment of fetal heart rate)

- Examination of body systems driven by prior and new signs, symptoms, and diagnoses including incident HIV and other STIs
- Routine pelvic examination is not required unless clinically indicated or unless specimen collection required

At all visits, additional assessments may be performed at the discretion of the examining clinician. For complaints of gynecologic symptoms, this may include a pelvic examination, with speculum if appropriate and available. Documentation of exam and medical management should be in line with the site guidelines for management of sexually transmitted infections.

All exam findings should be source documented in participant study records, and weight and height (at Entry only) should be entered into eCRFs. Temperature and blood pressure should be entered into eCRFs if abnormal; other abnormal findings may also be entered into eCRFs if required as specified in Section 7.2.

6.10 Maternal DXA Scans

Maternal participants in the PrEP Comparison Component will undergo DXA scans of the hip and lumbar spine at their Labor & Delivery Visit and Week 26 postpartum as well as early discontinuation visits, if applicable. Scans will be performed according to standardized procedures described in the study-specific MOP. Scan images will be transferred electronically for centralized reading and results will be transmitted electronically from the central readers to the Data Management Center (DMC); however, if a maternal participant experiences a bone fracture subsequent to her scan, the result of her scan will be provided to the site as soon as available.

6.11 Infant Medical and Medication History

Collection of medical and medication history is required at each scheduled infant visit during the PK and PrEP Comparison Components.

Medical and medication history at birth will consist of information recorded on medical records at birth as well as information obtained at the Birth Visit. The Birth Visit should take place as soon as possible after birth, but may take place up to 14 days after birth; as such, information recorded at birth may differ from information obtained at the Birth Visit. Thereafter, history information may be obtained based on maternal report but available medical records should also be obtained when possible to supplement maternal report.

The following should be source documented as part of medical and medications history at the Birth Visit:

- Date and time of birth
- Sex at birth
- Length, weight, and head circumference at birth (obtain from medical records; these measurements will also be performed by study staff at the infant Birth Visit)
- Apgar scores at one and five minutes (obtain from medical records)
- Medications taken since birth

The following should be source documented as part of interval medical and medication histories:

- Current status of conditions that were ongoing at the previous visit
- Current status of medications that were ongoing at the previous visit
- Occurrence of any new conditions (signs, symptoms, illnesses, and other diagnoses) since the last visit
- Use of any new medications since the last visit

Refer to Sections 7.2 and 6.8, respectively, for information on entering medical and medications history information into eCRFs.

6.12 Infant Feeding History

An infant feeding history is required at each scheduled visit during the PK and PrEP Comparison Components. At the Birth Visit, feeding history (since birth, to include the date and time of first breastfeeding, if applicable) will be collected. Thereafter, interval (since the last visit) histories will be collected. At each time point, data will be collected on feeding method, including whether the infant has been breastfed or formula fed, and date of last exposure to breast milk. Any therapeutic foods received will be recorded as concomitant medications (see Section 5.4).

6.13 Infant Physical Examinations

A physical examination is required at each scheduled infant visit during the PK and PrEP Comparison Components. In the PK Component, for Group 1 infants a complete exam is required at the Birth Visit and a targeted exam is required at the Postpartum Week 6 Visit; for Group 2 infants a complete exam is required at the Entry Visit and targeted exams are required at the Week 6 and Week 12 Visits. In the PrEP Comparison Component, complete exams are required at the Birth and PP Week 26 Visits; targeted exams are required at the PP Week 6 and PP Week 14 Visits. Complete exams are required at the Early Study Discontinuation Visit for all infants in both Components.

Complete exams should include the following:

- Length measurement
- Weight measurement
- Head circumference measurement
- Assessment of anterior fontanel closure
- Auscultation of chest (heart and lungs)
- Examination of skin, head, mouth, neck, abdomen, extremities
- Neurologic assessment

Targeted exams should include the following:

- Length measurement
- Weight measurement
- Head circumference measurement
- Assessment of anterior fontanel closure
- Auscultation of chest (heart and lungs)
- Examination of body systems driven by prior and new signs, symptoms, and diagnoses

At all visits, additional assessments may be performed at the discretion of the examining clinician. Also at all visits, the anthropometric growth measurements listed above should be charted on standard infant growth charts and weight-for-length should be assessed in relation to WHO growth standards.

All exam findings should be source documented in participant study records. Weight, length, and head circumference should be entered into eCRFs. Temperature should be entered into eCRFs if abnormal; other abnormal findings may also be entered into eCRFs if required as specified in Section 7.2.

6.14 Infant DXA Scans

Infants in the PrEP Comparison Component will undergo DXA scans of the whole body and lumbar spine at their Birth Visit and of the lumbar spine Week 26 postpartum. Scans will be performed according to standardized procedures described in the study-specific MOP. Scan images will be transferred electronically for centralized reading and results will be transmitted electronically from the central readers to the Data Management Center (DMC). The centralized readings of all scans will be made available to the study sites after the last scan has been read centrally. However, if an infant experiences a bone fracture subsequent to his or her scan, the result of his or her scan will be provided to the site as soon as available.

6.15 Fetal Ultrasound

If sonographic results from the current pregnancy are not available from medical records at the time of screening during the PK and PrEP Comparison Components, a fetal ultrasound scan will be performed to confirm (or used to re-calculate) gestational age at entry into the study. Scans should be performed during the 30-day study screening period, and results must be available at the time of enrollment.

Ultrasound scans may be performed at the study site or at off-site facilities. A result report that minimally documents the following must be obtained for filing in participant study charts and entry into eCRFs:

- Date of scan
- Number of fetuses
- Biometry measures for
  - If ≤ 14 weeks gestation:
    - Crown-rump length
  - If > 14 weeks gestation:
    - Femur length, abdominal circumference, and either or both head circumference and biparietal diameter
- Calculated gestational age on the date of the scan or estimated date of delivery

6.16 Questionnaires and Computer-Assisted Self-Interview (CASI)

6.16.1 PK Component

During enrollment and at Study Exit of the PK Component, maternal participants will be queried on demographic and behavioral HIV risk characteristics; this information will be recorded on an eCRF. Items from the following domains will be included:

- Sociodemographic characteristics
    - Current living situation
    - Household composition
- HIV risk perception for mother and infant
- Sexual behaviors

6.16.2 PrEP Comparison Component

6.16.2.1 COVID-19

Throughout follow-up of the PrEP Comparison Component, a COVID-19 questionnanire will be administered to maternal participants at select study visits (see Appendices IIA and IIB and Section 6.2). This questionnaire will cover topics such as the potential impact of COVID-19 on participants' life and study participation; this information will be recorded on an eCRF.

6.16.2.2 CASI

During enrollment and follow-up of the PrEP Comparison Component, maternal participants will be queried on the contextual factors, that have been shown to impact differential uptake and sustained use of PrEP in prior trials. Items from the following domains will be programmed into the CASI:

- Sociodemographic characteristics
    - Current living situation
    - Household composition
    - Private space for product storage
- HIV risk perception for mother and infant
- HIV stigma
- PrEP stigma
- Sexual behaviors
- Substance use
- PrEP beliefs and PrEP readiness
- Future orientation and aspirations; self-efficacy
- Mental health and post-traumatic stress symptoms
- Disclosure to peers, family members, and partner(s) about PrEP use and participation in the study
- Gender-based violence (GBV)
- Partnership characteristics: partner's HIV status

6.17 Additional Considerations for Laboratory Procedures

Each study site and laboratory involved in this study will comply with the DAIDS policy on Requirements for DAIDS Funded and/or Sponsored Laboratories in Clinical Trials Policy, which is available at:
https://www.niaid.nih.gov/research/daids-clinical-research-laboratory-specimens-management

6.17.1 Specimen Collection

Specimens will be collected for this study as indicated in the Schedule of Evaluations and per detailed guidance provided in the Laboratory Processing Chart (LPC), which will be available on the IMPAACT web site: www.impaactnetwork.org.

In accordance with U.S. National Institutes of Health (NIH) recommendations, adult blood collection will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

In accordance with U.S. National Institutes of Health (NIH) recommendations, pediatric blood collection will not exceed 5 mL/kg in a single day or 9.5 mL/kg in any eight-week period.

In the event that blood collection must be limited, available specimens should be prioritized for use in the following order: (1) HIV testing, (2) Creatinine, (3) Drug levels, (4) stored plasma.

6.17.2 Specimen Preparation, Testing, Storage, and Shipping

All specimens collected for this study will be labeled, transported, processed, tested, stored and/or shipped in accordance with the DAIDS policy referenced in Section 6.17.1, site and local laboratory SOPs, and the LPC. The frequency of specimen collection and testing will be directed by the Schedule of Evaluations and specifications for clinical management provided in Section 8. The Laboratory Data Management System (LDMS) will be used to document specimen collection, testing, storage, and shipping as specified in LPC.

Safety tests will be conducted in real time at the site laboratories.

Dried blood spot samples will be shipped to regional laboratories for TVF-DP drug levels. Samples will be shipped at a frequency which permits a site to receive results by the next study visit for the PrEP Comparison participants.

Infant specimens for HIV nucleic acid testing for infant HIV diagnosis and maternal specimens for HIV testing must be tested in a VQA-certified laboratory. HIV antibody tests must be performed according to GCLP guidelines and the laboratory must participate in an appropriate external QA program.

Maternal and infant sample swabs for microbiome analyses will be stored and batch shipped for analysis. No data from these samples will be available to the sites during the study.

Maternal vaginal secretions samples for drug level may be obtained with a soft plastic aspirator without speculum placement or by another appropriate technique and can be collected by the participant or by a clinician. Samples will be stored and batch shipped for testing. Since target drug concentrations for vaginal samples have not been determined, real time testing and reporting of vaginal specimen results to the sites will not be done. No vaginal specimens will be collected if

contraindicated by a pregnancy complication such as placenta previa or premature rupture of membranes.

After all protocol-specified laboratory testing has been performed, residual maternal and infant specimens may be of interest for future research use. Maternal participants (or other authorized guardians if applicable) will be asked to provide written informed consent for future research use of these specimens, if permitted by site IRBs/ECs and other applicable review bodies. Participants may choose to provide or to decline informed consent for future research use of residual specimens with no impact on other aspects of participation in the study. With permission, human genetic tests may be performed on these samples. The genetic testing may be used to help understand variability in enzymes responsible for tenofovir and emtricitabine disposition and drug response including, drug transporters, kinases, phosphotransferases, or enzymes involved in drug response.

6.17.3 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 and shipping and handling of all specimens for this study as currently recommended by the U.S. Centers for Disease Control and Prevention, NIH, and other applicable agencies. All specimens will be shipped using packaging that meets requirements specified by the International Air Transport Association Dangerous Goods Regulations for UN 3373, Biological Substance, Category B, and Packing Instruction 650. Culture isolates, if obtained in this study, are to be shipped as specified for UN 2814 Category A Infectious Substances.

7 SAFETY MONITORING, ASSESSMENT AND REPORTING

7.1 Safety-Related Roles and Responsibilities

Participant safety will be carefully assessed, monitored, and reported at multiple levels throughout this study. Sections 7.1, 7.2, and 7.3 describe safety-related roles, responsibilities, and procedures for site investigators. The safety monitoring roles of the Clinical Management Committee (CMC) and the IMPAACT Study Monitoring Committee (SMC) are briefly referenced in Section 7.1 and described in greater detail in Section 9.3.

7.1.1 Site Investigators

Site investigators are responsible for continuous monitoring of all study participants and for alerting the CMC if unexpected concerns arise. Site investigators will record safety-related data on eCRFs as indicated in Section 7.2 and complete expedited adverse event (EAE) reporting as indicated in Section 7.3. Site investigators are also responsible for prompt reporting to their IRBs/ECs and other applicable review bodies of any unanticipated problems involving risks to participants or others.

7.1.2 Clinical Management Committee (CMC)

The following Protocol Team members comprise the CMC: Protocol Chair and Vice Chairs, Medical Officers, Pharmacologists, Statisticians, Epidemiologist, Data Managers, Laboratory Data Managers, Clinical Trial Specialists, at least one international Protocol Investigator, or their

designees. The CMC will provide guidance as needed to site investigators regarding all aspects of participant management, including but not limited to questions of participant eligibility and management of adverse events, study drug administration, and other concomitant medications. Refer to Section 8 for more information on participant management.

On behalf of the full Protocol Team, the CMC will monitor participant safety through routine review of study data reports as described in Section 9.3.

7.1.3 Study Monitoring Committee (SMC)

An independent IMPAACT Study Monitoring Committee (SMC) will monitor participant safety through routine and as needed reviews of study data. Refer to Section 9.3.3 for more information on the composition and role of the SMC in monitoring of this study.

7.2 Safety-Related Data and Pre-Existing Conditions Data Collection

Note: This section describes eCRF data recording for pre-existing conditions and adverse events. As part of this description, reference is made to severity grading and criteria for EAE reporting; refer to Sections 7.3.3 and 7.3.2, respectively, for detailed information on these topics.

The definition of the term adverse event provided in Version 2.0 of the Manual for Expedited Reporting of Adverse Events to DAIDS (DAIDS EAE Manual) will be used in this study. This definition will be applied to all maternal and infant participants, in both the PK Component and in the PrEP Comparison Component, beginning at the time of enrollment, regardless of subsequent administration of or exposure to study drug. Any untoward medical conditions (including abnormal laboratory test results, signs, symptoms, or diseases) identified prior to enrollment will be considered pre-existing conditions. Refer to Section 4.4 for more information on defining the effective point of enrollment in the study.

Pre-existing conditions and adverse events will be entered into eCRFs as specified in Sections 7.2.1 (for maternal participants) and 7.2.2 (for infants). Additionally, in the PrEP Comparison Component all reported social harms will likewise be documented on eCRFs as specified in Section 13.6.

7.2.1 Safety-Related Data Collection for Maternal Participants

Pre-Existing Conditions

All pre-existing conditions identified among maternal participants during the 30 days prior to study entry will be recorded on maternal medical history eCRFs.

Adverse Events

The following adverse events— inclusive of abnormal laboratory test results and clinical signs, symptoms, and diagnoses — will be entered into maternal adverse events eCRFs:

For the PK Component:

- All Grade 3 or higher adverse events

For the PrEP Comparison Component:
- All Grade 2 or higher adverse events

For both the PK Component and PrEP Comparison Component:
- All adverse events that lead to a change of study drug
- All complications and sequela of adverse pregnancy outcomes.
- All serious adverse events (SAEs) as defined in Version 2.0 of the Manual for Expedited Reporting of Adverse Events to DAIDS (DAIDS EAE Manual)

Laboratory Test Results

In addition to the recording specified above, the following laboratory test results will be entered into the relevant maternal laboratory eCRFs, regardless of whether the test was protocol-specified or ordered by the site investigator for clinical purposes:

For the PK Component:
- All Grade 3 or higher hemoglobin, white blood cell count, absolute neutrophil count, and platelet count results

For the PrEP Comparison Component:
- All Grade 2 or higher hemoglobin, white blood cell count, absolute neutrophil count, and platelet count results

For both the PK Component and PrEP Comparison Component:
- All HIV test results
- All creatinine and creatinine clearance results regardless of severity grade
- All Grade 2 or higher ALT results
- All results that lead to a discontinuation of study drug
- All results that are serious as defined in Version 2.0 of the DAIDS EAE Manual

7.2.2 Safety-Related Data Collection for Infants

Pre-Existing Conditions

Given that infants in Group 1 of the PK Component, Cohort 1 and Cohort 2/Step 2 (specifically, infants of mothers who undergo the step change while pregnant) of the PrEP Comparison Component will be exposed to study drug *in utero*, all abnormal conditions identified during and after birth will be considered adverse events. No pre-existing conditions will be entered into eCRFs for infants.

Adverse Events

The following adverse events — inclusive of abnormal laboratory test results and clinical signs, symptoms, and diagnoses — will be entered into infant adverse events eCRFs:

For the PK Component:
- All Grade 3 or higher clinical signs, symptoms and diagnosis

For the PrEP Comparison Component:

- All Grade 2 or higher clinical signs, symptoms and diagnosis

For both the PK Component and PrEP Comparison Component:

- Suspected congenital anomalies
- All SAEs as defined in Version 2.0 of the DAIDS EAE Manual

Laboratory Test Results

In addition to the recording specified above, the following laboratory test results will be entered into the relevant infant laboratory eCRFs, regardless of whether the test was protocol-specified or ordered by the site investigator for clinical purposes:

- All HIV test results
- All creatinine results regardless of severity grade
- All results that are serious as defined in Version 2.0 of the DAIDS EAE Manual

7.3 Expedited Adverse Event (EAE) Reporting

EAEs will be reported only for participants in this study who are exposed to study drug.

7.3.1 Adverse Event Reporting to DAIDS

Requirements, definitions, and methods for expedited reporting of adverse events (AEs) are outlined in Version 2.0 of the DAIDS EAE Manual, which is available on the RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/manual-expedited-reporting-adverse-events-daids.

The DAIDS Adverse Experience Reporting System (DAERS), an internet-based reporting system, must be used for EAE reporting to DAIDS. In the event of system outages or technical difficulties, EAEs may be submitted via the DAIDS EAE Form. This form is available on the DAIDS RSC website at https://rsc.niaid.nih.gov/clinical-research-sites/paper-eae-reporting

For questions about DAERS, please contact NIAID Clinical Research Management System Support via email at CRMSsupport@niaid.nih.gov. Site queries may also be sent from within the DAERS application itself.

For questions about expedited reporting, please contact DAIDS RSC Safety Office via email at DAIDSRSCSafetyOffice@tech-res.com.

7.3.2 EAE Reporting Requirements for this Study

- EAEs will be reported only for maternal and infant participants in this study who are exposed to study drug.

- The serious adverse event (SAE) reporting category, as defined in Version 2.0 of the DAIDS EAE Manual, will be used for this study.

- The study agent for which expedited reporting is required is FTC/TDF.

7.3.3 Grading Severity of Events (applies to EAEs and all other adverse events)

The Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Corrected Version 2.1, dated July 2017 will be used in this study. This table is available on the RSC website at: https://rsc.niaid.nih.gov/clinical-research-sites/daids-adverse-event-grading-tables. For maternal participants in the PrEP Comparison Component, grading of creatinine and CrCl should be based on absolute value only, not on percentage change from baseline. In addition, the following protocol-specific grading scheme for axillary-measured fever will be used in this study:

Grade 1 37.4 to <38.0˚C
Grade 2 38.0 to <38.7˚C
Grade 3 38.7 to <39.4˚C
Grade 4 ≥39.4˚C

Note: The DAIDS AE Grading Table parameter for unintentional weight loss excludes postpartum weight loss. Therefore, maternal weight loss will not be graded in this study.

7.3.4 EAE Reporting Period

For maternal and infant participants exposed the study drug, the EAE reporting period begins at first exposure to study drug and ends on the date of last exposure to study drug.

For mothers, exposure to study drug is based on ingestion of FTC/TDF. The EAE reporting period begins on the day of first FTC/TDF tablet ingestion and ends on the last day of FTC/TDF tablet ingestion.

For infants, exposure to study drug may occur *in utero* and/or through breast milk while the mother is taking FTC/TDF. The EAE reporting period begins on the day of the first exposure *in utero* or through breast milk and ends after the day of last exposure *in utero* or through breast milk. For example, for an infant whose mother takes FTC/TDF during pregnancy but not postpartum, the infant's EAE reporting period would begin on the day of the mother's first FTC/TDF tablet ingestion during pregnancy and ends after delivery. For an infant, whose mother takes FTC/TDF during pregnancy and postpartum, and breastfeeds the infant, the infant's EAE reporting period would begin on the day of the mother's first FTC/TDF tablet ingestion during pregnancy and end after the mother's date of last FTC/TDF tablet ingestion.

After the above-specified period, only suspected, unexpected, serious adverse reactions (SUSARs), as defined in Version 2.0 of the DAIDS EAE Manual, will be reported to DAIDS if the study staff become aware of such events on a passive basis (e.g., from publicly available information).

8 PARTICIPANT MANAGEMENT

8.1 Maternal and Infant Adverse Events

All maternal and infant adverse events identified in this study will be source documented in participant research records, consistent with the policies and procedures referenced in Section 11. Among other details, source documentation will include the severity of each event (graded as

described in Section 7.3.3) and its relationship to study drug assessed by the site clinician according to the following categories and definitions:

Related: There is a reasonable possibility that the AE may be related to FTC/TDF

Not related: There is not a reasonable possibility that the AE is related to FTC/TDF

Maternal participants who choose to take PrEP are considered exposed to study drug immediately after initiation of dosing through ingestion of the last dose of study drug. Infant participants are considered exposed to study drug *in utero* and through breast milk, if their mothers choose to take PrEP and to breastfeed. Infant exposure begins immediately after initiation of maternal dosing and ceases following ingestion of the last maternal dose of study drug or breast milk feed (or after birth, if not breastfed).

All adverse events must be followed to resolution (return to baseline) or stabilization, with the frequency of repeat evaluations determined by the clinical significance of each event. Additional evaluations beyond those listed in the Schedule of Evaluations may be performed at the discretion of the site investigator to determine the etiology of a given event and/or further assess its severity or relationship to study drug. Clinical management of all adverse events should be provided consistent with the best medical judgment of the site investigator and local clinical practice standards.

8.1.1 Management of Maternal Adverse Events

Maternal adverse events will be managed based on their severity and assessed relationship to study drug. Refer to management table in Appendix V for detailed guidance on management of reduced creatinine clearance. All other adverse events should be managed as shown in Appendix IV for general toxicities. Site investigators will consult with the CMC as directed in the Maternal Toxicity Management Tables in Appendices IV and V and otherwise at their discretion as needed. The protocol Clinical Management Committee (CMC) will be notified of all events resulting in a change in the administration of the study drug. Clinical or laboratory AEs that are not related to FTC/TDF need not result in study drug interruption, unless the site investigator deems interruption necessary due to the specific circumstances. All maternal participants will remain on study and complete all follow-up visits, even if FTC/TDF is discontinued early due to toxicity or other reasons. Maternal participants with an ongoing AE at the time of the End of Study visit of Grade 3 or higher will continue to be followed until resolution or stabilization of the event. The CMC may request additional follow-up of selected AEs of lower grade based on the clinical context.

For maternal participants with other ongoing adverse events at End of Study visit, sites will continue to manage these conditions per local guidelines until the participant has been transitioned to a non-study primary care provider for continued monitoring, care and treatment. For adverse events of any severity assessed to be related to study-supplied study drug FTC/TDF that are ongoing at week 26 postpartum, sites will inform the CMC of their plan for continued clinical management

8.1.2 Compromised Renal Function

Further action is required if reduced creatinine clearance is recorded at any time during follow-up to evaluate potential compromise of a maternal participant's renal function. Appendix V provides general guidance for management of the FTC/TDF in response to reduced creatinine clearance.

8.1.3 Maternal Bone Mineral Density Concerns

There is no clarity on which bone density normative ranges should apply to women in Sub-Saharan Africa, given that available data suggests American Caucasian reference ranges are not appropriate and there are no accepted normative ranges for the region. Additionally, the degree of bone lost during periods of lactation is highly variable and maternal participants are likely to continue lactating for several months' post study exit. Thus, bone density scans conducted in this setting after delivery and prior to breastfeeding cessation are difficult to interpret clinically for the individual woman. Hence, maternal DXA scan data obtained in this study will only be of use for research purposes alone. The site investigator may specifically request these data for an individual participant at their discretion.

8.1.4 Infant Bone Mineral Density Concerns

There are no normal ranges for infant bone mineral content in current use. Infant DXA scan data obtained for this study are for research purposes alone and are not readily interpreted for clinical use. If normal values for infants become established while implementing this protocol, the DXA results will be communicated to the site investigator who will use his or her clinical judgment regarding medical consultation and referral.

8.2 Management of Participants Who Become Pregnant on Study

Pregnancy testing will be performed according to the relevant Schedule of Evaluations and when clinically indicated at any time during maternal follow-up and participants will be provided with contraception counseling per local standards of care, see Section 3.3.

Participants enrolled in Group 2 of the PK Component or in the PrEP Comparison Component who become pregnant during the study will be maintained in follow-up through scheduled study exit with no change to their study drug regimen. When the new pregnancy is identified, participants in Cohort 2 of the PrEP Comparison Component will be offered PrEP; if they choose to start PrEP, they will enter Cohort 2/Step 2 (see Section 5.1). Given that these participants will exit the study prior to delivery, those receiving PrEP will be referred to standard of care maternity services and efforts will be made to transition participants to locally available sources of PrEP to avoid gaps in PrEP coverage.

Participants who are pregnant at the time of study exit, will be contacted by study staff to ascertain their pregnancy outcomes (completion/termination of the pregnancy) and this information will be entered onto eCRFs. Outcomes may be ascertained based on participant report but medical records should be obtained whenever possible to supplement these reports.

8.3 Criteria for Premature Discontinuation of Study Drug

Study drug will be permanently discontinued before study exit in the following scenarios:

- Confirmed maternal HIV infection
- Confirmed maternal Grade 4 laboratory abnormality related to study drug
- Confirmed maternal Grade 3 laboratory abnormality related to study drug that does not resolve to Grade 2 or lower within 14 days of study drug hold
- Confirmed maternal Grade 3 or higher laboratory abnormality or clinical AE that recurs during re-challenge with the study drug after a hold
- Confirmed maternal creatinine clearance <50mL/minute

9 STATISTICAL CONSIDERATIONS

9.1 PK Component

9.1.1 General Design Issues-PK Component

The IMPAACT 2009 PK Component aims to establish drug thresholds for adequate adherence in pregnancy and postpartum when FTC/TDF is administered daily during pregnancy and in the postpartum period under directly observed conditions. All maternal participants will be administered once daily FTC/TDF. All doses will be directly observed for the 12-week duration. DBS specimens will be collected at weekly intervals for analysis. Approximately 40 maternal participants will be recruited to obtain at least 30 evaluable maternal participants with 20 participants who are recruited in the antepartum group (14-24 weeks of gestation), and 20 who are recruited in the postpartum group (6-12 weeks postpartum). A maternal participant is considered evaluable if she fulfils the following criteria; a) completes PK Component follow-up, b) has adequate documentation that PrEP doses were directly observed, c) has demonstrated adequate adherence to PrEP, defined as no more than 12 total directly observed doses missed over 12 weeks (maternal participants with more than three consecutively missed doses will be reviewed by the CMC to determine evaluability), and (d) had adequate DBS samples shipped to the PK testing laboratory, defined as no more than one missed specimen in any given two-week interval over 12 weeks with a minimum of seven samples correctly collected, processed, handled and shipped. The rationale for this design is to collect as many weekly measurements as feasible to enable the most robust estimates, but also to allow flexibility for occasional missed visits and doses to enable practicability for participants and sites.

9.1.2 Outcome Measures-PK Component

9.1.2.1 Primary Outcome Measure

- Steady state TFV-DP concentrations at Week 12.

9.1.2.2 Secondary Outcome Measures

- Steady state TFV-DP concentrations at Week 12.

9.1.3 Randomization and Stratification-PK Component

There is no randomization in this study. The enrollment will be stratified by:

- Group 1 (Antepartum women 14-24 weeks' gestation) vs. Group 2 (Postpartum women who delivered 6-12 weeks prior to enrollment)

9.1.4 Sample Size and Accrual-PK Component

An important objective of the PK Component is to determine the TFV-DP concentration corresponding to optimal adherence. We will estimate the 25th percentile of the TFV-DP distribution in each of the two groups. Table 2 shows estimated 95% CIs around the 25th percentile based on the normal distribution assumption at various sample sizes. With a sample size of 15 per group, the bounds of the 95% confidence interval are the 12th and 43rd percentile.

Table 2: 95% Confidence Intervals of Estimated 25th percentile of TFV-DP Steady State Concentration (Css)

Sample Size per Group	95% Confidence Interval (percentile)
15	(12th, 43th)
20	(13th, 41th)
30	(15th, 38th)
50	(17th, 35th)
80	(18.5th, 33th)
100	(19th, 32th)

Table 3 shows the precision of the estimate of minimum detectable difference in means for comparisons of mean TFV-DP steady state concentrations in DBS between the antepartum and postpartum group, given different sample sizes per group, alpha 0.05 using a two sample t-test. For example, with a sample size of 15 per group the half width of the 95% confidence interval around the difference in means is .75 of the standard deviation.

Table 3: Precision of Estimated Differences in Mean Css in DBS: PK Component

Sample Size per Group	Precision of estimated difference in mean Css (between AP and PP group)
20	**271** (64% of SD*)
18	**287** (68% of SD)
15 (protocol targeted)	**316** (75% of SD)
12	**358** (85% of SD)
10	**397** (94% of SD)

*SD=423 (*DOT-DBS Study, CROI 2017)*

9.1.5 Monitoring-PK Component

Reports summarizing data on accrual, missed weekly visits, missed PrEP doses, doses with inadequate source documentation of direct observation, number and adequacy of DBS specimens reaching the testing laboratory, mode of delivery, and safety and tolerability of FTC/TDF will be compiled by the Data Management Center (DMC) and reviewed and discussed by the CMC. Reports will be sent at least monthly. Adverse events will be monitored throughout the follow-up period. If the CMC identifies any potentially treatment-related toxicities, which may compromise participant safety, it will determine whether the study needs to be suspended or modified**.** Refer to Section 9.3 for details on monitoring by the CMC and Safety Monitoring Committee (SMC).

9.1.6 Analysis-PK Component

Prior to conducting analyses for the PK Component, the CMC (to include a minimum of the Protocol Epidemiologist and/or Statistician and the Pharmacologist), will review the pattern of completeness of each maternal participant's TFV-DP concentration data to determine suitability for inclusion in the analysis. Weekly TFV-DP concentration levels in DBS will be plotted for each maternal participant. In each of the two groups (antepartum and postpartum groups), the distributions of steady state concentration (Css) defined as the TFV-DP concentration observed at Week 12 corresponding to 6-7 day/week adherence ("excellent adherence"), will be summarized. Estimates of median, IQR, mean, standard deviation, and 95% confidence intervals will be calculated for each of the two groups. Differences in mean Css between the antepartum and postpartum groups will be evaluated using two sample t tests.

Estimated concentrations corresponding to numbers of doses/week (or percentages of doses taken) in the antepartum and postpartum periods will be derived from the two measures above (antepartum Css and postpartum Css after 12 weeks of daily dosing), assuming dose proportionality (in cases that doses were missed). The drug threshold corresponding to "excellent adherence" will be defined as TFV-DP levels meeting or exceeding the 25th percentile of the distribution of TFV-DP concentrations at Week 12 of the PK Component. "Excellent adherence" is defined as the TFV-DP concentration corresponding to 6/7 doses per week on average, based on Cottrell et al who modeled that at least 6/7 doses per week is needed for adequate drug concentrations in women. The PK testing laboratory will provide sites with a drug level result and reference ranges as it relates to the categorized level of adherence to use during adherence counselling sessions (24). The reference ranges will include approximately three adherence categories, aligned with other similar studies in the field. For example, excellent adherence (equivalent to six to seven doses per week), good adherence with room for improvement (four to five doses per week), poor adherence (one to three doses per week), and not taking any doses (undetected TFV-DP levels).

If in the PK Component, TFV-DP variability at Week 12 appears high, this will be discussed among the CMC. For example, the team will decide if the threshold corresponding to adequate adherence should be set at the median or a different percentile instead of the 25th percentile.

In addition, antepartum and postpartum TFV-DP concentration thresholds corresponding to "excellent adherence" and the adherence categories for Weeks 4, 8 of the PrEP Comparison Component will be derived using the methods described above but from the distributions observed at Weeks 4 and 8 of the PK Component. For additional details on PK data analysis please refer to Section 10.2.

9.1.7 Decision to open PrEP Comparison Component

The CMC will use the PK data (see Section 9.1.6) and monitoring reports (see Section 9.1.5) to come to a decision regarding whether PK Component data are sufficient to recommend opening the PrEP Comparison Component. The CMC will then convene to review the matrix and present the decision to the SMC whether or not to open enrollment to the PrEP Comparison Component according to Table 4. The decision will be communicated to study staff by way of a notification which will include detail of the adherence target identified in the PK Component.

Table 4: Criteria to open enrollment to the PrEP Comparison Component

	Acceptable	Requires CMC review group discussion
Evaluable Maternal Participants	15 or more in each Group in the PK Component who meet the evaluability criteria set out in Section 9.1.1	14 or less in any Group in the PK Component who meet the evaluability criteria set out in Section 9.1.1
PK Assay	15 or more maternal participants in each Group in the PK Component with valid and complete TVF-DP concentration data	
Css	Distribution of steady state concentration data available	
Target Threshold	Defined for both Groups in the PK Component	Not defined or defined for only one Group in the PK Component

9.2 PrEP Comparison Component

Primary outcome measures listed below will be addressed in the study's primary Statistical Analysis Plan, which will define the content of the Primary Analysis Report. This report will form the basis for the primary study manuscript and results reporting to ClinicalTrials.gov. Outcomes of interest for additional objectives intended for subsequent publications are listed under "Other" and "Exploratory" Outcome Measures.

9.2.1 General Design Issues: PrEP Comparison Component

The IMPAACT 2009 PrEP Comparison Component is a prospective non-randomized open label study. The objectives are to 1) estimate and describe PrEP adherence during pregnancy and post-partum among HIV-uninfected adolescent and young adult women (aged 16-24 years) who initiate once-daily FTC/TDF in pregnancy and are followed through 26 weeks postpartum when offered adherence support through mobile technology and drug level-directed counseling 2) compare maternal and infant adverse events (including pregnancy outcomes) at delivery and Week 26 postpartum between women who initiate PrEP and women who decline PrEP over the observation period but receive HIV risk reduction and SMS interventions.

Two important design issues regarding the PrEP Comparison Component are as follows: 1) Assignment to each of the two cohorts is determined by maternal participant choice rather than by randomization. The team agreed that it would be unethical to deny women at high risk of acquiring HIV infection the opportunity to use PrEP, given the mounting evidence that PrEP use is an HIV prevention strategy with proven effectiveness. Due to the non-randomized design, potential confounders of the association between PrEP use and adverse pregnancy or safety outcomes may be distributed differently in the two groups, introducing bias. For example, HIV-uninfected women in serodiscordant relationships may be more willing to initiate PrEP and may also be at higher risk of adverse pregnancy outcomes than women with partners of known HIV-uninfected status. To address the first challenge, potential confounders at entry and follow up will be measured and adjusted for in multivariable models. An important limitation is that particularly for endpoints with low event rates, the small sample size will limit the number of confounders that can be included in multivariable models. 2) A second important design issue is that statistical power is limited for making conclusions regarding the safety of PrEP in pregnancy or postpartum. This study will yield estimates of the incidence and prevalence of selected safety outcomes in each of the two groups, with 95% confidence intervals for descriptive purposes. The study allows adequate power to detect large cohort differences, depending on the true background rate of the event.

9.2.2 Outcome Measures: PrEP Comparison Component

9.2.2.1 Primary Outcome Measures

Table 5 summarizes the primary outcome measures for each study objective of the PrEP Comparison Component:

Table 5: Primary Outcome Measures – PrEP Comparison Component

Primary Outcome Measures – PrEP Comparison Component	
Objective	**Outcome Measures**
2.3.1	**PrEP adherence**: TFV-DP drug concentration level in Dried Blood Spots (DBS)
2.3.2	**Maternal Adverse Events:** • Maternal Grade 3 or higher adverse events (signs, symptoms, labs, and diagnoses) • Maternal Grade 2 or higher chemistry abnormalities
2.3.2	**Adverse Pregnancy Outcomes:** Composite outcome of • Spontaneous abortion (occurring at <20 weeks gestation) • Stillbirth (occurring at ≥20 weeks gestation) • Preterm delivery (<37 completed weeks' gestation) • Small for gestational age (<10th percentile using **INTERGROWTH-21st** norms and ultrasound derived gestational age at delivery) **Infant Safety:** • Infant death within the first 26 weeks of life • Infant Grade 3 or higher adverse events (signs, symptoms, labs, diagnoses) reported between birth and exit • Infant bone mineral content based on DXA scan of the whole body (WB-BMC) at birth and lumbar spine (LS-BMC) at birth and 26 weeks postpartum • Infant creatinine and CrCl rate at birth and 26 weeks postpartum • Infant length for age z-score at birth and exit

9.2.2.2 Other Outcome Measures

Table 6 summarizes the other outcome measures for each study objective of the PrEP Comparison Component:

Table 6: Other Outcome Measures – PrEP Comparison Component

Other Measures – PrEP Comparison Component	
Objective	**Outcome Measure**
2.4.1	**PrEP adherence:** TFV-DP drug concentration level in DBS
2.4.2	**Sexual risk behavior** • Any condomless vaginal or anal sex • Incident sexually transmitted infection defined as any new HSV, syphilis, chlamydia or gonorrhea infections during follow-up • Incident HIV infection
2.4.3	• Antiretroviral drug resistance: presence of virus resistant to TDF at time of HIV diagnosis
2.4.4	**Bone mineral density** – Mean maternal bone mineral density (g/m2) based on DXA scan of the lumbar spine (LS-BMD) and hip (H-BMD) at delivery and 26 weeks postpartum

9.2.2.3 Exploratory Outcome Measures

Table 7 summarizes the exploratory outcome measures for each study objective of the PrEP Comparison Component:

Table 7: Exploratory Outcome Measures – PrEP Comparison Component

Exploratory Outcome Measures – PrEP Comparison Component	
Objective	**Outcome Measures**
2.5.1	**Microbiome – PrEP or no PrEP exposure** • Compare the maternal vaginal/rectal microbiome, differences in diversity (continuous), bacterial composition (continuous) and relationship to blood and cervicovaginal drug levels (continuous) longitudinally at study entry, late pregnancy, six weeks post-partum and 26 weeks post-partum • Compare the infant microbiome differences in diversity and bacterial composition initially at six weeks post-partum and 26 weeks post-partum

9.2.3 Randomization and Stratification

There will be no randomization for this study. To monitor the balance of the maternal participants enrolled among various groups, the enrollment will be stratified by:

- Cohort: PrEP vs. No PrEP at enrollment
- Trimester of enrollment during pregnancy: 2nd trimester vs. 3rd trimester
- Country
- Age at enrollment: 16-17 years vs. 18 years of age and older

Stratifying by the above factors in this non-randomized study is for the purpose of monitoring the accrual to ensure that there are not large differences between the two cohorts (PrEP and No PrEP) in the proportion of women who are 16-17 years old, who are from a specific country, or who enroll in the 2nd (vs. 3rd) trimester of pregnancy, and to ensure a proportion of enrolled women are in the 16-17 year old age group.

9.2.4 Sample Size and Accrual

The PrEP Comparison Component will enroll a total of 350 (233 in Cohort 1 and 117 in Cohort 2) women over a 12-month period. Women will be followed through 26 weeks postpartum. Expected duration of follow-up for a given maternal participant is between 7 and 12 months depending on the gestational age of the pregnancy at enrollment. The following sample size and power calculations assume a final analysis sample of 300 women (200 in Cohort 1 and 100 in Cohort 2). The primary reason for the 2:1 enrollment into the two cohorts was to balance the need for a "No PrEP" comparison group (to provide the background rates for safety outcome measures) with the need for a large enough PrEP group to examine outcomes that would be restricted to women in that group (Adherence). To achieve this sample size at the analysis phase, this study will enroll ~350 women to accommodate a decrease of ~15% due to maternal attrition.

Table 8 shows the expected precision of a range of PrEP adherence estimates across different sample sizes in Cohort 1. For example, with a sample size of 200 women in Cohort 1, the width of the 95% confidence interval for an estimated adherence rate of 75% is 13%.

Table 8: Precision (shown as 2-sided 95% confidence intervals) of different estimates of the proportion with good adherence (for illustration, defined as meeting or exceeding the threshold corresponding to protective drug levels of TFV-DP) at different sample sizes for Cohort 1.

	Sample Size in Cohort 1			
% Adherent	150	200	250	300
50	42-58%	43-57%	44-56%	44-56%
60	52-68%	53-67%	54-66%	54-66%
65	57-73%	58-72%	59-71%	59-70%
70	62-77%	63-76%	64-76%	65-75%
75	67-82%	68-81%	69-80%	70-80%
80	73-82%	74-85%	75-85%	75-84%

Table 9 below shows the precision of estimates of safety outcomes (e.g. preterm birth) expected with a range of event rates and a sample size of 200 women in Cohort 1 and 100 women in Cohort 2. It is expected that the rates of these outcomes will be low particularly since this is a young group of healthy HIV uninfected women. To that end, a range of AE rates with corresponding confidence intervals is included in the protocol.

Table 9: 95% Confidence Intervals for different possible event rates of safety outcomes in Cohort 1 (n=200) and Cohort 2 (n=100)

Event Rate	95% Confidence Interval Cohort 1 (n=200)	95% Confidence Interval Cohort 2 (n=100)
0.5%	(0, 2.8)	(0, 3.6)
2.0%	(0.5, 5.0)	(0.2, 7.0)
4.0%	(1.7, 7.7)	(1.1, 9.9)
10.0%	(6.2, 15.0)	(4.9, 17.6)
15.0%	(10.4, 20.7)	(8.6, 23.5)
20.0%	(14.7, 26.2)	(12.7, 29.2)
30.0%	(23.7, 36.9)	(21.2, 40.0)

The study allows adequate power to detect moderate cohort differences from 8.5% to 16%, depending on the true background rate of the event, as summarized below in Table 10. The table shows minimum detectable differences in safety outcomes between Cohort 1 and Cohort 2 which could be achieved with 80% power and alpha=0.05. For example, based on a sample size of 200 in Cohort 1, and assuming a 2:1 enrollment among women agreeing to and declining PrEP, differences of at least 13% will be detectable between the two cohorts in the proportion of maternal adverse events for events with a 10% background rate (10% in Cohort 2, 23% in Cohort 1).

Table 10: Minimum detectable differences in safety outcomes based on evaluable sample size of 300 for Cohort 1(n=200) and Cohort 2 (n=100), 80% power, alpha=0.05

% with event in Cohort 2 (N=100)	% with event in Cohort 1 (N=200)	Odds Ratio
2%	10.5%	5.8
5%	15.6%	3.5
10%	23%	2.67
15%	29%	2.36
20%	35%	2.19
25%	41%	2.10

To address primary objective 2.3.2, the mean bone mineral content of infants at birth and at age 26 weeks will be estimated and differences in mean bone mineral content between infants in Cohort 1 and Cohort 2 will be evaluated using two sample t tests. Table 11 shows the statistical

power the study would have to detect a range of estimated differences in means. For example, with a sample size of 200 in Cohort 1 and 100 in Cohort 2 this study will be able to detect differences in mean bone mineral content of at least 0.35 SD with 80% power. Based on the SMARTT study in the U.S., assuming a standard deviation of 16.6 (77) this would be equivalent to a difference in means of 5.81g. Assuming a standard deviation of 14.9 found in the P1084s study (79) this would be equivalent to a difference in means of 5.22 g.

Table 11: Estimated Power to Detect the Mean Differences in Infant Growth Outcomes Infant DXA, Whole Body Bone Mineral Content (BMC) (g) at Birth (Mean=63.8, SD=16.6)**

Difference to be Detected	Effect Size (SD)	**Power** (based on sample sizes*)		
		N1=200 N2=100	**N1=180 N2=90**	**N1=160 N2=80**
4% (of 63.8)	2.55 (16.6)	24%	22%	20%
5%	3.19	35%	32%	29%
6%	3.83	47%	43%	39%
7%	4.47	59%	55%	50%
8%	5.10	71%	66%	61%
9%	5.74	80%	76%	71%
10%	6.38	88%	84%	80%
0.25*SD	4.15	53%	49%	44%
0.30*SD	4.98	68%	64%	59%
0.35*SD	5.81	81%	77%	72%
0.40*SD	6.64	90%	87%	83%
0.45*SD	7.47	96%	94%	91%
0.50*SD	8.30	98%	97%	95%
0.75*SD	12.45	> 99%	> 99%	> 99%

*Sample Sizes: **N1 --** Cohort 1 (PrEP), N2 – Cohort 2 **(**no PrEP)
**Data were from PHACS SMARTT Study (Siberry, 2015)

To address other objective 2.4.4, the mean bone mineral density of the lumbar spine and of the hip of women at delivery and at 26 weeks post-delivery will be estimated and differences in mean bone mineral density between women in Cohort 1 and Cohort 2 will be evaluated using two sample t tests. Table 12 shows estimated statistical power to detect a range in differences in mean maternal bone mineral density that the study could detect given different sample sizes. For example, with a sample size of 300 (200 PrEP and 100 no PrEP) the study would have at least 80% power to detect a minimum 4% difference in mean BMD and .35 SD difference in means between groups at delivery and 26 weeks postpartum.

Table 12: Estimated Power to Detect the Mean Differences in Maternal Growth Outcomes Maternal DXA, Whole Body Bone Mineral Content (BMC) (g) (Mean=0.979, SD=0.111)**

BMD	Difference to be Detected	Effect Size (SD*)	Power (based on sample sizes**)		
			N1=200 N2=100	N1=180 N2=90	N1=160 N2=80
Lumbar Spine	1% (of 0.979*)	0.0098 (0.111*)	11%	10%	10%
	2%	0.0196	30%	28%	25%
	3%	0.0294	58%	53%	49%
	4%	0.0392	82%	78%	73%
	5%	0.0490	95%	93%	89%
	6%	0.0587	99%	98%	97%
	0.10*SD	0.0111	13%	12%	11%
	0.15*SD	0.0167	23%	21%	19%
	0.20*SD	0.0222	37%	34%	31%
	0.25*SD	0.0278	53%	49%	44%
	0.30*SD	0.0333	69%	64%	59%
	0.35*SD	0.0389	81%	77%	72%
	0.40*SD	0.0444	90%	87%	83%
Total Hip	1% (of 0.968*)	0.0097 (0.119*)	10%	10%	9%
	2%	0.0194	26%	24%	22%
	3%	0.0290	51%	47%	43%
	4%	0.0387	75%	71%	66%
	5%	0.0484	91%	88%	84%
	6%	0.0581	98%	97%	94%
	0.10*SD	0.0119	13%	12%	11%
	0.15*SD	0.0179	23%	21%	19%
	0.20*SD	0.0238	37%	34%	31%
	0.25*SD	0.0298	53%	49%	44%
	0.30*SD	0.0357	68%	64%	59%
	0.35*SD	0.0417	81%	77%	72%
	0.40*SD	0.0476	90%	87%	83%

*Data were from JAIDS (Mirembe, 2015): mean (SD) =0.979 (0.111) for lumbar spine; mean (SD) = 0.968 (0.119) for total hip.
Sample Sizes: **N1 -- PrEP Arm, **N2** -- non-PrEP Arm

9.2.5 Monitoring – PrEP Comparison Component

Since this study is not randomized, some potential concerns are differential enrollment into the two cohorts, either by country, gestational period of enrollment, or in the proportion of women in the youngest age group (16-17 years old). Large differences in cohort enrollment – by country, age or by gestational period of enrollment could introduce bias or make some analyses no longer feasible. To manage those potential risks, the balance between Cohorts 1 and 2 during enrollment at each site will be monitored by the CMC and active measures undertaken to minimize such biases. The accruals and updated status of women in Cohort 1 and Cohort 2 will be closely monitored and reported regularly to participating sites for each site and across all sites to guide

the recruitment strategies. If the CMC observes significant divergence between cohorts in these enrollment characteristics then the team will discuss and agree on plans for notifying sites to adopt remedial measures, which may include curtailing enrollment to the Cohort more commonly chosen while enrollment into the other Cohort proceeds.

9.2.6 Analyses-PrEP Comparison Component

The primary analyses to address the objectives of the PrEP Comparison Component are outlined below. For a more detailed description of the analyses to be conducted, please refer to the Statistical Analysis Plan.

Descriptive summaries:

Entry demographic, pregnancy, pregnancy history, psychosocial, sexual behavior and partner characteristics of women in Cohort 1 and women in Cohort 2 will be summarized and compared using chi square tests (or Fishers exact test as appropriate) for discrete variables and t tests or Wilcoxon rank sum tests for continuous variables. Logistic regression models will be fit to identify predictors of PrEP initiation.

Primary Objective 2.3.1:

- Women in Cohort 1 will comprise the analysis sample. A binary indicator, "optimal adherence" will be created, as defined above as TFV-DP levels in DBS meeting or exceeding the threshold determined in the PK Component as corresponding to protective levels of PrEP (24). At each visit that TFV-DP levels are obtained (see Appendix IIA), the proportion of women with optimal adherence will be calculated based on their TFV-DP levels at each visit. The proportion of visits with optimal adherence in the pregnancy vs. postpartum periods will be calculated and compared.

- Exploratory analyses identifying baseline and time-varying predictors of optimal adherence will be conducted with multivariable regression models using generalized estimating equations with a logistic link (to account for repeated measures across time for each participant).

- Adherence (as measured in TFV-DP levels) will also be summarized as a continuous outcome (median, IQR and mean [SD]) at each time point noted above when drug levels are measured, and "average" adherence across pregnancy and postpartum periods, will be calculated, using generalized estimating equations. We will focus on periods during which the participant has fomally elected to be on PrEP. If a woman elects to formally stop PrEP (by notifying study personnel), periods of being formally off study drug will not be considered in adherence calculations.

- Other approaches to summarizing the adherence outcome will be considered. This includes an ordinal variable with categories corresponding to different dosing levels. For this type of outcome, at each visit that drug levels are measured in DBS, descriptive analyses will summarize the proportion of women in each adherence category, refer to categories discussed in Section 9.1.6.

- Baseline characteristics, and patterns of adherence and duration on PrEP will be summarized separately for women in Cohort 2 who later decide to initiate PrEP using the approaches outlined above.

- The following additional descriptive statistics will be calculated: The proportion of women a) with at least one visit with TFV-DP levels corresponding to optimal adherence, b) who had TFV-DP levels corresponding to optimal adherence at all visits, c) who had no detectable TFV-DP levels at any visit.

Primary Objective 2.3.2:

Analyses of adverse pregnancy outcomes: The proportion of women in Cohort 1 and Cohort 2 with adverse pregnancy outcomes (a composite indicator defined as at least one of the following: spontaneous abortion (occurring at <20 weeks gestation), stillbirth (occurring at ≥20 weeks gestation), preterm delivery (<37 completed weeks gestation), or small for gestational age (<10th percentile using **INTERGROWTH-21st** norms and ultrasound (US)- derived gestational age at delivery) will be estimated and compared. Univariable and multivariable logistic regression methods will be used to evaluate associations of PrEP use and the composite outcome indicating presence vs. absence of any adverse pregnancy outcomes, controlling for potential confounders. The same analyses will be conducted for each of the pregnancy outcomes which comprise the composite outcome separately.

Analyses of maternal adverse events and infant adverse events: Cumulative rates of maternal adverse events and infant adverse events will be compared, respectively, in Cohort 1 and Cohort 2. Where appropriate, there will be stratification according to time period (i.e., antepartum (AP) and postpartum (PP), and within AP by gestational age category of enrollment). Maternal adverse events will be considered separately from infant adverse events. Cox proportional hazards regression will be used to evaluate differences in the rate of adverse events in the two cohorts. For the maternal adverse events, among women in Cohort 1, person time will begin accruing when PrEP is initiated and for women in Cohort 2 it will begin accruing after study entry. Rates of maternal and infant adverse events in AP and PP periods will also be calculated and compared across levels of PrEP adherence (based on TFV-DP levels in DBS). Models will be fit adjusting for potential confounders.

Analyses of infant safety outcomes re: bone health and creatinine clearance: To address Primary Objective 2.3.2, to evaluate infant safety outcomes, (CrCl measured by Schwartz equation at birth and 26 weeks anthropometric growth measures at birth and 26 weeks, bone mineral content measured by dual energy X-ray absorptiometry (DXA) at birth and 26 weeks), mean length-for-age z-scores for the growth parameters and mean grams for BMC will be calculated, and means will be compared between infants in Cohort 1 and infants in Cohort 2 using two sample t-tests at each time point of evaluation. If normality assumptions are not met the Wilcoxon Rank Sum test will be used to evaluate differences in these endpoints by Cohort.

Other Objective 2.4.1:

Exploratory analyses will be conducted to identify individual, social, and structural barriers and facilitators to PrEP uptake during pregnancy, and to adherence and continued use during pregnancy and breastfeeding. Women in both cohorts (Cohort 1 and Cohort 2) will be included in analyses of predictors of PrEP uptake at enrollment. Logistic regression methods will be used to evaluate univariate and multivariate associations of baseline demographic, psychosocial, sexual behavior and partner characteristics noted below with PrEP uptake and adherence.

Primary Covariates and Potential Confounders:

Behavioral and Contextual Factors (both cohorts)
demographic, psychosocial, sexual behavior and partner characteristics

- Sociodemographic characteristics
    - Current living situation
    - Household composition
    - Private space for product storage
- HIV risk perception for mother and infant
- HIV stigma
- PrEP stigma
- Sexual behaviors
- Substance use
- Mental health and post-traumatic stress symptoms
- Disclosure to peers, family members, teachers, and partner(s) about PrEP use and participation in the study
- Gender-based violence (GBV)
- Partnership characteristics: partner's HIV status

To evaluate factors associated with PrEP use, a similar approach will be used, but analyses will be restricted to Cohort 1 with the binary indicator of "optimal adherence" (based on TFV-DP levels) as the outcome.

To complement both exploratory analyses described above, key themes will be summarized from the qualitative data regarding how aspects of PrEP, women's perception of risk, the context of pregnancy, and interpersonal and structural influences contribute to PrEP uptake, use, and discontinuation. Additional details regarding the qualitative data analysis are at the end of this analysis section.

Other Objective 2.4.2:

Analyses to address this objective will include women in Cohort 1 and women in Cohort 2 in the analysis sample. Estimates of the proportion of women (at each visit) reporting condomless vaginal or anal sex at each visit that it is measured, and the proportion of visits with condomless vaginal or anal sex during pregnancy and during the postpartum period will be compared between Cohort 1 and Cohort 2 using chi square tests. To evaluate the association of Cohort (Cohort 1 vs. Cohort 2) with sexual risk behavior, GEE models will be fit, including adjustment for sociodemographic and behavioral factors that could confound the association of Cohort with risky sexual behavior. Cox regression methods will be used to estimate and compare the incidence rates of STIs in the antepartum period, the postpartum period, and throughout the study, among women with no STIs at enrollment in Cohort 1 and Cohort 2, adjusting for baseline confounders.
Cox regression methods will be used to estimate and compare HIV incidence rates in pregnant and breastfeeding women who have initiated PrEP, in comparison to women who decline PrEP during the antenatal period.

Other Objective 2.4.3:

Among women and infants who acquire HIV infection, the proportion with antiretroviral drug resistance to FTC/TDF-at the time of HIV diagnosis will be calculated. In addition, testing of entry specimens will be performed to see if resistance was transmitted or selected by the study drugs.

Other Objective 2.4.4:

Student t tests will be used to compare the mean maternal bone mineral density of the lumbar spine and of the hip for Cohort 1 and Cohort 2 at delivery and week 26 post-delivery.

Exploratory Objective 2.5.1:

The distribution of maternal microbiome parameters in the PrEP and no PrEP groups will be described at the maternal (vaginal, gut) and infant (gut) sites. The changes in these parameters from entry to delivery will be evaluated according to PrEP use, and the relationship between vaginal microbiota and drug (PrEP) levels via univariate and multivariate regression models will be assessed. The potential effect of other factors, including recent antibiotic use, contraception use (including methods), and mode of delivery (i.e., vaginal vs. Caesarean delivery) will also be assessed.

Qualitative Analysis

All interviews will be audio-recorded and transcribed and translated into English by trained staff at each respective study site. Qualitative interviewers will also complete a debriefing summary report to facilitate more rapid "real-time" summary and analysis of qualitative data prior to formal analysis of interview transcripts. Quality assurance of each transcript will be conducted at the site level and centrally (across sites), by listening to the digital recordings, comparing the content to that in the transcriptions, noting the tone of the maternal participant when appropriate, and making appropriate corrections. Sites will also be responsible for ensuring that transcriptions are appropriately stripped of identifying information before they are transmitted to the DMC.

A team composed of study staff and Protocol Team members with expertise in qualitative research will code and analyze the qualitative data using Atlas-ti or a comparable analysis software package. Since this study seeks to learn about the range of factors that impact young women's uptake of and adherence to PrEP, the qualitative analysis will utilize a psychological phenomenological framework. Phenomenology is specifically focused on describing what a given group of people have in common as they experience a particular phenomenon, and it is an inductive analytic approach that allows the patterns, themes, and categories of analysis to emerge from the data (63, 174). Given that influences on PrEP uptake may be different from influences on PrEP adherence, PrEP uptake and adherence among pregnant and post-partum women as separate (but related) phenomena will be investigated.

Data coding and analysis will be both iterative and interactive processes. The analysis team will first read all interview transcripts to increase familiarity with the data. Next, the team will assign *a priori* codes and create emergent codes. Transcripts will then be re-read to create pattern codes that connect subsequent concepts under larger headings. Consistent patterns in meaning, concepts, and themes across all interviews will be identified, and data matrices will be created as visual representations of the findings. Comparative analyses will be conducted to clarify differences that may exist for any subgroups of young women. Coding and analytic activities will

be discussed during qualitative data analysis meetings, and discrepancies in coding and interpretation will be resolved through consensus.

9.3 Monitoring

Implementation of this study will be monitored at multiple levels, consistent with standard IMPAACT procedures. A Study Progress, Safety, and Data Monitoring Plan (SPDSMP) that details monitoring roles and responsibilities and data to be reviewed at each level will be prepared before the study opens to accrual. Please refer to Sections 11 and 12 for more information on on-site monitoring and quality management at the site level. Further information on monitoring of study progress, quality of study conduct, and participant safety across sites is provided below.

9.3.1 Monitoring by the Protocol Team

The Protocol Team is responsible for continuous monitoring of study progress, including timely achievement of key milestones, and the quality of study conduct. As indicated in Sections 4.4 and 4.5, the Protocol Team will closely monitor participant accrual and retention based on reports that will be generated at least monthly by the SDMC. If accrual or retention rates fall below target, team members will work with study sites to identify operational issues or problems and to take appropriate action to address below-target rates.

Team members will similarly review other key indicators of the quality of study conduct (e.g., endpoint evaluability, data quality and completeness) based on reports generated by the SDMC and take action with study sites as needed to ensure high quality study conduct throughout the period of study implementation. As indicated in Section 5.1.1.1 the site staff will actively monitor the documentation of doses taken throughout the data collection period for the PK Component only. If the site staff identifies a participant having missed more than three consecutive doses, the Protocol Team will review the participant's data to determine if the participant may be considered evaluable.

9.3.2 Participant Safety

On behalf of the Protocol Team, the CMC will closely monitor participant safety through routine review of safety data reports generated by the SDMC. These reports will provide tabulations of adverse events (defined in Section 8.1) and social harms (refer to Sections 7.2 and 13.6) identified in enrolled participants, including abnormal laboratory test results, signs, symptoms, and diagnoses, pooled across arms. Further detail will be provided in the SPDSMP. The CMC will review these reports via conference call or other meeting at least monthly. Prior to each call, the DAIDS Medical Officer will review any AEs ≥Grade 3 and EAEs (defined in Section 7.3) reported to the DAIDS Safety Office that are not yet reflected in the data reports. The CMC will continually evaluate the pattern and frequency of reported events and assess for any individual occurrences or trends of concern.

9.3.3 Monitoring by the SMC

An independent IMPAACT Study Monitoring Committee (SMC) will review this study regularly, following policies described in the IMPAACT Manual of Procedures. The composition of the SMC will include the SMC Chair; IMPAACT Chair or Vice-Chair; IMPAACT Prevention Scientific representative; representatives of the IMPAACT Operations Center and Statistical and Data Management Center; and representatives of NIAID and NICHD.

SMC reviews will occur at least annually and on a more frequent or *ad hoc* basis if any safety issues or concerns arise. *Ad hoc* reviews may also be triggered per the safety monitoring guidelines specified below. Based on any of its reviews, the SMC may recommend that the study proceed as currently designed, proceed with design modifications, or be discontinued. The SMC may also provide operational recommendations to help address any study implementation challenges that may be identified during their reviews.

The SMC will monitor study progress, quality of study conduct, and participant safety. The SMC will generally review the same types of data reports as the Protocol Team and CMC. For *ad hoc* or triggered safety reviews, more limited data may be reviewed, focusing on the events that triggered the reviews.

Adverse events in both groups and proportion of women with Week 12 TFV-DP drug levels below the limit of quantification in Cohort 1 (as well as decisions to initiate PrEP later in the study among women in Cohort 2) will be monitored and summaries prepared at regular intervals for review by the SMC.

The following events will trigger a SMC review. These events and further details will be described in the SPDSMP.

Transition from PK Component to PrEP Comparison Component: Following completion of the PK Component, the CMC reviewed data from the PK Component (see Section 1.3.4), determined that the criteria outlined in protocol Section 9.1.7 had been met, and provided a recommendation to proceed to the PrEP Comparison Component to the SMC. The SMC reviewed the recommendation, which included the recommended TFV-DP threshold for adherence monitoring, and after consulting with the IMPAACT Scientific Leadership Group and convening reviews of the PK Component data by an independent PK Panel and independent Ethics review panel, determined that the protocol may begin enrollment into the PrEP Comparison Component.

PrEP Comparison Component:

- Monitoring the proportion of women in Cohort 1 without quantifiable levels of TFV-DP at Week 12 (excluding women who have decided to stop PrEP):
    - At any site, if this proportion at Week 12 is greater than 40% after 15 maternal participants in Cohort 1 have completed that study visit, the CMC will review the data and consult with site investigators, and make a recommendation to the SMC regarding the need for remedial actions or discontinuation of enrollment at the site. The SMC will convene to consider the CMC's recommendation.
- Monitoring participant safety outcomes (for women or infants in Cohort 1 and Cohort 2, Step 2). *Ad hoc* SMC reviews will be convened:
    - If the proportion of women experiencing Grade 3 or higher adverse events is greater than 15%, after 50 women have been enrolled
    - If the rate of adverse pregnancy outcomes is greater than 20%, after 25% of pregnancies have been completed.
    - If the proportion of livebirth infants experiencing Grade 3 or higher adverse events is greater than 15%, after 50 completed pregnancies resulting in live births.

Please refer to the SPDSMP for further details regarding the above conditions that would meet criteria for triggering an additional SMC review. The SMC will convene as part of its standard regularly scheduled review to review data from the PrEP Comparison Component.

10 CLINICAL PHARMACOLOGY PLAN

10.1 Pharmacology Overview and Objectives

PK Component: The primary objective is to determine TFV-DP thresholds associated with optimal adherence to FTC/TDF among women who initiate PrEP during pregnancy and postpartum.

PrEP Comparison Component: The primary objective is to determine TFV-DP to assess adherence to FTC/TDF among women who initiate PrEP.

10.2 Study Design, Modeling and Data Analysis

PK Component:

All maternal participants will be administered once daily FTC/TDF. All doses will be DOT for the 12-week duration. DBS specimens will be collected at weekly intervals for analysis.

The goal is to quantify steady state TFV-DP in DBS. Css (drug concentration at steady state) will be determined by visual inspection of the plateauing of concentrations on the concentration time curve. Additionally, the following expression will be fit to the concentration time data, $Ct=Css*(1-e^{-K*t})$, where Ct is the drug concentration at time t, Css is the fitted steady state drug concentration, K is the fitted first-order elimination rate constant. Half-life will be determined as ln(2)/K. This model assumes first-order pharmacokinetics, which appears to be followed by TFV-DP in DBS (175). In addition, all available drug concentration samples will be utilized in a population pharmacokinetic model, thereby accounting for possible non-linearity and missed doses due to protocol deviations. Predicted Css from modeling will be subjected to the same analyses as above.

PrEP Comparison Component:

All maternal participants will be prescribed once daily FTC/TDF. Visits will occur at Weeks 4, 8, 12 and then every 12 weeks (while pregnant) as well as the time of delivery, and 6, 14, and 26 weeks post-delivery. At each of these visits DBS specimens will be collected for analysis.

The goal for the PrEP Comparison Component is to use the expected TFV-DP levels in DBS from PK Component to define a threshold for optimal adherence. This threshold will be incorporated into the study-specific MOP for the PrEP Comparison Component. This threshold may be the 25^{th} percentile of six or more doses per week on average, which would be 6/7ths of expected level determined in the PK Component. The percentile used (i.e. 25^{th} percentile versus median, etc.) will be determined by the CMC team (described above) and will be based upon the variability in the TFV-DP concentrations in the PK component.

10.2.1 Number of Participants

PK Component:

Approximately 40 women will be enrolled to obtain at least 30 evaluable with 20 who are in Group 1, the antepartum period (14-24 weeks of gestation), and 20 in Group 2, the postpartum period (6-12 weeks postpartum).

PrEP Comparison Component:

Approximately 350 women will be enrolled to obtain at least 300 evaluable (200 Cohort 1 and 100 in Cohort 2).

10.2.2 Phlebotomy and PK Sampling

DBS will be collected by spotting EDTA blood from venipuncture (refer to the LPC for procedure).

10.2.3 Processing, Handling, and Storage of PK Specimens

Details regarding collection, processing, handling and storage of PK samples will be provided in the study-specific LPC.

10.2.4 Laboratory Performing the Assays

It is expected that the University of Cape Town Laboratory will test samples from both the PK Component and the PrEP Comparison Component to provide testing consistency between study components. In addition, the University of Colorado Antiviral Pharmacology Laboratory will co-assay approximately 50 samples from the PK Component to provide a quality assurance assessment.

11 DATA HANDLING AND RECORD KEEPING

11.1 Data Management Responsibilities

As described in Section 4.4, data on screening and enrollment in this study will be collected using the DMC Subject Enrollment System.

Study sites must maintain adequate and accurate research records containing all information pertinent to the study for all screened and enrolled mother-infant pairs, including paper-based CRFs (if used), eCRFs and supporting source data. In maintaining these records, sites must comply with the standards of source documentation specified in the DAIDS policy on Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (available on the web site referenced in Section 11.2). All DAIDS policies referenced in this section are available at:
https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures

eCRFs and an eCRF completion guide will be made available to study sites by the DMC. Study staff will enter required data into eCRFs, with system checks applied and data queries generated immediately upon saving the entered data. Data must be entered within the timeframes specified

by the DMC; queries must also be resolved in a timely manner. Selected laboratory data will be transferred electronically to the DMC through the LDMS.

Further information on eCRFs and IMPAACT data management procedures, will be provided by the DMC. A User Manual for the Subject Enrollment System is available on the DMC portal at www.fstrf.org.

11.2 Essential and Source Documents and Access to Source Data

All DAIDS policies referenced in this section are available at: https://www.niaid.nih.gov/sites/default/files/daids-sourcedocpolicy.pdf.

Study sites must comply with DAIDS policies on Requirements for Essential Documents at Clinical Research Sites Conducting DAIDS Funded and/or Sponsored Clinical Trials and Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. In its policy on Requirements for Manual of Operational Procedures, DAIDS requires sites to establish SOPs for maintaining essential and source documents in compliance with these policies. Site SOPs should be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study, and site SOPs should be followed throughout the study.

Per the DAIDS policy on Storage and Retention of Clinical Research Records, study records must be stored in a manner that ensures privacy, confidentiality, security, and accessibility during the conduct of the study and after the study is completed. Records must be retained for a minimum of three years after the completion of the study. Per 21 CFR 312.62, records must be maintained for two years after the date a marketing application is approved for the study drug for the indication for which it is evaluated in this study; or, if no application is filed, or if the application is not approved for this indication, records must be retained two years after the study is discontinued and the FDA is notified.

All study records must be accessible for inspection, monitoring, and/or auditing during and after the conduct of the study by authorized representatives of the study sponsors and their contracted monitors, IMPAACT, Gilead Sciences, the FDA, site drug regulatory authorities, site IRBs/ECs, OHRP, and other U.S., local, and international regulatory entities. Records must be kept on-site throughout the period of study implementation; thereafter, instructions for off-site storage may be provided by NIAID. No study records may be removed to an off-site location or destroyed prior to receiving approval from NIAID.

11.3 Quality Control and Quality Assurance

Study sites must ensure that essential documents and participant research records are subject to continuous quality control and quality assurance procedures consistent with the DAIDS policy on Requirements for Clinical Quality Management Plans, which is available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

12 CLINICAL SITE MONITORING

Site monitors under contract to NIAID will visit study sites to inspect study facilities and review participant study records including consent forms, eCRFs, medical records, laboratory records, and pharmacy records, to ensure protection of study participants, compliance with the IRB/EC approved protocol, and accuracy and completeness of records. The monitors also will review essential document files to ensure compliance with all applicable regulatory requirements. Site investigators will make study facilities and documents available for inspection by the monitors.

13 HUMAN SUBJECTS PROTECTIONS

13.1 Institutional Review Board/Ethics Committee Review and Approval

Prior to study initiation, site investigators must obtain IRB/EC review and approval of this protocol and site-specific ICFs in accordance with 45 CRF 46; subsequent to initial review and approval, IRBs/ECs must review the study at least annually. Site investigators must also promptly report to the IRB/EC any changes in the study and any unanticipated problems involving risks to participants or others.

All IRB/EC policies and procedures must be followed and complete documentation of all correspondence to and from the IRBs/ECs must be maintained in site essential document files. Sites must submit documentation of both initial review and approval and continuing review to the DAIDS Protocol Registration Office (PRO) in accordance with the DAIDS Protocol Registration Manual (see also Section 14.2).

13.2 Vulnerable Participants

The NIH is mandated by law to ensure that pregnant women and children be included in clinical research when appropriate (176, 177). This study responds to that mandate and will provide clinical research data to inform PrEP guidelines for pregnant and lactating women. Nonetheless, the pregnant women, fetuses, and children who take part in this study are considered vulnerable participants per the U.S. Code of Federal Regulations, and site IRBs/ECs must consider the potential risks and benefits to maternal, fetal, and infant participants as described in 45 CFR 46 Subpart B (for pregnant women, fetuses, and neonates) and 45 CFR 46 Subpart D (for children).

With respect to 45 CFR 46 Subpart B, the specifications of 45 CFR 46.204 (d) are considered to apply; therefore, maternal participants will be asked to provide written informed consent for their own and their children's study participation.

With respect to 45 CFR 46 Subpart D, IRBs/ECs must determine the level of risk to children in the categories specified in 45 CFR 46.404-407. Documentation of this determination is required to complete the DAIDS protocol registration process described in Section 14.2, and the risk category assigned by the IRB/EC further determines the parental informed consent requirements for the study at each site. Per 45 CFR 46.408 (b), the IRB/EC may find that the consent of one parent is sufficient for research to be conducted under 46.404 or 46.405. If the IRB/EC finds that the research is covered by 46.406 or 46.407, both parents must give their consent, unless one parent is deceased, unknown, incompetent, or not reasonably available or when only one parent has legal responsibility for the care and custody of the child (as determined locally). IRBs/ECs must document their risk determination, and study sites should adapt the signature pages of their

site-specific ICFs as needed to accommodate the parental consent requirements associated with the IRB/EC determination.

Study sites must comply with the requirements of the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research, which is available at: https://www.niaid.nih.gov/sites/default/files/enrollingchildrenrequirements.pdf

13.3 Informed Consent

Refer to Section 4 and the study-specific MOP for further information on informed consent procedures for this study.

Written informed consent for maternal and infant study participation will be obtained before any study-specific procedures are performed. The informed consent process will include information exchange, detailed discussion, and assessment of understanding of all required elements of informed consent, including the potential risks, benefits, and alternatives to study participation. Maternal participants will be extensively counseled on the importance of obstetrical and newborn care, as well as HIV prevention strategies while pregnant and postpartum.

Maternal participants who have reached the legal age of independent informed consent for research (typically 18 years of age) are expected to provide written informed consent for their own and their infant's study participation. For maternal participants who have not reached the legal age of independent consent, two options may be considered:

- Parental consent with participant assent: In this case, (i) the parent or legal guardian of the minor maternal participant must provide informed consent for the maternal participant's and her infant's study participation and (ii) the minor maternal participant must provide written assent for her and her infant's study participation.

- Waiver of parental consent: In this case, if site ethical review bodies (IRBs/ECS) provide a waiver of parental consent, the minor maternal participant may provide written informed consent for her and their infant's study participation. For this study, IRBs/ECs may find that a waiver of parental consent is ethically justified for 16-17 year-old participants, given the perceived favorable risk-benefit profile of study participation. If such a waiver is obtained, then minors will provide consent and parental or legal guardian consent will not be sought. If this were to occur at any site, specific requirements would be developed in collaboration with local regulatory authorities and be reflected in the site-specific consent forms.

All sites must establish the SOPs that document the locally-applicable legal age of independent informed consent for research, noting whether this age differs for young women who are pregnant. Site SOPs must also specify procedures to be followed when obtaining informed consent and assent for this study, reflecting all applicable IRB/EC determinations and all other IRB/EC requirements.

Sites will adapt the consent forms to clarify for minor participants what information will and will not be shared with parents/guardians based on local regulations. Even under a consent/assent requirement, minors can consent independently to some procedures in the study depending on local laws; for example, minors may be allowed to consent independently to:

- Medical treatment, including STI and HIV treatment

- Contraceptives access and contraceptive advice and information, including emergency contraceptives
- Pregnancy testing and counseling.

In accordance with the DAIDS policy on Enrolling Children (including Adolescents) in Clinical Research (available at the website referenced in Section 13.2), all study sites must establish and maintain written procedures describing the standards that will be followed to identify who may serve as guardian for an enrolled infant, reflective of applicable IRB/EC guidance for conduct of human subjects research within the context of available local law, regulation, or government policy.

13.4 Potential Benefits

For all participants, the services provided to support antenatal and early newborn care may result in improved health-seeking behaviors. Those who initiate and adhere to daily oral PrEP could have the benefit of reduced HIV acquisition. From a broader perspective, however, the information learned from this study will help program managers and policymakers understand the feasibility of this intervention among pregnant, HIV-uninfected women in sub-Saharan Africa.

13.5 Potential Risks

13.5.1 Venipuncture

Venipuncture is sometimes associated with discomfort. Venipuncture may lead to discomfort, dizziness, bruising, swelling, and rarely, an infection at the venipuncture site.

13.5.2 HIV and STI Testing

Site will collect from women vaginal swabs or urine (*PrEP Comparison Component only*) for chlamydia and gonorrhea testing. Maternal participants may become embarrassed, worried, or anxious when completing their STI testing, HIV risk assessment and/or receiving HIV counseling. They also may become worried or anxious while waiting for their HIV and additional STI test results. Individuals who learn that they have an HIV infection or other STI may experience anxiety or depression related to their test results. Staff members will be trained to provide counseling following these tests, to provide the necessary information for future prevention (if negative) or for care and treatment (if positive).

13.5.3 Dual-Energy X-ray Absorptiometry Scan

Bone mineral density is measured by dual-energy x-ray absorptiometry (DXA) which involves exposure to radiation. For this protocol, a maternal DXA scan will be conducted for both Cohorts in the PrEP Comparison Component per Appendices IIA and IIB for a total radiation exposure of up to 1.36 mrem (0.22 mrem for each scan of the spine, 0.46 mrem for each scan of the hip). This is comparable to the dose of radiation received from naturally occurring sources over a two to three-day period at sea level (approximately 0.8 mrem per day).

For infant participants in both Cohorts of the PrEP Comparison Component, a DXA scan of the whole body is conducted at birth and a DXA scan of the lumbar spine is done at birth and study exit. Assuming that up to three attempts may be required to complete scans of the whole body and lumbar spine in infants who undergo DXA scans, the total radiation exposure will be a maximum

of 4.08 mrem (0.89 mrem for each scan of the whole body, 0.47 mrem for each scan of the spine). This is comparable to the dose of radiation received from naturally occurring sources over a five-day period at sea level. For both maternal and infant participants, the exposure to radiation associated with the DXA scans is well below limits set forth by the FDA in 21 CFR 361.1(b)(3).

13.5.4 PrEP (FTC/TDF)

FTC/TDF may have side effects, some of which are listed below. This list includes the more serious or common side effects with known or possible relationship. Maternal participants taking FTC/TDF will be monitored closely for any side effects, and are asked to report all side effects to the study site clinician. Refer to Section 1.3.2 for information of the safety of PrEP during pregnancy and breastfeeding.

The following side effects have been commonly associated with the use of FTC/ TDF as treatment in HIV-infected adults:

- Gastrointestinal intolerance (such as nausea, abdominal pain, diarrhea, or vomiting), most commonly in the first month and typically resolves without treatment
- Flatulence (gas), most commonly in the first month and typically resolves without treatment
- Headache, dizziness, tiredness, or inability to sleep

Rare, but serious side effects include:

- Rash
- Worsening or new kidney damage
- Bone pain and bone changes such as thinning and softening
- Allergic reaction
- Lactic acidosis (buildup of too much acid in the body). Lactic acidosis can cause shortness of breath, nausea and liver failure.
- Depression and unusual dreams have been reported in HIV infected persons taking Truvada

13.5.5 HIV Resistance

It is possible that a maternal participant who is taking PrEP and becomes infected with HIV during this study may develop resistance to FTC/TDF. Multiple steps will be taken to minimize the risk of drug resistance. HIV testing will be performed frequently throughout follow-up for all maternal participants. If acute HIV infection is suspected after enrollment for any participant accepting PrEP, the participant will undergo evaluation using tests described in Appendix IID. These steps should minimize the risk of drug resistance occurrence by identifying HIV infection in its early stages and stopping study drug. If a participant becomes infected during the study, real-time resistance testing will be conducted for clinical management (see Section 6.3).

13.5.6 Sensitive Questions

Maternal participants will be asked questions about their sexual behavior that may make them feel uneasy. Maternal participants do not have to answer any question that they do not want to and can stop answering the questions at any time.

13.5.7 Mobile Phones

As described in Section 3.2, all maternal participants enrolled in the PrEP Comparison Component will receive SMS texts on antenatal, delivery, and early infant care, and maternal participants in Cohort 1 will also participate in SMS messaging to promote PrEP adherence. It is possible that timing or volume of text messages will be inconvenient to maternal participants. Because the costs for text messaging will be covered by the project, it should not incur additional expense for the maternal participant.

Information entered by maternal participants into mobile phone programs will be maintained on a 21CFR Part 11 compliant secure server via a secure, password-protected log-in. However, it is possible that someone who has access to the maternal participant's phone may see this information, revealing participation in the study. It will not be possible for the study team to access any private information on maternal participants' mobile phones. Lost or stolen phones are a practical reality. Maternal participants will be trained to secure their phones with a password or PIN and to delete old messages. Messages will not disclose individual level information so that if accessed will not compromise confidentiality around health status or medication use.

13.6 Potential Social Impact

Participation in clinical research includes the risk of loss of confidentiality. Although study sites make every effort to protect participant privacy and confidentiality, it is possible that participants' involvement in the study could become known to others, and that social harms – non-medical adverse consequences – may result (e.g., because participants could be perceived as being HIV-infected or at "high risk" for HIV infection, or because a participant's choice to participate or use PrEP is not supported). For example, participants could be treated unfairly, or could have problems being accepted by their families and/or communities or could experience problems in their relationships with their partner(s). The Protocol Team believes this risk is small, given that the target population is HIV-uninfected women; however, study staff will work to minimize such potential risks both at the clinic and community levels.

At every follow-up visit in the PrEP Comparison Component, sites will assess for social harms experienced since the last visit. All reported social harms will be source documented and entered into eCRFs for maternal participants. As part of routine study monitoring, social harms will be tabulated and reviewed as described in Sections 7.2 and 9.3.2. In the event that a participant reports a social harm, every effort will be made by study staff to provide appropriate follow-up support and counseling to the participant as necessary; referrals to appropriate non-study resources may also be provided. Each site will provide such support in accordance with local standards of care and site SOPs.

13.7 Reimbursement/Compensation

Pending IRB/EC approval, maternal participants will be reimbursed for costs associated with completing study visits (e.g., transport costs). Reimbursement amounts will be specified in site-specific ICFs or other materials if applicable per IRB/EC policies and procedures.

13.8 Privacy and Confidentiality

All study procedures will be conducted in private and every effort will be made to protect participant privacy and confidentiality to the extent possible. Participant information will not be

released without written permission to do so except as necessary for review, monitoring, and/or auditing as described in Section 11.2.

All study-related information will be stored securely. Participant research records will be stored in locked areas with access limited to study staff. Participant information necessary for implementation of the mHealth component (SMS messages), such as names and phone numbers, will be maintained on a General Data Protection Regulation (GDPR) compliant cloud server. All laboratory specimens, eCRFs, and other documents that may be transmitted off-site (e.g., EAE report forms, photographs of observed reactions, SMS message logs) will be identified by PID only. Likewise, communications between study staff and Protocol Team members regarding individual participants will identify participants by PID only.

Study sites are encouraged but not required by DAIDS policies to store study records that bear participant names or other personal identifiers separately from records identified by PID. All local databases must be secured with password protected access systems. Lists, logbooks, appointment books, and any other documents that link PID numbers to personal identifying information should be stored in a separate, locked location in an area with limited access.

13.9 Communicable Disease Reporting

Study staff will comply with local requirements to report communicable diseases including HIV infection identified among study participants to health authorities. Participants will be made aware of all applicable reporting requirements as part of the study informed consent process.

13.10 Management of Incidental Findings

Site clinicians will inform maternal participants (or other authorized guardians if applicable) of all clinically meaningful physical exam findings and laboratory test results, including results of HIV tests and hematology and chemistry tests. When applicable, site clinicians will provide referrals to non-study sources of medical care for further evaluation and/or treatment of these findings.

The results of protocol-specified DXA scans for infants are not planned to be provided to maternal participants, as there are no normal values for this test in infants and it is not expected to be relevant to clinical care and management. If, however, new information becomes available during the course of the study indicating that the results of the DXA scan are of clinical relevance, the results of this test will be provided prior to study exit.

13.11 Management of New Information Pertinent to Study Participation

Maternal participants will be provided any new information learned over the course of the study that may affect their willingness to continue receiving study drug, their decision to take up PrEP and/or remain in follow-up in the study.

13.12 Post-Trial Access to Study Drug

Maternal participants who choose to continue PrEP after exiting the study will be referred to appropriate non-study PrEP programs, if available, for continued services. If a PrEP program is not in place, maternal participants will be required to stop PrEP at the end of the study period. Participants will be counseled and referred to all HIV prevention methods available, outside of

the study, consistent with local standards of care. Each site will be required to engage applicable stakeholders and outline locally relevant procedures.

14 ADMINISTRATIVE PROCEDURES

14.1 Regulatory Oversight

This study is sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD), and National Institute of Mental Health (NIMH), which are part of the United States National Institutes of Health (NIH). Study drug FTC/TDF is provided by Gilead Sciences, Inc.; however, this organization is not involved in sponsorship or regulatory oversight of this study.

The Division of AIDS (DAIDS) within the NIAID is responsible for regulatory oversight of this study. DAIDS will distribute safety-related information pertaining to the study drug prior to and during the conduct of the study, in accordance with its sponsor obligations.

NIAID provides funding to the clinical research sites at which this study will be conducted and contracts with independent clinical site monitors who will perform monitoring visits as described in Section 12. As part of these visits, monitors will inspect study-related documentation to ensure compliance with all applicable U.S. and local regulatory requirements.

14.2 Protocol Registration

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol ICFs approved, as appropriate, by their local IRB/EC, local IBC, and any other applicable regulatory entity. Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all required documents have been received.

Site-specific ICFs will be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

For any future protocol amendments, upon receiving final IRB/EC and any other applicable regulatory entity approvals, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICFs will not be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual, which is available at: https://www.niaid.nih.gov/research/daids-clinical-site-implementation-operations

14.3 Study Implementation

This study will be conducted in accordance with the protocol, international good clinical practice guidelines, and all applicable US and local regulations. Study implementation will also be guided by the IMPAACT MOP, study-specific MOP, LPC, and other study implementation materials, which will be available on the IMPAACT web site: www.impaactnetwork.org.

Study implementation at each site will also be guided by site-specific SOPs. The DAIDS policy on *Requirements for Manual of Operations* specifies the minimum set of SOPs that must be established at sites conducting DAIDS funded and/or sponsored clinical trials (available on the website referenced in Section 11.2). These SOPs should be updated and/or supplemented as needed to describe roles, responsibilities, and procedures for this study.

14.4 Protocol Deviation Reporting

Per the policy for Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials (available at the website referenced in Section 11.2), all protocol deviations must be documented in participant research records. Reasons for the deviations and corrective and preventive actions taken in response to the deviations should also be documented.

All DAIDS policies referenced in this section are available at:
https://www.niaid.nih.gov/research/daids-clinical-research-policies-standard-procedures

Deviations should be reported to site IRBs/ECs and other applicable review bodies in accordance with the policies and procedures of these review bodies. Serious deviations that are associated with increased risk to one or more study participants and/or significant impacts on the integrity of study data must also be reported within IMPAACT, following procedures specified in the IMPAACT Manual of Procedures.

14.5 Critical Event Reporting

Per the DAIDS policy on Identification and Classification of Critical Events, a critical event is defined as an unanticipated study-related incident that is likely to cause harm or increase the risk of harm to participants or others or has a significant adverse impact on study outcomes or integrity. All such events must be reported following procedures specified in the DAIDS Critical Events Manual, which is available at: https://www.niaid.nih.gov/research/daids-clinical-research-event-reporting-safety-monitoring

14.6 ClinicalTrials.gov

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

15 PUBLICATIONS

All presentations and publications of data collected in this study are governed by NIH public access policy and IMPAACT policies, which are available in the IMPAACT Manual of Procedures.

16 REFERENCES

1. Bekker LG GR, Hughes J, Amico R, Roux S, Hendrix C, Anderson P, Dye B, Elharrar V, Stirratt M. HPTN 067/ADAPT Cape Town: a comparison of daily and nondaily dosing in African women [Abstract 978LB]. Paper presented at: Conference on Retroviruses and Opportunistic Infections 2015; Seattle, WA. 2015.
2. PEPFAR. DREAMS Innovation Challenge Fact Sheet. In: DREAMS, editor. 2016.
3. Venter WD, Cowan F, Black V, Rebe K, Bekker LG. Pre-exposure prophylaxis in Southern Africa: feasible or not? J Int AIDS Soc. 2015;18(4 Suppl 3):19979.
4. Shisana O, Rehle T, Simbayi LC, Zuma K, Jooste S, Zungu N. South African National HIV Prevalence, Incidence and Behaviour Survey. South Africa: HSRC Press; 2014.
5. UNAIDS. Global AIDS Update. Switzerland; 2016.
6. Drake AL, Wagner A, Richardson B, John-Stewart G. Incident HIV during pregnancy and postpartum and risk of mother-to-child HIV transmission: a systematic review and meta-analysis. PLoS Med. 2014;11(2):e1001608.
7. Mellins CA, Chu C, Malee K, Allison S, Smith R, Harris L, et al. Adherence to antiretroviral treatment among pregnant and postpartum HIV-infected women. AIDS Care. 2008;20(8):958-68.
8. Parekh BS, Hu DJ, Vanichseni S, Satten GA, Candal D, Young NL, et al. Evaluation of a sensitive/less-sensitive testing algorithm using the 3A11-LS assay for detecting recent HIV seroconversion among individuals with HIV-1 subtype B or E infection in Thailand. AIDS ResHumRetroviruses. 2001;17(5):453-8.
9. Fonner VA, Dalglish SL, Kennedy CE, Baggaley R, O'Reilly K R, Koechlin FM, et al. Effectiveness and safety of oral HIV pre-exposure prophylaxis (PrEP) for all populations: A systematic review and meta-analysis. AIDS. 2016.
10. Baeten JM, Donnell D, Ndase P, Mugo NR, Campbell JD, Wangisi J, et al. Antiretroviral Prophylaxis for HIV Prevention in Heterosexual Men and Women. N Engl J Med. 2012;367(5):399-410.
11. Thigpen MC, Kebaabetswe PM, Paxton LA, Smith DK, Rose CE, Segolodi TM, et al. Antiretroviral Preexposure Prophylaxis for Heterosexual HIV Transmission in Botswana. N Engl J Med. 2012;367(5):423-34.
12. Choopanya K, Martin M, Suntharasamai P, Sangkum U, Mock PA, Leethochawalit M, et al. Antiretroviral prophylaxis for HIV infection in injecting drug users in Bangkok, Thailand (the Bangkok Tenofovir Study): a randomised, double-blind, placebo-controlled phase 3 trial. The Lancet. 2013;381(9883):2083-90.
13. Grant RM, Lama JR, Anderson PL, McMahan V, Liu AY, Vargas L, et al. Preexposure Chemoprophylaxis for HIV Prevention in Men Who Have Sex with Men. N Engl J Med. 2010;363(27):2587-99.
14. Marrazzo JM, Ramjee G, Richardson BA, Gomez K, Mgodi N, Nair G, et al. Tenofovir-Based Preexposure Prophylaxis for HIV Infection among African Women. N Engl J Med. 2015;372(6):509-18.
15. Rees H D-MS, Lombard C, Baron D, Panchia R, Myer L, Schwartz J, Doncel G, Gray G. FACTS 001 Phase III trial of pericoital tenofovir 1% gel for HIV prevention in women [Abstract 26LB]. Paper presented at: Conference on Retroviruses and Opportunistic Infections 2015; Seattle, WA. 2015.
16. Van Damme L, Corneli A, Ahmed K, Agot K, Lombaard J, Kapiga S, et al. Preexposure Prophylaxis for HIV Infection among African Women. N Engl J Med. 2012;367:411-22.
17. Abdool Karim Q, Abdool Karim SS, Frohlich JA, Grobler AC, Baxter C, Mansoor LE, et al. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science. 2010;329(5996):1168-74.
18. Molina J-M, Capitant C, Spire B, Pialoux G, Cotte L, Charreau I, et al. On-Demand Preexposure Prophylaxis in Men at High Risk for HIV-1 Infection. N Engl J Med. 2015;373(23):2237-46.

19. McCormack S, Dunn DT, Desai M, Dolling DI, Gafos M, Gilson R, et al. Pre-exposure prophylaxis to prevent the acquisition of HIV-1 infection (PROUD): effectiveness results from the pilot phase of a pragmatic open-label randomised trial. The Lancet.387(10013):53-60.
20. Grant RM, Anderson PL, McMahan V, Liu A, Amico KR, Mehrotra M, et al. Uptake of pre-exposure prophylaxis, sexual practices, and HIV incidence in men and transgender women who have sex with men: a cohort study. The Lancet Infectious Diseases. 2014;14(9):820-9.
21. McCormack S DD. Pragmatic open-label randomised trial of preexposure prophylaxis: the PROUD study [Abstract 22LB]. Paper presented at: Conference on Retroviruses and Opportunistic Infections 2015; Seattle, WA. 2015.
22. Preexposure prophylaxis for the prevention of HIV infection in the United States - 2014 clinical practice guideline. In: CDC, editor. 2014. p. 1-67.
23. World Health Organization. Guideline on when to start Antiretroviral Therapy and on Pre-Exposure Prophylaxis for HIV. 2015.
24. Cottrell ML, Yang KH, Prince HM, Sykes C, White N, Malone S, et al. A Translational Pharmacology Approach to Predicting HIV Pre-Exposure Prophylaxis Outcomes in Men and Women Using Tenofovir Disoproxil Fumarate+/-Emtricitabine. J Infect Dis. 2016.
25. Miller LG, Hays RD. Measuring adherence to antiretroviral medications in clinical trials. HIV Clin Trials. 2000;1(1):36-46.
26. Murphy DA, Wilson CM, Durako SJ, Muenz LR, Belzer M. Antiretroviral medication adherence among the REACH HIV-infected adolescent cohort in the USA. AIDS Care. 2001;13(1):27-40.
27. La Greca AM, Auslander WF, Greco P, Spetter D, Fisher EB, Jr., Santiago JV. I get by with a little help from my family and friends: adolescents' support for diabetes care. J Pediatr Psychol. 1995;20(4):449-76.
28. Vaz MJ, Barros SM, Palacios R, Senise JF, Lunardi L, Amed AM, et al. HIV-infected pregnant women have greater adherence with antiretroviral drugs than non-pregnant women. Int J STD AIDS. 2007;18(1):28-32.
29. Henegar CE, Westreich DJ, Maskew M, Miller WC, Brookhart MA, Van Rie A. Effect of pregnancy and the postpartum period on adherence to antiretroviral therapy among HIV-infected women established on treatment. J Acquir Immune Defic Syndr. 2015;68(4):477-80.
30. Bardeguez AD, Lindsey JC, Shannon M, Tuomala RE, Cohn SE, Smith E, et al. Adherence to antiretrovirals among US women during and after pregnancy. J AcquirImmuneDeficSyndr. 2008;48(4):408-17.
31. Nachega JB, Uthman OA, Anderson J, Peltzer K, Wampold S, Cotton MF, et al. Adherence to antiretroviral therapy during and after pregnancy in low-income, middle-income, and high-income countries: a systematic review and meta-analysis. AIDS. 2012;26(16):2039-52.
32. Phillips T, Thebus E, Bekker LG, McIntyre J, Abrams EJ, Myer L. Disengagement of HIV-positive pregnant and postpartum women from antiretroviral therapy services: a cohort study. J Int AIDS Soc. 2014;17:19242.
33. Matthews LT, Heffron R, Mugo NR, Cohen CR, Hendrix CW, Celum C, et al. High medication adherence during periconception periods among HIV-1-uninfected women participating in a clinical trial of antiretroviral pre-exposure prophylaxis. J Acquir Immune Defic Syndr. 2014;67(1):91-7.
34. Matthews LT SS, Mansoor LE, Yende-Zuma N, Bangsberg DR, Karim QA. Women with pregnancies had lower adherence to 1% tenofovir vaginal gel as HIV preexposure prophylaxis in CAPRISA 004, a phase IIB randomized-controlled trial. PLoS One. 2013(8(3):e56400).
35. Joint United Nations Programme on HIV/AIDS (UNAIDS). The Gap Report [Report]. Geneva, Switzerland: UNAIDS; 2014 [Available from: http://www.unaids.org/en/media/unaids/contentassets/documents/unaidspublication/2014/UNAIDS_Gap_report_en.pdf.

36. Baeten JM, Palanee-Phillips T, Brown ER, Schwartz K, Soto-Torres LE, Nel A, et al. A phase III trial of the dapivirine vaginal ring for HIV-1 prevention in women. Conference on Retroviruses and Opportunistic Infections; Boston, MA2016.
37. Kapiga S, Bekker LG, Borremans M, Rosenberg Z, editors. Safety and efficacy of dapivirine vaginal ring for HIV-1 prevention in African women. Conference on Retroviruses and Opportunistic Infections 2016; Boston, MA.
38. Corneli AL, Deese J, Wang M, Taylor D, Ahmed K, Agot K, et al. FEM-PrEP: Adherence Patterns and Factors Associated With Adherence to a Daily Oral Study Product for Pre-exposure Prophylaxis. Journal of Acquired Immune Deficiency Syndromes (1999). 2014;66(3):324-31.
39. Corneli A, Perry B, Agot K, Ahmed K, Malamatsho F, Van Damme L. Facilitators of adherence to the study pill in the FEM-PrEP clinical trial. PLoS One. 2015;10(4):e0125458.
40. Corneli AL, McKenna K, Perry B, Ahmed K, Agot K, Malamatsho F, et al. The science of being a study participant: FEM-PrEP participants' explanations for overreporting adherence to the study pills and for the whereabouts of unused pills. J Acquir Immune Defic Syndr. 2015;68(5):578-84.
41. Corneli A, Perry B, McKenna K, Agot K, Ahmed K, Taylor J, et al. Participants' Explanations for Nonadherence in the FEM-PrEP Clinical Trial. J Acquir Immune Defic Syndr. 2016;71(4):452-61.
42. van der Straten A, Brown ER, Marrazzo JM, Chirenje MZ, Liu K, Gomez K, et al. Divergent adherence estimates with pharmacokinetic and behavioural measures in the MTN-003 (VOICE) study. J Int AIDS Soc. 2016;19(1):20642.
43. van der Straten A, Stadler J, Luecke E, Laborde N, Hartmann M, Montgomery ET. Perspectives on use of oral and vaginal antiretrovirals for HIV prevention: the VOICE-C qualitative study in Johannesburg, South Africa. J Int AIDS Soc. 2014;17(3 Suppl 2):19146.
44. van der Straten A, Montgomery ET, Musara P, Etima J, Naidoo S, Laborde N, et al. Disclosure of pharmacokinetic drug results to understand nonadherence. AIDS. 2015;29(16):2161-71.
45. Baeten J HR, Kidoguchi L, et al. Near elimination of HIV transmission in a demonstration project of PrEP and ART [Abstract 24]. Paper presented at: Conference on Retroviruses and Opportunistic Infections 2015; Seattle, WA. 2015.
46. Amico KR, editor HPTN 067 ADAPT: 'PrEP Ubuntu' and Experiences with open-label PrEP Among South African Women. 8th IAS Conference on HIV Pathogenesis, Treatment & Prevention; 2015 19-22 July 2015; Vancouver, Canada.
47. K. Gill, R. Marcus, J. Dietrich, T. Bennie, S. Hosek, G. Gray, et al., editors. An analysis of baseline and early data from the Plus Pills study: an open-label trial of pre-exposure prophylaxis for South African adolescents. 21st International AIDS Conference; 2016; Durban.
48. Bekker L-G. PlusPills principal investigator In: Hosek S, editor. 2017.
49. Amico KR MV, Marcus J, Goicochea P, Vargas L, Grant R, Liu A. Integrated Next Step Counseling (iNSC): A discussion based sexual health promotion conversation to support men who have sex with men using PrEP in the iPrEx open label extension [80467]. Oral presentation at the 7th Annual HIV Treatment and Prevention Adherence Conference of the International Association of Providers in AIDS Care, Miami, FL. 2012.
50. Kofron R. Real time plasma TFV levels to support adherence in a pre-exposure prophylaxis demonstration project. 9th International Conference on HIV Treatment and Prevention Adherence IAPAC, Miami, FL. 2014.
51. Amico KR. Evidence for Technology Interventions to Promote ART Adherence in Adult Populations: a Review of the Literature 2012-2015. Curr HIV/AIDS Rep. 2015;12(4):441-50.
52. Muessig KE, Nekkanti M, Bauermeister J, Bull S, Hightow-Weidman LB. A systematic review of recent smartphone, Internet and Web 2.0 interventions to address the HIV continuum of care. Curr HIV/AIDS Rep. 2015;12(1):173-90.
53. Poushter J, Oates R. Cell Phones in Africa: Communication Lifeline: Texting Most Common Activity, but Mobile Money Popular in Several Countries. Pew Research Center; 2015.

54. Hightow-Weidman LB, Muessig KE, Bauermeister J, Zhang C, LeGrand S. Youth, Technology, and HIV: Recent Advances and Future Directions. Curr HIV/AIDS Rep. 2015;12(4):500-15.
55. Swahn MH, Braunstein S, Kasirye R. Demographic and psychosocial characteristics of mobile phone ownership and usage among youth living in the slums of Kampala, Uganda. West J Emerg Med. 2014;15(5):600-3.
56. Ybarra ML, Mwaba K, Prescott TL, Roman NV, Rooi B, Bull S. Opportunities for technology-based HIV prevention programming among high school students in Cape Town, South Africa. AIDS Care. 2014;26(12):1562-7.
57. Lim MS, Hocking JS, Aitken CK, Fairley CK, Jordan L, Lewis JA, et al. Impact of text and email messaging on the sexual health of young people: a randomised controlled trial. J Epidemiol Community Health. 2012;66(1):69-74.
58. van Velthoven MH, Brusamento S, Majeed A, Car J. Scope and effectiveness of mobile phone messaging for HIV/AIDS care: a systematic review. Psychol Health Med. 2013;18(2):182-202.
59. Mobile Alliance for Maternal Action. The power of health in every mama's hand [Available from: http://www.mobilemamaalliance.org/mobile-messages.
60. Celum C, Mgodi N, Bekker L-G, Hosek S, Donnell D, Anderson PL, et al. PrEP adherence and effect of drug level feedback among young African women in HPTN 082. International AIDS Conference; 2019 July 21-24; Mexico City, Mexico2019.
61. Scorgie F, Khoza N, Delany-Moretlwe S, Mangxilana N, Atujuna M, Musara P, et al. Measuring perceptions of sexual risk among adolescent girls and young women taking PrEP: A new qualitative method using visual timelines in HPTN 082. International AIDS Conference; 2019 July 21-24; Mexico City, Mexico2019.
62. Zimbabwe: Standard DHS, 2015 [Available from: http://dhsprogram.com/what-we-do/survey/survey-display-475.cfm.
63. Creswell JW. Qualitative inquiry and research design: Choosing among five approaches. Research Design. . Thousand Oaks, CA: Sage Publications 2012.
64. Stadler J, Scorgie F, van der Straten A, Saethre E. Adherence and the Lie in a HIV Prevention Clinical Trial. Med Anthropol. 2015:1-14.
65. Corneli A, Perry B, McKenna K, Agot K, Ahmed K, Taylor J, et al. Participants' explanations for non-adherence in the FEM-PrEP clinical trial. Journal of acquired immune deficiency syndromes (1999). 2015.
66. Mofenson LM. Tenofovir Pre-exposure Prophylaxis for Pregnant and Breastfeeding Women at Risk of HIV Infection: The Time is Now. PLoS Med. 2016;13(9):e1002133.
67. Mofenson LM, Baggaley RC, Mameletzis I. Tenofovir Disoproxil Fumarate Safety for Women and their Infants during Pregnancy and Breastfeeding: Systematic Review. AIDS. 2016.
68. Plosker GL. Emtricitabine/tenofovir disoproxil fumarate: a review of its use in HIV-1 pre-exposure prophylaxis. Drugs. 2013;73(3):279-91.
69. Mugwanya KK, Hendrix CW, Mugo NR, Marzinke M, Katabira ET, Ngure K, et al. Pre-exposure prophylaxis use by breastfeeding HIV-uninfected women: a prospective short-term study of antiretroviral excretion in breast milk and infant absorption. PLoS Med. 2016;13(9):e1002132.
70. Smith DJ. Modern Marriage, Men's Extramarital Sex, and HIV Risk in Southeastern Nigeria. Am J Public Health. 2007;97(6):997-1005.
71. Mugwanya K CC, Baeten J, et al. Reversibility of kidney function decline in HIV-1 uninfected men and women using pre-exposure prophylaxis [Abstract 981]. Paper presented at: Conference on Retroviruses and Opportunistic Infections 2015; Seattle, WA. 2015.
72. Fowler MG, Qin M, Fiscus SA, Currier J, Makanani B, Martinson F, et al. PROMISE: efficacy and safety of 2 strategies to prevent perinatal HIV transmission [Abstract 31LB]. Conference on Retroviruses and Opportunistic Infections; Seattle, WA2015.
73. Pan CQ, Duan Z, Dai E, Zhang S, Han G, Wang Y, et al. Tenofovir to Prevent Hepatitis B Transmission in Mothers with High Viral Load. N Engl J Med. 2016;374(24):2324-34.

74. Liu AY, Vittinghoff E, Sellmeyer DE, Irvin R, Mulligan K, Mayer K, et al. Bone mineral density in HIV-negative men participating in a tenofovir pre-exposure prophylaxis randomized clinical trial in San Francisco. PLoS One. 2011;6(8):e23688.
75. Mirembe BG, Kelly CW, Mgodi N, Greenspan S, Dai JY, Mayo A, et al. Bone Mineral Density Changes Among Young, Healthy African Women Receiving Oral Tenofovir for HIV Preexposure Prophylaxis. Journal of acquired immune deficiency syndromes (1999). 2016;71(3):287.
76. Stranix-Chibanda L, Tierney C, Marr C, George K, Sebikari D, Dadabhai S, et al. Impact of tenofovir-containing triple antiretroviral therapy (ART) on bone mineral density in HIV-infected breastfeeding women in sub-Saharan Africa. International Workshop on HIV Pediatrics; 2016 Jul 15-16; Durban, South Africa2016.
77. Siberry GK, Jacobson DL, Kalkwarf HJ, Wu JW, DiMeglio LA, Yogev R, et al. Lower newborn bone mineral content associated with maternal use of tenofovir disoproxil fumarate during pregnancy. Clin Infect Dis. 2015;61(6):996-1003.
78. Jao J ML, Phillips T, Petro G, Zerbe A, Abrams EJ. In utero tenofovir exposure is not associated with fetal long bone growth. Paper presented at: 8th IAS Conference on HIV Pathogenesis, Treatment, and Prevention 2015; Vancouver, Canada 2015.
79. Siberry G, Tierney C, Stranix-Chibanda L, Marr C, Shepherd JA, Browning R, et al. Impact of Maternal Tenofovir Use on HIV-Exposed Newborn Bone Mineral. Conference on Retroviruses and Opportunistic Infections; 2016 Feb 22-25; Boston, MA2016.
80. Makanani B, Balkus J, Palanee-Phillips T, Mbilizi Y, Piper J, Kintu K, et al., editors. Pregnancy incidence and outcomes among women using the dapivirine vaginal ring. Conference on Retroviruses and Opportunistic Infections (CROI); 2017 February 13–16, 2017; Seattle, Washington.
81. Heffron R, Mugo NR, Hong T, Bukusi EA, Katabira E, Asiimwe S, et al., editors. PrEP used in pregnancy does not increase poor birth outcomes. Conference on Retroviruses and Opportunistic Infections (CROI); 2017; Seattle, Washington.
82. Brown RS, Jr., McMahon BJ, Lok AS, Wong JB, Ahmed AT, Mouchli MA, et al. Antiviral therapy in chronic hepatitis B viral infection during pregnancy: A systematic review and meta-analysis. Hepatology. 2016;63(1):319-33.
83. Jourdain G, Ngo-Giang-Huong N, Cressey TR, Hua L, Harrison L, Tierney C, et al. Prevention of mother-to-child transmission of hepatitis B virus: a phase III, placebo-controlled, double-blind, randomized clinical trial to assess the efficacy and safety of a short course of tenofovir disoproxil fumarate in women with hepatitis B virus e-antigen. BMC Infect Dis. 2016;16:393.
84. Jourdain G. personal communication. 2016.
85. Weis J MC, Matsen F, et al. PrEP-selected drug resistance fades by six months following drug cessation [Abstract 983]. Paper presented at: Conference on Retroviruses and Opportunistic Infections 2015; Seattle, WA. 2015.
86. Larmarange JI, Collins; Orne-Gliemann, Joanna; McGrath, Nuala; Plazy, Mélanie; Baisley, Kathy; Bärnighausen,Till; Dabis, Francois; Pillay, Deenan Measuring the Impact of Test & Treat on the HIV Cascade: The Challenge of Mobility. Conference on Retroviruses and Opportunistic Infections. 2016.
87. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. Lancet Infect Dis. 2016.
88. Wilde GJS. The Theory of Risk Homeostasis: Implications for Safety and Health. Risk Anal. 1982;2(4):209-25.
89. Wilde G. Risk homeostasis theory: an overview. Inj Prev. 1998;4(2):89-91.
90. Hosek S RB, Landovitz R, Kapogiannis B, Siberry G, Liu N, Rutledge B, Brothers J, Rooney J, Wilson CM. An HIV pre-exposure prophylaxis demonstration project and safety study for young men who have sex with men in the United States (ATN 110). J Int AIDS Soc. 2015;18.
91. Amico KR, Stirratt MJ. Adherence to preexposure prophylaxis: current, emerging, and anticipated bases of evidence. Clin Infect Dis. 2014;59 Suppl 1:S55-60.

92. Liu AY, Vittinghoff E, Chillag K, Mayer K, Thompson M, Grohskopf L, et al. Sexual risk behavior among HIV-uninfected men who have sex with men participating in a tenofovir preexposure prophylaxis randomized trial in the United States. J Acquir Immune Defic Syndr. 2013;64(1):87-94.
93. Marcus JL, Glidden DV, Mayer KH, Liu AY, Buchbinder SP, Amico KR, et al. No evidence of sexual risk compensation in the iPrEx trial of daily oral HIV preexposure prophylaxis. PLoS One. 2013;8(12):e81997.
94. Kimberly Koester RA, Albert Liu, Vanessa McMahon, Sybil Hosek, Kenneth Mayer, Robert Grant. Qualitative findings from the iPrEx Open Label Extension (OLE) in the US. International AIDS Conference,; Melbourne, Australia 2014.
95. Hosek SG, Siberry G, Bell M, Lally M, Kapogiannis B, Green K, et al. The acceptability and feasibility of an HIV preexposure prophylaxis (PrEP) trial with young men who have sex with men. J Acquir Immune Defic Syndr. 2013;62(4):447-56.
96. McCormack S. Sexually Transmitted Infection Control in the Era of PrEP. Conference on Retroviruses and Opportunistic Infections; Boston, Massachusetts2016.
97. Bessesen M, Ives D, Condreay L, Lawrence S, Sherman KE. Chronic active hepatitis B exacerbations in human immunodeficiency virus-infected patients following development of resistance to or withdrawal of lamivudine. Clin Infect Dis. 1999;28(5):1032-5.
98. Mondou E, Sorbel J, Anderson J, Mommeja-Marin H, Rigney A, Rousseau F. Posttreatment exacerbation of hepatitis B virus (HBV) infection in long-term HBV trials of emtricitabine. Clin Infect Dis. 2005;41(5):e45-7.
99. Bellini C, Keiser O, Chave JP, Evison J, Fehr J, Kaiser L, et al. Liver enzyme elevation after lamivudine withdrawal in HIV-hepatitis B virus co-infected patients: the Swiss HIV Cohort Study. HIV Med. 2009;10(1):12-8.
100. Solomon MM, Schechter M, Liu AY, McManhan VM, Guanira JV, Hance RJ, et al. The Safety of Tenofovir-Emtricitabine for HIV Pre-Exposure Prophylaxis (PrEP) in Individuals With Active Hepatitis B. J Acquir Immune Defic Syndr. 2016;71(3):281-6.
101. Peterson L, Taylor D, Roddy R, Belai G, Phillips P, Nanda K, et al. Tenofovir disoproxil fumarate for prevention of HIV infection in women: a phase 2, double-blind, randomized, placebo-controlled trial. PLoS Clin Trials. 2007;2(5):e27.
102. Nuriel-Ohayon M, Neuman H, Koren O. Microbial Changes during Pregnancy, Birth, and Infancy. Front Microbiol. 2016;7:1031.
103. O'Hanlon DE, Moench TR, Cone RA. Vaginal pH and microbicidal lactic acid when lactobacilli dominate the microbiota. PLoS One. 2013;8(11):e80074.
104. Hyman RW, Fukushima M, Jiang H, Fung E, Rand L, Johnson B, et al. Diversity of the vaginal microbiome correlates with preterm birth. Reprod Sci. 2014;21(1):32-40.
105. Shiozaki A, Yoneda S, Yoneda N, Yonezawa R, Matsubayashi T, Seo G, et al. Intestinal microbiota is different in women with preterm birth: results from terminal restriction fragment length polymorphism analysis. PLoS One. 2014;9(11):e111374.
106. Vinturache AE, Gyamfi-Bannerman C, Hwang J, Mysorekar IU, Jacobsson B. Maternal microbiome - A pathway to preterm birth. Semin Fetal Neonatal Med. 2016;21(2):94-9.
107. Turner AN, Morrison CS, Munjoma MW, Moyo P, Chipato T, van de Wijgert JH. Vaginal practices of HIV-negative Zimbabwean women. Infect Dis Obstet Gynecol. 2010;2010.
108. Alcaide ML, Chisembele M, Malupande E, Arheart K, Fischl M, Jones DL. A cross-sectional study of bacterial vaginosis, intravaginal practices and HIV genital shedding; implications for HIV transmission and women's health. BMJ Open. 2015;5(11):e009036.
109. Kinuthia J, Richardson BA, Drake AL, Matemo D, Unger JA, McClelland RS, et al. Sexual Behavior and Vaginal Practices During Pregnancy and Postpartum: Implications for HIV Prevention Strategies. J Acquir Immune Defic Syndr. 2017;74(2):142-9.

110. Romero R, Hassan SS, Gajer P, Tarca AL, Fadrosh DW, Nikita L, et al. The composition and stability of the vaginal microbiota of normal pregnant women is different from that of non-pregnant women. Microbiome. 2014;2(1):4.
111. Huang YE, Wang Y, He Y, Ji Y, Wang LP, Sheng HF, et al. Homogeneity of the vaginal microbiome at the cervix, posterior fornix, and vaginal canal in pregnant Chinese women. Microb Ecol. 2015;69(2):407-14.
112. Zevin AS, Xie IY, Birse K, Arnold K, Romas L, Westmacott G, et al. Microbiome Composition and Function Drives Wound-Healing Impairment in the Female Genital Tract. PLoS Pathog. 2016;12(9):e1005889.
113. Zevin AS, McKinnon L, Burgener A, Klatt NR. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Curr Opin HIV AIDS. 2016;11(2):182-90.
114. Cohen J. INFECTIOUS DISEASE. Vaginal microbiome affects HIV risk. Science. 2016;353(6297):331.
115. Gosmann C, Anahtar MN, Handley SA, Farcasanu M, Abu-Ali G, Bowman BA, et al. Lactobacillus-Deficient Cervicovaginal Bacterial Communities Are Associated with Increased HIV Acquisition in Young South African Women. Immunity. 2017;46(1):29-37.
116. Hillier S, Meyn LA, Bunge K, Austin M, Moncla BJ, Dezzutti CS, et al., editors. Impact of vaginal microbiota on genital tissue and plasma concentrations of tenofovir. Conference on Retroviruses and Opportunistic Infections (CROI); 2017; Seattle, Washington.
117. Heffron R, McClelland RS, Balkus J, Celum C, Cohen CR, Mugo NR, et al., editors. Daily oral PrEP is effective among women with abnormal vaginal microbiota. Conference on Retroviruses and Opportunistic Infections (CROI); 2017; Seattle, Washington.
118. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. HIV-induced alteration in gut microbiota. Gut Microbes. 2014;5(4):562-70.
119. Noguera-Julian M, Rocafort M, Guillen Y, Rivera J, Casadella M, Nowak P, et al. Gut Microbiota Linked to Sexual Preference and HIV Infection. EBioMedicine. 2016;5:135-46.
120. Nowak P, Troseid M, Avershina E, Barqasho B, Neogi U, Holm K, et al. Gut microbiota diversity predicts immune status in HIV-1 infection. AIDS. 2015;29(18):2409-18.
121. Vujkovic-Cvijin I, Dunham RM, Iwai S, Maher MC, Albright RG, Broadhurst MJ, et al. Dysbiosis of the gut microbiota is associated with HIV disease progression and tryptophan catabolism. Sci Transl Med. 2013;5(193):193ra91.
122. Lozupone CA, Li M, Campbell TB, Flores SC, Linderman D, Gebert MJ, et al. Alterations in the gut microbiota associated with HIV-1 infection. Cell Host Microbe. 2013;14(3):329-39.
123. McHardy IH, Li X, Tong M, Ruegger P, Jacobs J, Borneman J, et al. HIV Infection is associated with compositional and functional shifts in the rectal mucosal microbiota. Microbiome. 2013;1(1):26.
124. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. Gut Microbes. 2014;5(4):562-70.
125. Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, et al. Altered Virome and Bacterial Microbiome in Human Immunodeficiency Virus-Associated Acquired Immunodeficiency Syndrome. Cell Host Microbe. 2016;19(3):311-22.
126. Owen BN, Elmes J, Silhol R, Dang Q, McGowan I, Shacklett B, et al. How common and frequent is heterosexual anal intercourse among South Africans? A systematic review and meta-analysis2017.
127. Nguyen QN, Himes JE, Martinez DR, Permar SR. The Impact of the Gut Microbiota on Humoral Immunity to Pathogens and Vaccination in Early Infancy. PLoS Pathog. 2016;12(12):e1005997.
128. Gordon HA, Pesti L. The gnotobiotic animal as a tool in the study of host microbial relationships. Bacteriol Rev. 1971;35(4):390-429.
129. Hu J, Nomura Y, Bashir A, Fernandez-Hernandez H, Itzkowitz S, Pei Z, et al. Diversified microbiota of meconium is affected by maternal diabetes status. PLoS One. 2013;8(11):e78257.

130. Collado MC, Rautava S, Aakko J, Isolauri E, Salminen S. Human gut colonisation may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid. Sci Rep. 2016;6:23129.
131. Hansen R, Scott KP, Khan S, Martin JC, Berry SH, Stevenson M, et al. First-Pass Meconium Samples from Healthy Term Vaginally-Delivered Neonates: An Analysis of the Microbiota. PLoS One. 2015;10(7):e0133320.
132. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. Nat Med. 2016;22(7):713-22.
133. Bender JM, Li F, Martelly S, Byrt E, Rouzier V, Leo M, et al. Maternal HIV infection influences the microbiome of HIV-uninfected infants. Sci Transl Med. 2016;8(349):349ra100.
134. Marrazzo J RG, Nair G, et al. Pre-exposure prophylaxis for HIV in women: daily oral tenofovir, oral tenofovir/emtricitabine, or vaginal tenofovir gel in the VOICE study (MTN 003) [Abstract 26LB]. Paper presented at: 20th Conference on Retroviruses and Opportunistic Infections 2013; Atlanta, GA. 2013.
135. Baeten JM, Palanee-Phillips T, Brown ER, Schwartz K, Soto-Torres LE, Govender V, et al. Use of a Vaginal Ring Containing Dapivirine for HIV-1 Prevention in Women. N Engl J Med.0(0):null.
136. Graybill LA, Kasaro M, Freeborn K, Walker JS, Poole C, Powers KA, et al. Incident HIV among pregnant and breast-feeding women in sub-Saharan Africa: a systematic review and meta-analysis. AIDS. 2020;34(5):761-76.
137. Gray RH, Li X, Kigozi G, Serwadda D, Brahmbhatt H, Wabwire-Mangen F, et al. Increased risk of incident HIV during pregnancy in Rakai, Uganda: a prospective study. Lancet. 2005;366(9492):1182-8.
138. Mugo NR, Heffron R, Donnell D, Wald A, Were EO, Rees H, et al. Increased risk of HIV-1 transmission in pregnancy: a prospective study among African HIV-1-serodiscordant couples. AIDS. 2011;25(15):1887-95.
139. Moodley D, Esterhuizen TM, Pather T, Chetty V, Ngaleka L. High HIV incidence during pregnancy: compelling reason for repeat HIV testing. AIDS. 2009;23(10):1255-9.
140. Keating MA, Hamela G, Miller WC, Moses A, Hoffman IF, Hosseinipour MC. High HIV incidence and sexual behavior change among pregnant women in Lilongwe, Malawi: implications for the risk of HIV acquisition. PLoS One. 2012;7(6):e39109.
141. Matthews L, Moore L, Crankshaw T, Milford C, Mosery F, Greener R, et al. South Africans with recent pregnancy rarely know partner's HIV serostatus: implications for serodiscordant couples interventions. BMC Public Health. 2014;14(1):843.
142. Schwartz SR, Bassett J, Sanne I, Phofa R, Yende N, Van Rie A. Implementation of a safer conception service for HIV-affected couples in South Africa. AIDS. 2014;28 Suppl 3:S277-85.
143. Mutambirwa J. Pregnancy, childbirth, mother and child care among the indigenous people of Zimbabwe. Int J Gynaecol Obstet. 1985;23(4):275-85.
144. Kinuthia J, Drake AL, Matemo D, Richardson BA, Zeh C, Osborn L, et al. HIV acquisition during pregnancy and postpartum is associated with genital infections and partnership characteristics. AIDS. 2015;29(15):2025-33.
145. Ngure K, Heffron R, Mugo NR, Celum C, Cohen CR, Odoyo J, et al. Contraceptive method and pregnancy incidence among women in HIV-1-serodiscordant partnerships. AIDS. 2012;26(4):513-8.
146. Odutola A, Baisley K, Hayes RJ, Rusizoka M, Tanton C, Weiss HA, et al. Pregnancy and contraceptive use among women participating in an HIV prevention trial in Tanzania. Sex Transm Infect. 2012;88(6):436-43.
147. Mugo NR, Hong T, Celum C, Donnell D, Bukusi EA, John-Stewart G, et al. Pregnancy incidence and outcomes among women receiving preexposure prophylaxis for HIV prevention: a randomized clinical trial. JAMA. 2014;312(4):362-71.

148. Prevention CfDCa. Pre-exposure prophylaxis for the prevention of HIV infection in the United States. 2014.
149. Dettinger JC, Kinuthia J, Pintye J, Abuna F, Begnel E, Mugwanya K, et al. Perinatal outcomes following maternal pre-exposure prophylaxis (PrEP) use during pregnancy: results from a large PrEP implementation program in Kenya. J Int AIDS Soc. 2019;22(9):e25378.
150. Gibb DM, Kizito H, Russell EC, Chidziva E, Zalwango E, Nalumenya R, et al. Pregnancy and Infant Outcomes among HIV-Infected Women Taking Long-Term ART with and without Tenofovir in the DART Trial. PLoS Med. 2012;9(5):e1001217.
151. Organization WH. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection: recommendations for a public health approach. 2013.
152. Greenup AJ, Tan PK, Nguyen V, Glass A, Davison S, Chatterjee U, et al. Efficacy and safety of tenofovir disoproxil fumarate in pregnancy to prevent perinatal transmission of hepatitis B virus. J Hepatol. 2014;61(3):502-7.
153. Hu YH, Liu M, Yi W, Cao YJ, Cai HD. Tenofovir rescue therapy in pregnant females with chronic hepatitis B. World J Gastroenterol. 2015;21(8):2504-9.
154. Joseph Davey DL, Pintye J, Baeten JM, Aldrovandi G, Baggaley R, Bekker LG, et al. Emerging evidence from a systematic review of safety of pre-exposure prophylaxis for pregnant and postpartum women: where are we now and where are we heading? J Int AIDS Soc. 2020;23(1):e25426.
155. Baeten JM, Haberer JE, Liu AY, Sista N. Preexposure prophylaxis for HIV prevention: where have we been and where are we going? J Acquir Immune Defic Syndr. 2013;63 Suppl 2:S122-9.
156. Heffron R, Pintye J, Matthews LT, Weber S, Mugo N. PrEP as Peri-conception HIV Prevention for Women and Men. Curr HIV/AIDS Rep. 2016;13(3):131-9.
157. Hodges-Mameletzis I, Fonner VA, Dalal S, Mugo N, Msimanga-Radebe B, Baggaley R. Pre-Exposure Prophylaxis for HIV Prevention in Women: Current Status and Future Directions. Drugs. 2019;79(12):1263-76.
158. van der Straten A, Ryan JH, Reddy K, Etima J, Taulo F, Mutero P, et al. Influences on willingness to use vaginal or oral HIV PrEP during pregnancy and breastfeeding in Africa: the multisite MAMMA study. J Int AIDS Soc. 2020;23(6):e25536.
159. Dettinger JC, Kinuthia J, Pintye J, Mwongeli N, Gómez L, Richardson BA, et al. PrEP Implementation for Mothers in Antenatal Care (PrIMA): study protocol of a cluster randomised trial. BMJ Open. 2019;9(3):e025122.
160. Pintye J, Kinuthia J, Abuna F, Mugwanya K, Lagat H, Dettinger JC, et al. Frequency and predictors of tenofovir-diphosphate detection among young Kenyan women in a real-world pre-exposure prophylaxis implementation program. Clin Infect Dis. 2020.
161. Pintye J, Davey DLJ, Wagner AD, John-Stewart G, Baggaley R, Bekker LG, et al. Defining gaps in pre-exposure prophylaxis delivery for pregnant and post-partum women in high-burden settings using an implementation science framework. Lancet HIV. 2020;7(8):e582-e92.
162. Liu AY, Cohen SE, Vittinghoff E, Anderson PL, Doblecki-Lewis S, Bacon O, et al. Preexposure Prophylaxis for HIV Infection Integrated With Municipal- and Community-Based Sexual Health Services. JAMA Intern Med. 2016;176(1):75-84.
163. Anderson PL, Glidden DV, Liu A, Buchbinder S, Lama JR, Guanira JV, et al. Emtricitabine-tenofovir concentrations and pre-exposure prophylaxis efficacy in men who have sex with men. Sci Transl Med. 2012;4(151):151ra25.
164. Benaboud S, Hirt D, Launay O, Pannier E, Firtion G, Rey E, et al. Pregnancy-Related Effects on Tenofovir Pharmacokinetics: a Population Study with 186 Women. Antimicrob Agents Chemother. 2012;56(2):857-62.
165. Lurie S, Mamet Y. Red blood cell survival and kinetics during pregnancy. Eur J Obstet Gynecol Reprod Biol. 2000;93(2):185-92.

166. Anderson P, Liu AY, Castillo-Mancilla J, Seifert S, McHugh C, Wagner T, et al., editors. TFV-DP in dried blood spots (DBS) following directly observed therapy: DOT-DBS Study. Conference on Retroviruses and Opportunistic Infections (CROI); 2017; Seattle, Washington.
167. Anderson PL, Stranix-Chibanda L, Huang S, Hosek S, Kacanek D, Nematadzira T, et al. TFV-DP in DBS for pregnant/postpartum adolescent and young women on PrEP in Africa. Conference on Retroviruses and Opportunistic Infections; Boston, MA2020.
168. Anderson PL, Liu AY, Castillo-Mancilla JR, Gardner EM, Seifert SM, McHugh C, et al. Intracellular Tenofovir-Diphosphate and Emtricitabine-Triphosphate in Dried Blood Spots following Directly Observed Therapy. Antimicrob Agents Chemother. 2018;62(1).
169. Arpaia N, Rudensky AY. Microbial metabolites control gut inflammatory responses. Proc Natl Acad Sci U S A. 2014;111(6):2058-9.
170. Costantine MM. Physiologic and pharmacokinetic changes in pregnancy. Front Pharmacol. 2014;5:65.
171. De Sousa Mendes M, Hirt D, Urien S, Valade E, Bouazza N, Foissac F, et al. Physiologically-based pharmacokinetic modeling of renally excreted antiretroviral drugs in pregnant women. Br J Clin Pharmacol. 2015;80(5):1031-41.
172. Bekker L-G RK, Venter F. Southern African guidelines on the safe use of pre-exposure prophylaxis in persons at risk of acquiring HIV-1 infection. S Afr J HIV Med. 2016;17(1).
173. Office of Behavioral and Social Sciences Research NIoH. Qualititative methods in health research: opportunities and considerations in application and review.
174. Patton MQ. Qualitative Research and Evaluation Methods. 3rd ed. Thousand Oaks, CA: Sage Publications 2002.
175. Castillo-Mancilla JR, Zheng JH, Rower JE, Meditz A, Gardner EM, Predhomme J, et al. Tenofovir, emtricitabine, and tenofovir diphosphate in dried blood spots for determining recent and cumulative drug exposure. AIDS Res Hum Retroviruses. 2013;29(2):384-90.
176. National Institutes of Health. Guidelines for the Inclusion of Women and Minorities as Subjects in Clinical Research. Federal Register.; 1994 March 18.
177. National Institutes of Health. NIH Policy and Guidelines on the Inclusion of Children as Participants in Research Involving Human Subjects. 1998 March 6.

Appendix IA (Schedules of Evaluation): Maternal PK Component Group 1 and Group 2

MATERNAL PROCEDURES	SCR	Entry (Day 0)	Week (± 1 day)												Group 1 only
			1	2	3	4	5	6	7	8	9	10	11	12/ Exit and Early D/C[1]	L&D[2]
Informed Consent	X														
Medical and Drug History	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X	X				X				X				X	X
Study drug dispensing		X				X				X					
iNSC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy Outcome															X
Demographic/Behavioral Questionnaires		X												X[3]	
HIV RNA[4]; store plasma for HIV drug resistance testing[5]	4mL	6mL				6mL				6mL				6mL	6mL
Rapid HIV[4,6]	X	X				X				X				X	X
TFV-DP level (DBS)		4mL	4 mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL
Complete Blood Count	3mL													3mL	
ALT, creatinine, CrCl	4mL					4mL								4mL	
Hepatitis B surface antigen	3mL													3mL	
Glucose (random)	1mL														
Chlamydia/gonorrhea (swab)		X													
Urinalysis (protein and glucose)	X														
Ultrasound (Group 1 only)	X														
Pregnancy Test (Group 2 only)[7]														X	
Total maximum blood volume	15mL	10mL	4mL	4mL	4mL	14mL	4mL	4mL	4mL	10mL	4mL	4mL	4mL	21mL	10mL

[1] If the participant has not delivered at 12 weeks of follow-up, participants may choose to continue in follow-up until delivery, refer to Section 5.1.2.1
[2] If within the window of one of the weekly visits, visits may be combined.
[3] Questionnaire is done at Week 12 visit only; questionnaire is not required at early D/C.
[4] If positive refer to Section 6.3 and Appendix IID for additional procedures.
[5] Store plasma for HIV drug resistance testing at all visits indicated EXCEPT Screening.
[6] Blood collected for other tests will be used for the rapid HIV test. Collection of additional blood is not required.
[7] Urine (5mL) or blood (1mL) tests are acceptable. The total blood volume shown above accommodates collection of 1mL of blood, if needed.

Appendix IB (Schedule of Evaluations): Infant PK Component Group 1

INFANT PROCEDURES: Group 1	Birth	PP Wk 6	Early D/C
Medical and Medications History	X	X	X
Physical Examination	X	X	X
Infant Feeding History	X	X	X
LABORATORY EVLUATIONS			
Creatinine	1mL	1mL	1mL
TFV-DP level (DBS)	0.5mL		
Total maximum blood volume	1.5mL	1mL	1mL

Appendix IC (Schedule of Evaluations): Infant PK Component Group 2

INFANT PROCEDURES: Group 2	Entry (Day 0)	Wk 6	Wk 12 and Early D/C
Medical and Medications History	X	X	X
Physical Examination	X	X	X
Infant Feeding History	X	X	X
LABORATORY EVLUATIONS			
Creatinine	1mL	1mL	1mL
TFV-DP level (DBS)	0.5mL	0.5mL	
Total maximum blood volume	1.5mL	1.5mL	1mL

Appendix IIA (Schedule of Evaluations): Maternal PrEP Comparison Component: Cohort 1, Cohort 2/Step 2

MATERNAL PROCEDURES:	SCR	Entry	Wk 4	Wk 8	Wk 12	Wk 24	Wk 36	L&D	PP Wk 6	PP Wk 14	PP Wk 26 / Exit and Early D/C	Interim Visit
Informed Consent	X											As per Section 6.5
Medical and Drug History	X	X	X	X	X	X	X	X	X	X	X	
Physical Examination	X	X	X	X	X	X	X	X	X	X	X	
Study drug dispensing		X	X	X	X	X	X	X	X	X		
Assess for social harms			X	X	X	X	X	X	X	X	X	
iNSC	X	X									X	
iNSC with drug level feedback			X	X	X	X	X	X	X	X		
CASI evaluations and COVID-19 questionnaire		X	X	X	X	X	X			X	X[1]	
Qualitative IDI					X					X		
HIV RNA[2]; store plasma for HIV drug resistance testing[3]	4mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	
Rapid HIV[2, 4]	X	X	X	X	X	X	X	X	X	X	X	
TFV-DP level (DBS)		4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	4mL	
Complete Blood Count	3mL									3mL		
ALT, creatinine, CrCl	4mL		4mL		4mL	4mL	4mL	4mL		4mL	4mL	
Hepatitis B surface antigen	3mL										3mL	
Syphilis		1mL									1mL	
HSV2		1mL									1mL	
Glucose (random)	1mL											
Chlamydia/gonorrhea (vaginal swab or urine)		X									X	
Microbiome (rectal swabs); if consent provided		X	X[5] (EGA 34-38/40 weeks)						X		X	
Microbiome (vaginal swab)		X	X[5] (EGA 34-38/40 weeks)						X		X	
Vaginal secretion sampling for drug level			X[5](EGA 34 -38/40 weeks)						X		X	
Urinalysis (protein and glucose)	X											
Ultrasound	X											
DXA scan (Hip, Lumbar Spine)[6]								X			X	
Pregnancy Test[7]										X	X	
Total maximum blood volume	15mL	12mL	14 mL	10mL	14mL	14mL	14mL	14mL	10mL	18mL[7]	20mL[7]	

[1] CASI is done at Week 26 visit only; CASI is not required at early D/C.
[2] If positive refer to Section 6.3.1 and Appendix IID for additional procedures.
[3] Store plasma for HIV drug resistance testing at all visits indicated EXCEPT Screening.
[4] Blood collected for other tests will be used for the rapid HIV test; collection of additional blood is not required.
[5] Collect sample at any visit which occurs at an estimated 34 – 38/40 weeks' gestation.
[6] DXA scan at Wk 26/Exit and early D/C (as applicable) must be preceded by a pregnancy test.
[7] Urine (5mL) or blood (1mL) tests are acceptable. The total blood volume shown above accommodates collection of 1mL of blood, if needed. *Note: Pregnancy testing may be done prior to the DXA scan at Wk 26/Exit and Early D/C and does not need to be repeated.*

Appendix IIB (Schedule of Evaluations): Maternal PrEP Comparison Component Cohort 2

MATERNAL PROCEDURES:	SCR	Entry	Wk 4	Wk 8	Wk 12	Wk 24	Wk 36	L&D	PP Wk 6	PP Wk 14	PP Wk 26 / Exit and Early D/C	Step Change	
												Visit 1	Visit 2
Informed Consent	X												
Medical and Drug History	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical Examination	X	X	X	X	X	X	X	X	X	X	X	X	X
Study drug dispensing													X
Assess for social harms			X	X	X	X	X	X	X	X	X	X	X
iNSC	X	X	X	X	X	X	X	X	X	X	X	X	X
CASI evaluations and COVID-19 questionannire		X	X	X	X	X	X			X	X[1]	X	X
Qualitative IDI					X					X			
HIV RNA[2]; store plasma for HIV drug resistance testing[3]	4mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	6mL	
Rapid HIV[2,4]	X	X	X	X	X	X	X	X	X	X	X		X
TFV-DP level (DBS)		4mL						4mL					4mL
Complete Blood Count	3mL									3mL		3mL	
ALT, creatinine, and CrCl	4mL		4mL		4mL	4mL	4mL	4mL		4mL	4mL	4mL	
Hepatitis B surface antigen	3mL										3mL	3mL	
Syphilis		1mL									1mL		
HSV2		1mL									1mL		
Glucose (random)	1mL											1mL	
Chlamydia/gonorrhea (vaginal swab or urine)		X									X		
Microbiome (rectal swabs); if consent provided		X	X[5](EGA 34-38/40 weeks)						X		X		X
Microbiome (vaginal swab)		X	X[5](EGA 34-38/40 weeks)						X		X		X
Urinalysis (protein and glucose)	X											X	
Ultrasound	X												
DXA scan (Hip, Lumbar Spine)[6]								X			X		
Pregnancy Test[7]										X	X		
Total maximum blood volume	15mL	12mL	10mL	6mL	10mL	10mL	10mL	14mL	6mL	14mL[7]	16mL[7]	17mL	4mL

[1]CASI is done at Week 26 visit only; CASI is not required at early D/C.

[2] If positive refer to Section 6.3.1 and Appendix IID for additional procedures.

[3]Store plasma for HIV drug resistance testing at all visits indicated EXCEPT Screening.

[4]Blood collected for other tests will be used for the rapid HIV test; collection of additional blood is not required.

[5]Collect sample at any visit which occurs at an estimated 34 – 38/40 weeks' gestation.

[6]DXA scan at Wk 26/Exit and Early D/C (as applicable) must be preceded by a pregnancy test.

[7]Urine (5mL) or blood (1mL) tests are acceptable. The total blood volume shown above accommodates collection of 1mL of blood, if needed. *Note: Pregnancy testing may be done prior to the DXA scan at Wk 26/and Early D/C and does not need to be repeated.*

Appendix IIC (Schedule of Evaluations): Infant PrEP Comparison Component (both cohorts)

INFANT PROCEDURES	Birth	PP Wk 6	PP Wk 14	PP Wk 26	Early D/C
CLINICAL EVALUATIONS					
Medical and Medications History	X	X	X	X	X
Physical Examination	X	X	X	X	X
Infant Feeding History	X	X	X	X	X
OTHER EVALUATIONS					
DXA scan: Whole Body	X				
Lumbar spine	X			X	
LABORATORY EVALUATIONS					
Creatinine	1mL			1mL	1mL
TFV- DP level (DBS)	0.5mL				
Microbiome (rectal swab or fresh stool)	X	X	X	X	X
Total maximum blood volume	1.5mL			1mL	1mL

Appendix IID (Schedule of Evaluations): Maternal Suspected or Confirmed HIV Infection

Note: Maternal participants continue to follow the relevant Schedule of Evaluations in Appendices IA, IIA, and IIB. These procedures are considered additional procedures and visits can be combined.

	Both PK and PrEP Comparison Components	
MATERNAL PROCEDURES	**Confirmatory Visit**	**Confirmed Infected 4 weeks after date of initial HIV positive blood draw**
CLINICAL EVALUATIONS		
Referral for HIV care and treatment (and PMTCT if pregnant or breastfeeding)	X	X
Interim medical and medication history		X
iNSC	X	
LABORATORY EVALUATIONS		
Confirmatory HIV Test[1]	[Up to 10mL]	
Store plasma and non-viable PBMCs for HIV drug resistance testing	12mL	12mL
CD4 Count	3mL	3mL
Total maximum blood volume	25mL	15mL

1. HIV confirmatory testing will follow the site-specific approved HIV testing algorithm and Section 6.3.1.

Appendix IIE (Schedule of Evaluations): Infant HIV Exposure and Infection

Note: Infant participants continue to follow the relevant Schedule of Evaluations in Appendices IB, IC or IIC. These procedures are considered additional procedures and visits can be combined.

	HIV Exposed		HIV Infected	
INFANT PROCEDURES	**At Maternal Confirmatory Visit (at delivery if positive test was during pregnancy)**	**At every study visit thereafter**	**Confirmatory Testing (obtain as soon as possible after initial positive result)**	**At every study visit thereafter**
CLINICAL EVALUATIONS				
Referral to primary care provider	X			
Referral to HIV care and treatment			X	
LABORATORY EVALUATIONS				
HIV nucleic acids testing (NAT)	3mL	3mL	3mL	
Complete Blood Count				0.5mL
Lymphocyte subsets				1mL
Stored plasma and non-viable PBMCs for resistance testing			2mL	2mL
Total maximum blood volume	3mL	3mL	5mL	3.5mL

Appendix III: Targeted Medications List

All medications specified below must be entered into eCRFs.

- **Antimicrobial agents** when administered by the following routes:
  - Oral
  - Vaginal
  - Intramuscular
  - Intravenous

- **Drugs that may Interfere with Tenofovir Excretion**
  - Adefovir
  - Antineoplastics
  - Cidofovir
  - Cyclosporin A
  - Indomethacin
  - Penicillins
  - Probenecid
  - Rifampin
  - Sirolimus
  - Ursodiol
  - (Val)ganciclovir hydrochloride

- **Hormonal Contraceptives** (Including hormones used for feminization)

- **Other Hormonal Therapy**
  - Thyroid replacement
  - Anabolic agents

- **Inhaled Steroids**

- **Systemic Glucocorticoids**

- **Antiepileptics**

- **Antidepressants**
  - Serotonin release inhibitors
  - NRI

- **Lipid Lowering Agents**

- **Appetite Stimulants**

- **Herbal Medicine**
  - St. John's Wort
  - Garlic supplements
  - Echinacea

- **Over the Counter Agents** (sold under a variety of trade names)
  - Androstenedione
  - Dihydroepiandrosterone, DHEA
  - Fish oil (n-3 fatty acids)
  - GH-releasers (arginine, ornithine, lysine)
  - β-hydroxy-β-methylbutyrate (betaHMB)

Appendix IV: General Management Guidelines for Maternal Toxicities

General Toxicities		
SEVERITY	**STUDY DRUG USE**	**FOLLOW-UP AND MANAGEMENT**
Grade 1 and Grade 2 Clinical and laboratory adverse events regardless of relatedness	Study drug may be continued at the discretion of the site clinician	Monitor consistent with the SoE (no additional evaluations are required). Consultation with the CMC is available but not required.
Grade 3 and Grade 4 Clinical adverse events assessed as not related to study drug	Continue study drug	Inform the CMC. Monitor every 7-10 days for improvement to Grade 2 or lower, unless instructed otherwise by the CMC. If severity worsens, or does not improve to Grade 2 or lower within 14 days, inform and request guidance from the CMC.
Grade 3 and Grade 4 Clinical adverse events assessed as related to study drug	Hold study drug	Consult the CMC for further guidance on study drug management. Monitor every 7-10 days for improvement to Grade 2 or lower, unless instructed otherwise by the CMC.

General Toxicities		
SEVERITY	**STUDY DRUG USE**	**FOLLOW-UP AND MANAGEMENT**
Grade 3 laboratory adverse event	Study drug may be continued at the discretion of the site clinician pending receipt of confirmatory test results.	• Repeat the laboratory test within 7-10 days. • If the repeat test result is Grade 2 or lower, manage per the grade of the repeat test. • If the repeat test is Grade 3, inform the CMC and continue to monitor every 7-10 days until improvement to Grade 2 or lower, unless instructed otherwise by the CMC. – If the confirmed Grade 3 event is assessed as <u>not related</u> to study drug, study drug may be continued. If severity worsens, or does not improve to Grade 2 or lower within 14 days, inform and request guidance from the CMC. If the confirmed Grade 3 event is assessed as <u>related</u> to study drug, <u>hold</u> study drug until improvement to Grade 2 or lower, at which time re-challenge with study drug may be considered in consultation with the CMC. Following re-challenge (if applicable), if the Grade 3 event recurs, permanently discontinue study drug. If severity worsens, or does not improve to Grade 2 or lower within 14 days, inform CMC and permanently discontinue study drug.
Grade 4 laboratory adverse event	Hold study drug pending receipt of confirmatory test results.	• Repeat the laboratory test within 3 business days. • If the repeat test result is Grade 3 or lower, manage per the grade of the repeat test. • If the repeat test is Grade 4, continue to hold study drug. Inform the CMC and continue to monitor every 7-10 days until improvement to Grade 2 or lower, unless instructed otherwise by the CMC. • If severity does not improve to Grade 2 or lower within 14 days, inform the CMC and permanently discontinue study drug. • If severity improves to Grade 2 or lower, re-challenge may be considered in consultation with the CMC. Following re-challenge (if applicable), if the event recurs at Grade 3 or higher, permanently discontinue study drug.

Appendix V: General Management Guidelines for Maternal Increased Creatinine and Reduced Creatinine Clearance

Maternal Increased Creatinine and Reduced Creatinine Clearance		
CONDITION AND SEVERITY	**STUDY DRUG USE**	**FOLLOW-UP AND MANAGEMENT**
All grades		• Assess participant for other potential causes of decreased renal function per site standard of care. • Management action should be taken based on the absolute value of each participant's creatinine value and estimated CrCl (not change from study baseline). • Management should be based on the most severe result (creatinine or creatinine clearance).
Grade 1 increased creatinine	Continue study drug	Follow-up per SOE
Grade 2 increased creatinine OR reduced creatinine clearance (estimated CrCl <90 to ≥60 mL/min)	Continue study drug	Repeat the test and perform urinalysis within 1 week of site awareness. • If the repeat test does not confirm Grade 2 severity, follow the management guidance for that severity grade. • If the repeat test confirms Grade 2 severity: o Inform the CMC, and o Monitor creatinine and CrCl every 2 weeks until creatinine and CrCl are stable or improving, and o Update the CMC with each new result
Grade 3 or Grade 4 increased creatinine OR reduced creatinine clearance (estimated CrCl <60mL/min)	Hold study drug while awaiting confirmatory test result.	Repeat the test and perform urinalysis within 3 business days of site awareness. • If the repeat test does not confirm Grade 3 or Grade 4 severity: o Resume study drug at the site clinician's discretion, and o Follow the management guidance for that severity grade • If the repeat test confirms Grade 3 or Grade 4 severity: o Inform the CMC, and o Permanently discontinue study drug, and o Monitor creatinine and CrCl at the frequency set by the CMC, and o Update the CMC with each new result

Appendix VI: Sample Informed Consent Form for PK Component (Groups 1 & 2)

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

Introduction

We invite you to take part in a research study with your baby named IMPAACT 2009. There are two parts to this study, and this form is for the first part – the "PK Component."

This form gives information about both parts of the study. Please read it, or have it read to you, and ask any questions you may have. You can take as much time as you need to fully understand the study. We will ask you questions to see if we have explained the study clearly.

After you understand the study, if you decide that you will participate with your baby, we will ask you to sign or make your mark on this form. You will be offered a copy to keep.

About the Study

The International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) and *[insert site name]* are doing this study to learn more about a medication called "PrEP." PrEP stands for "pre-exposure prophylaxis." For people who are not infected with HIV, but may be at risk of becoming infected with the HIV virus, taking medication called PrEP can help protect them. Other studies have shown that PrEP works very well in preventing HIV infection. We also know that the level of protection PrEP provides depends on whether someone takes PrEP every day. So far, not many women have taken PrEP while they are pregnant and just after delivery, even though women may be at heightened risk of becoming infected with HIV during this period.

The purpose of the IMPAACT 2009 study is to find out whether young women in sub-Saharan Africa at heightened risk of becoming infected with HIV are interested and would be willing to take a PrEP pill once a day while they are pregnant and breastfeeding. The purpose of the first part of the study (PK Component) is to find out how much PrEP medication can be measured in the blood of women who take PrEP every day without missing any doses while they are pregnant and after delivery.

We invite you to join the PK Component of this study because you are not infected with HIV and are pregnant or have just delivered. The PK Component of this study will include up to 40 mothers from clinical sites in Sub-Saharan Africa. Mothers who join the study after delivery will be in the study with their babies for twelve weeks. Mothers who join the study while pregnant will be in the study for at least twelve weeks, through labor and delivery. Their baby will also be seen when they are six weeks old.

The person in charge of the study at *[insert site name]* is *[insert name of IoR]*. The United States National Institutes of Health is paying for the study.

1. There are two parts to this study. You and your baby are being asked to join the first part – the PK Component.

The two parts of the study are called the "PK Component" and the "PrEP Comparison Component." The PK Component will be done first. This part will include up to 40 mothers with their babies; half will join when they are pregnant (Group 1) and the others will join in the period after their delivery (Group 2).

If you join the PK Component, you will take pills every day and have a study check-up every week for 12 weeks.

When the PK Component has been completed, the PrEP Comparison Component will be done and this part will include about 350 mothers and their babies. If you participate in the PK Component, you will not be able to participate in the PrEP Comparison Component.

2. The study will assess PrEP use in women who are pregnant and just after delivery and their babies.

The medicine being used for PrEP in this study is called Truvada®. Truvada® is a pill that contains two medications called "emtricitabine" and "tenofovir" that are commonly used to treat HIV infection and sometimes to treat Hepatitis. We know that Truvada® is safe and effective in reducing the risk of HIV infection when taken by people who are HIV uninfected. But, not many studies about PrEP have involved HIV uninfected women while they are pregnant and after delivery, so we need more information during this period.

Truvada® is approved for PrEP in many countries around the world. In *[insert site country]*, PrEP is approved for *[insert details as relevant, e.g. of population in which PrEP is approved and if PrEP is national policy]*.

3. The PK Component part will assess how much PrEP medication can be measured if a woman takes it daily.

The PK Component will be done to look at how much of the PrEP drug is in your blood when you take PrEP every day for 12 weeks, during pregnancy (Group 1) or after delivery (Group 2). We will also measure how much PrEP is passed on to your baby.

We need to do these measurements because the body changes during pregnancy, so the amount of PrEP drug found in the blood of pregnant women may be different from non-pregnant women. The information we gather from this part of the study will be used later to help us counsel women about how well they are taking PrEP and how well they are protected from HIV.

4. Only mothers who qualify can participate in the study.

If you decide to join the study with your baby, we will first do some tests to see if you qualify. More information about the tests is given in #5 (see below). If you qualify, you and your baby will enter the study. If you do not qualify, you and your baby cannot enter the study.

If you qualify, you can choose if you want to join the study or not. You are free to join or not join. If you join, you can change your mind later and leave the study. Your decision will have no effect on the medical care that you and your baby would normally receive from your usual clinic. Your access to services, and the benefits and rights you normally have, will not be affected.

Take your time and consider your decision carefully. If you wish, you can talk to other people about the study. You can also bring other people here to learn about the study with you.

Finding out if You and Your Baby Qualify – Visit 1

5. We will ask questions, examine you, and discuss the study requirements with you.

To find out if you and your baby qualify for the study, we will:

- Review your medical records
- Ask about your health and any medicines you or your baby take
- Talk with you about the study requirements and if you meet them
- Have you talk with a counselor about your health behaviors
- Give you a physical examination (and your baby, if already delivered)
- Collect urine to check how well your kidneys are working and if there is sugar is in your urine
- Collect your blood (up to 15 mL or about 3 teaspoons) for tests. The tests will:
  - Check your blood cells
  - Check if you have Hepatitis B (a disease of the liver)
  - Check how well your liver and kidneys are working
  - Check your blood to confirm that you are not infected with HIV.
  - Check your blood sugar levels

These procedures will take about 2 hours *[here and throughout this form, sites may modify the expected visit duration as needed]*.

6. <u>If you are pregnant</u>, we will also do an ultrasound scan.

We need to know how long you have been pregnant when you enter the study and whether you may be pregnant with more than one baby. To do this, you will have an ultrasound scan and we will ask you the date of your last menstrual period *[sites may use locally appropriate terminology to refer to the scan]*. The scan uses sound waves to check the size of your baby, which shows how long you have been pregnant. Ultrasound scans are commonly done among pregnant women.

To do the scan, gel is placed on your belly. Then a small device is moved back and forth on your belly to send and receive sound waves. The scan will show a picture of your baby and measure the size.

[Sites to modify first three sentences as needed:] The scan will be done at *[insert clinic name and/or location]*. It will take less than 30 minutes. We will arrange for you to have the scan at a scheduled time before entering the study. There is no cost for having the scan done.

If you have already had an ultrasound and have the report, this may not be needed.

7. We will tell you if you and your baby qualify.

We will give you the results of all procedures and explain them to you.

If you or your baby have any medical problems, or you do <u>not</u> qualify for the study for any reason, we will tell you this. You will <u>not</u> be entered in the study.

If you do qualify for the study and you agree to join with your baby, you will enter the study.

Entering the Study – Visit 2

8. If you and your baby qualify, you will enter the study within a couple of weeks.

When you enter the study, we will:

- Ask about your health and the medicines you take.
- Have you talk with a counselor about your health behaviors and how to take PrEP.
- Ask you questions about your health behaviors.
- Give you a physical exam.
- Collect a vaginal swab to check if you have sexually transmitted infections such as chlamydia or gonorrhea. For this specimen, a small swab is inserted into the vagina to collect some mucus.
- Collect your blood (10 mL or about 2 teaspoons) for tests. These tests will:
  - Confirm you are not infected with HIV.
  - Check if any PrEP is in your blood.
- Give you a supply of PrEP pills.
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests.

It is very important that you take the PrEP pill once a day, <u>every day for 12 weeks</u>.

Being in the Study

9. You will take PrEP under Directly Observed Therapy (DOT)

This is **the most important** part about being in the PK Component. <u>We need to check</u> that you swallow the PrEP pill every day to understand the levels of PrEP medication we measure in your blood. To do this, study staff will contact you **every day** during the 12 weeks you take PrEP; we call this directly observed therapy or DOT. Some women may prefer to take their pill in front of a study staff member at the clinic or at home each day. Other women may choose to video record themselves taking their pill or take their pill while on a video call with study staff using a smartphone, tablet or computer to confirm that they have taken their pill. We will talk with you to work out a suitable DOT plan if you decide to join the study.

If you complete all your visits before delivering your baby, you may choose to continue taking PrEP until labor and delivery.

If you don't want us to contact you <u>every day for 12 weeks</u>, then you should not join the PK Component of the study.

10. You will have a visit every week for 12 weeks.

Each visit will take about 1-2 hours. You will need to return to the clinic for visits at Weeks 4, 8 and 12. For the other visits, you can either return to the clinic or the study staff can visit you at an off-site location which can include your home. The study staff will discuss the location and best time to visit you so that the visit is at a convenient time and the visit is done at a place you feel comfortable and confidentiality can be maintained. If you have already delivered when you enter the PK Component (Group 2), you will come with your baby.

At these visits for all <u>women</u> we will:

- Review your medical records
- Ask about your health and the medicines you take
- Give you a physical exam *(at Weeks 4, 8 and 12 only)*
- Collect your blood (up to 4-21 mL or about 1-4 teaspoons) for tests. At different visits, the tests will:
  - Check your blood cells
  - Check if you have Hepatitis B
  - Check how well your liver and kidneys are working
  - Confirm you remain HIV uninfected
  - Check how much of the PrEP drugs are in your blood
- Have you talk with a counselor about your health behaviors
- Ask questions about how easy it is to take your PrEP every day and about your health behavior (*at Week 12 only)*
- Give you a supply of PrEP pills
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests

11. Your baby will also have study visits

<u>If you are pregnant</u> when you join the study (Group 1), you and your baby will also have a study visit soon after delivery and at 6 weeks.

You should contact us when your labor begins to arrange for a study visit as soon as possible after delivery. It is important for this visit to take place close to the baby's birth. This visit will take up to 4 hours.

When you come to the clinic with your baby for the visit soon after delivery we will:

- Review your and your baby's medical records
- Have you talk with a counselor about your health behaviors
- Ask about your and your baby's health and the medicines you take
- Ask about how you are feeding your baby
- Give you and your baby a physical exam
- Collect blood from you (up to 10 mL or about 2 teaspoons) for tests:
  - To check how much PrEP is in your blood
  - To confirm you remain HIV uninfected
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests
- Collect blood from your baby (up to 1.5 mL or less than ½ teaspoon)
  - To check how much PrEP is in your baby's blood
  - To confirm your baby's kidneys are working well (at the first visit only)

Your next study visit for your baby only will be when your baby is about 6 weeks of age. This visit will take about 2 hours and will be a repeat of most of the procedures which took place soon after delivery.

If you join the study after delivery (Group 2) your baby will have a study visit on the day you enter the study, after 6 weeks and after 12 weeks.

When you come to the clinic with your baby for these visits we will:

- Review your baby's medical records
- Ask about your baby's health and medicines
- Ask about how you are feeding your baby
- Give your baby a physical exam
- Collect blood from your baby (up to 1.5 mL or less than ½ teaspoon)
  - To check how much PrEP is in your baby's blood
  - To confirm your baby's kidneys are working well (at the first, sixth and twelfth visit only)

While you are in the study, we will give you the results of the tests for you and your baby. We may need to repeat a blood test for you or your baby. If you become infected with HIV during the study, we still want you and your baby to complete all your study visits, but it is your choice. We will discuss with you how to get care and treatment for HIV outside the study and arrange it with the staff there *[site to revise as needed]*.

12. If you become infected with HIV during the study, you and your baby will have extra visits.

We will test you for HIV throughout the study using a rapid test in the clinic and another type of HIV test in the laboratory (RNA PCR) test. If your HIV test results are positive, we will take more blood to confirm the HIV infection and measure the HIV viral load (how much HIV is in the blood). It usually takes about a week for these results to come back.

These extra visits will take about 1 hour. At these visits, we will:

- Have you talk with a counselor about your health behaviors
- Refer you for HIV care and treatment.
- Collect your blood (15-25 mL or about 3.5 to 5 teaspoons) for tests.

The tests will:

- Confirm your HIV viral load
- Check your cells
- Check if the HIV in the blood is resistant to the medications in the PrEP pill
- Save some of the blood for additional tests about the HIV virus
- Collect any remaining PrEP pills from you

13. Tests will be done at our laboratory.

We will do tests of you and your baby's specimens at our laboratory. Most of the tests will be done right away at a local facility. We will give you the results of these tests and explain the results to you. However, some specimens will be kept and tested together at the end of the study. If the results show that you or your baby may need medical care or treatment that cannot be provided by the study, we will tell you where you can go for this care. Some tests, such as the PrEP drug levels, are special and must be sent to specialized laboratories outside the country. If there are any specimens left-over after all the tests for this study have been done for you and your baby, we would like to keep those specimens because they may be useful for studies in the future. We will talk to you about what you want to happen to these left-over specimens in another informed consent form.

14. We may stop your PrEP.

The examinations and laboratory tests we do during the study will help us to tell if the PrEP you are taking is causing any bad effects. If so, the PrEP may be stopped. PrEP may be stopped if you have certain illnesses or need to take other medicines that cannot be taken with PrEP. We will always discuss stopping PrEP with you. Please tell us about any problems you may have with taking PrEP.

We may stop the PrEP you are receiving from the study if:

- You are not able to come to study visits
- You are not able to take PrEP every day
- Taking PrEP may be harmful to you
- You ask to stop taking PrEP

15. We may take you and your baby off the study early.

We expect you to stay in the study until you and your baby have completed all the study visits; Group 1 – until the baby is six weeks old; Group 2 – until the baby is about 3 months old. However, we may take you and your baby off the study early if:

- The study is stopped for any reason
- We determine that you and your baby cannot meet the study requirements (for example, if you move away and cannot come to the clinic)
- We determine that staying in the study might harm you or your baby

We will ask you and your baby to come to the clinic for one last visit. At this visit, we will do the same types of procedures we do at the weekly study visits (see #10 and #11). We will answer any questions you may have and tell you how to contact us in the future, if you wish.

16. Please tell us if you want to leave the study.

You and your baby are free to leave the study at any time for any reason – you just need to tell us. The care that you and your baby would normally receive will not be affected, but it is important that we know your decision. We will ask you and your baby to come to the clinic for one last visit. At this visit, we will do the same types of procedures we do at the weekly study visits (see #10 and #11 above). We will answer any questions you may have and tell you how to contact us in the future, if you wish.

17. How to stay uninfected after leaving the study.

We cannot give you PrEP after you finish your study visits. Before you leave the study, we will talk again about how to protect yourself against HIV infection and give you information about other clinics that offer PrEP to women. If you want to continue taking PrEP, we will refer you there. *[Sites to modify as necessary and insert information about referral letters and sharing study laboratory results with the non-study PrEP provider, as appropriate]*

18. If you joined the study after delivery, we will test for pregnancy during the study.

If you were not pregnant when you entered the study, we will test for pregnancy during the study. If you become pregnant, you will continue in the study and may continue to take PrEP during the study. If you choose to continue taking PrEP after you leave the study, we will refer you to clinics that offer PrEP to women. We will contact you after your last study visit to find out the outcome of your pregnancy.

Risks of the Study

19. There may be risks from the study procedures.

Most procedures done in this study are routine medical procedures, with little risk to you and your baby.

Blood Collection: Collecting blood can cause pain, swelling, bruising, or bleeding where the needle is inserted. Rarely, drawing blood can cause fainting or infection where the needle enters the skin.

Urine Collection: There is no risk from collecting your urine

Ultrasound scan: There is no risk from ultrasound

Vaginal swab collection: You may feel a little discomfort when the swab is inserted into the vagina. The study staff will show you the swab and you can ask any questions about this procedure before it is done.

HIV Testing: We will perform an HIV test at about five visits, which is done to make sure that you are HIV uninfected before starting or continuing PrEP. You will be counseled before and after the test is done. *[Sites to insert reporting responsibilities in the country the site is located in. Also include whether if a participant tests positive, the results will become part of public health records, or any other record (medical file, etc.)]* You can come to the clinic and ask for an HIV test anytime you are worried.

STI Testing: You will be tested for STIs. *[Insert here any reporting responsibilities for your state or local jurisdictions or reporting of these infections to public health authorities].* If you have any of these infections, we will provide you with treatment.

Acquiring HIV Infection and Drug Resistance: You must understand this information about what PrEP can and can't do:

- PrEP cannot give you HIV infection
- Taking PrEP prevents HIV in most people who take it every day, but
- **You still may become infected with HIV while you are taking PrEP**
- **PrEP does not prevent pregnancy or STIs**
- You still need to use other methods to avoid HIV infection and other STIs while you are taking PrEP, like using condoms every time you have sex and keeping your number of sexual partners low. Study staff will discuss all the different ways you can keep yourself from getting these infections.

If you become infected with HIV while you are taking PrEP, the strain of the HIV virus in your blood might be "resistant" to Truvada® and some other medications used for HIV treatment (ART). "Resistance" means the virus can't be killed by that medication, which makes treating the HIV more difficult. If you become infected with HIV, we will test for HIV drug resistance. You will be able to discuss ART and resistance with the study staff.

If you have any questions about anything we have said here, please talk to us.

Other Risks: Also, there may be uncommon risks or risks we don't know about yet that might occur. You should report any problems to the study staff immediately. There may also be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions we will ask you, some of the procedures that will be done, or some of the test results that you will receive. You may also experience stigma as a result of being involved in a study about HIV because people may assume that you are HIV-infected. If you have HIV or other infections, knowing this could make you worried. Study staff are trained to help you deal with any feelings or questions you have.

20. There are risks from PrEP(Truvada®)

You or your baby may feel sick while participating in the study. These problems may be due to taking PrEP or due to an illness that has no relation to the study, like a cold or flu. You should tell us about any symptoms that you feel while you are participating in the study. You will be given a telephone number to contact the clinic.

You should contact us if you experience any symptoms at any time.

In PrEP research studies, nausea and diarrhea were the most common side effects, but happened in only about 10% or one in ten people. Nausea and diarrhea mainly happened in the first month and then went away. A small number (<1% or one in one hundred people) in PrEP studies showed a small decrease in how their kidneys work, but this stopped when the people stopped taking the drug.

Other side effects, such as changes in bone mineral density (how much calcium and other minerals are in your bone which keeps them strong) were very rare in people taking the drug who did not have HIV and have always gotten better when the drug was stopped.

You could have these side effects or other side effects that we do not know about. Please tell the staff here if you have any side effect that bothers you or does not go away.

Other Medications: Some medications should not be taken while you are taking Truvada®. When you visit the study clinic, we will ask you about other medications that you are taking.

Risks to your baby: The medicines in PrEP (Truvada®) are often used to treat women with HIV infection. Many HIV-infected women have taken these medicines while pregnant and breastfeeding. We know that these medicines pass through to the baby in the womb and are found in breastmilk in small amounts. The information we have so far suggests that these medicines do not cause serious problems in the baby. However, it is still possible that taking PrEP may cause some problems in the baby soon after birth or when they are older. The study staff will check for side effects in babies during study visits and tell you what to do if your baby has any side effects. You should contact the study staff at any time if you are worried about the baby's health.

Truvada® may have side effects that no one knows about yet. We will let you know if we learn anything that might make you change your mind about continuing to participate in the study.

21. There could be risks of disclosure of your or your baby's information.

We will make every effort to keep you and your baby's information private and confidential. Study records and specimens will be kept in secure locations. All specimens and most records will be labeled only with a code number. However, you and your baby's names will be written on some records that are kept in the clinic. Despite our best efforts to keep this information private, it is possible that the information could be obtained by someone who should not have it. If this were to happen, you or your baby could be treated badly or unfairly. You could feel stress or embarrassment.

While you are in the PK Component, you will be contacted every day for 12 weeks by the study staff. Because of this, the people you live with may realize that you are involved in a study and could ask you questions about it. You can talk to study staff about how to handle this situation before you decide to join.

Benefits of the Study

22. There may be no benefit from being in the study.

We will test you for HIV and other STIs throughout this study. If you take your Truvada® every day, it will help you to avoid HIV. Participating in this study may give you knowledge and skills that may help you to avoid HIV and other STIs. What we learn about PrEP use in young women may help women in the future decide if they want to take PrEP while they are pregnant and breastfeeding.

Other information about the study

23. There are no costs to you for being in the study.

There are no costs to you or your baby for study visits or procedures or the PrEP that is given by the study, and you will not receive payment for participating in the study.

[Insert information about compensation/reimbursement here, e.g., You will be reimbursed for the cost of transport to study visits. For each visit, you will be given (specify amount).*]*

24. Study records may be reviewed by study staff and groups that oversee the study.

Groups that oversee the study include:

- *[insert name of site IRB/EC]*
- *[insert name of site drug regulatory authority]*
- *[insert name of other site regulatory entities]*
- The United States National Institutes of Health and its study monitors
- The United States Food and Drug Administration (FDA)
- The Office for Human Research Protections (OHRP)
- Other U.S., local, and international regulatory entities
- The IMPAACT Network that is coordinating the study
- Gilead Sciences (the company that makes Truvada®)

The study staff and these groups are required to make every effort to keep study records private and confidential.

The results of the study may be presented publicly or published. However, no presentation or publication will use your name or your baby's name or identify you or your baby personally.
A description of this study will be available on a website called www.ClinicalTrials.gov.This website will not include information that can identify you or your baby. At most, the website will include a summary of the results of the study. You can search this website at any time.

Your and your baby's study information may be disclosed to other authorities if required by law.

25. If you or your baby gets sick or injured, contact us immediately.

Your and your baby's health is important to us. We will make every effort to protect your well-being and minimize risks. It is possible, however, that you or your baby could have an illness or injury that is study-related. This means the illness or injury occurred as a direct result of being in the study.

[Sites may modify this paragraph to reflect local institutional policies; information regarding coverage available through clinical trial insurance obtained by the site should be included if applicable; the statement regarding no program for compensation through the NIH may not be removed.] If a study-related illness or injury occurs, we will treat you or your baby or tell you where you can get the treatment you or your baby need. The cost for this treatment may be charged to you or your health insurance. There is no program to pay money or give other forms of compensation for study-related illness or injury through *[site name or]* the United States National Institutes of Health.

Who to Contact

26. If you have questions, concerns, or problems at any time, use these contacts.

- If you have questions about the study:
  [insert name and telephone number of investigator or other study staff]

- If you have questions about your rights or your baby's rights as research participants or concerns about how you or your baby are being treated in the study:
  [insert name and telephone number of IRB/EC contact person or other appropriate person/organization]

- If you or your baby have any health or other problems that may be related to study participation:
  [insert name and telephone number of investigator or other study staff]

- If you want to leave the study:
  [insert name and telephone number of investigator or other study staff]

Signature Page for Maternal Participants

Screening and Enrollment Consent for Maternal Participants of Legal Age to Provide Independent Consent

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

If you decide to join the PK Component of this study with your baby, sign or make your mark below.

Before deciding whether to join this study with your baby, make sure you have read this form, or had it read to you, and that all of your questions have been answered. You should feel that you understand the study, its risks and benefits, and what is expected of you and your baby if you decide to join.

We will tell you any new information from this study or other studies that may affect your willingness for you and your baby to stay in the study. You can ask questions or request more information at any time.

You do not give up any rights by signing this form.

[Insert signature blocks as required by site IRB/EC policies.]

Name of Participant (print)	Signature of Participant	Date
Name of Study Staff Conducting Consent Process Name (print)	Signature of Study Staff	Date
Name of Witness (as appropriate; print)	Signature of Witness	Date

Signature Page for Maternal Participants
Assent for Maternal Participants Not of Legal Age to Provide Independent Consent

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

If you decide to join the PK Component of this study with your baby, sign or make your mark below.

Before deciding whether to join this study with your baby, make sure you have read this form, or had it read to you, and that all of your questions have been answered. You should feel that you understand the study, its risks and benefits, and what is expected of you and your baby if you decide to join.

We will tell you any new information from this study or other studies that may affect your willingness for you and your baby to stay in the study. You can ask questions or request more information at any time.

You do not give up any rights by signing this form.

[Insert signature blocks as required by site IRB/EC policies.]

Name of Participant (print)	Signature of Participant	Date
Name of Study Staff Conducting Consent Process Name (print)	Signature of Study Staff	Date
Name of Witness (as appropriate; print)	Signature of Witness	Date

Appendix VII: Sample Informed Consent Form for PrEP Comparison Component

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

Introduction

We invite you to take part in a research study with your baby named IMPAACT 2009. There are two parts to this study, the first part was completed in 2019.This form is for the second part, called the "PrEP Comparison Component."

This form gives information about both parts of the study. Please read it, or have it read to you, and ask any questions you may have. You can take as much time as you need to fully understand the study. We will ask you questions to see if we have explained the study clearly.

Here is a summary of important information about the study:

- The study is testing a medicine called Truvada®, or pre-exposure prophylaxis (PrEP) used to prevent HIV among young women who are pregnant and not infected with HIV.
- Truvada® is a pill that contains 2 anti-HIV medicines (ARVs), called emtricitabine and tenofovir. These medicines are often used to treat HIV infection, but they can also be safe and effective in reducing the chance of becoming infected with HIV when taken by people who are not infected with HIV.
- PrEP is approved in many countries around the world, but not many studies have involved HIV uninfected women while they are pregnant and just after delivery.
- The first part of this study found that the amount of PrEP drug found in the blood was lower when pregnant women took the medication compared to those who had recently given birth. At this time, it is not known whether this means PrEP works less well during pregnancy. The World Health Organization still recommends Truvada® for pregnant and breastfeeding women at risk for HIV.
- Women will enter the study when they are pregnant and will be in the study for 26 weeks after their baby is born.
- To be in the study, women can choose to take PrEP or not take PrEP. Those who choose to take PrEP will take one pill **consistently at about the same time** every day.
- While in the study, women and their babies will have clinic visits including physical examinations, blood draws for laboratory tests and special x-rays that measure bone strength. Women will answer questions about themselves, their health, and their health behaviors and will receive weekly SMS messages.
- Visits will be scheduled to take place at the clinical research site; however, if it is not possible to conduct study visits in-person at the study site, then visit procedures may be done off-site or remotely (such as by phone).
- There are some possible risks for women and babies in the study. One possible risk for women who choose to take PrEP is that the medicine could cause side effects. Regardless of whether they chose to take or not take PrEP, women may experience stigma from being in a study about HIV because people might think that they have HIV.

- There are some possible benefits for women in the study. One possible benefit is that women could gain knowledge and skills that may help minimize the risk of getting HIV or other STIs in the future.
- Your decision on your participation in the study will have no effect on the medical care you and your baby would normally receive. Your access to services, and the benefits and rights you normally have, will not be affected.

More information is given in this form about the study, its risks and benefits. You should feel that you understand the study before deciding whether you will participate with your baby. If you decide to participate, we will ask you to sign or make your mark on this form. You will be offered a copy to keep.

About the Study

The International Maternal Pediatric Adolescent AIDS Clinical Trials Network (IMPAACT) and *[insert site name]* are doing this study to learn more about a medication called "PrEP." For people who are not infected with HIV, but may be at risk of becoming infected with the HIV virus, taking medication called PrEP can help protect them. Other studies in men and non-pregnant women have shown that PrEP works very well in preventing HIV infection. We also know that the level of protection PrEP provides depends on whether someone takes PrEP every day. So far, not many women have taken PrEP while they are pregnant and after delivery, even though women may be at heightened risk of becoming infected with HIV during this period.

The purpose of the IMPAACT 2009 study is to find out whether young women in sub-Saharan Africa at heightened risk of becoming infected with HIV are interested and would be willing to take a PrEP pill once a day while they are pregnant and breastfeeding. The purpose of the second part of the study (PrEP Comparison Component) is to find out how likely and safe it is for young women to take PrEP during pregnancy and just after delivery.

We invite you to join the PrEP Comparison Component of this study with your baby because you are pregnant and not infected with HIV. The PrEP Comparison Component of this study will include up to 350 mothers from clinical sites in Sub-Saharan Africa. Each mother and baby will be in this part of the study during pregnancy and up to 26 weeks (about six months) after the baby is born.

The person in charge of the study at *[insert site name]* is *[insert name of IoR]*. The United States National Institutes of Health is paying for the study.

1. There are two parts to this study. You and your baby are being asked to join the second part, called the PrEP Comparison Component.

The two parts of the study are called the "PK Component" and the "PrEP Comparison Component." The PK Component was done first. That part included 40 mothers with their babies to see how much PrEP medication can be measured in her blood if a woman takes it once a day. While none of the women in the PK Component got HIV during the study, the results did show some differences in the amount of PrEP medicine in women's blood during pregnancy and after birth. Women who were pregnant had drug levels about one third (33%) lower than women who recently had their baby. These results are similar to what has been found in other studies of the same medicine; however, at this time, it is not known whether this means PrEP works less well during pregnancy. We discussed these results with several expert groups, including representatives from the World Health Organization (WHO) and the U.S. Food and Drug Administration (FDA) and received approval from the IMPAACT Network to move to the second part of the study.

The PrEP Comparison Component will include about 350 mothers and their babies. If you join the PrEP Comparison Component, you will have a choice to begin taking a pill for PrEP once a day while you are pregnant and until your baby is about 26 weeks old. If you participated in the PK Component, you cannot participate in the PrEP Comparison Component.

2. The study will assess PrEP use in women who are pregnant and just after delivery and their babies.

The medicine being used for PrEP in this study is called Truvada®. Truvada® is a pill that contains two medications called "emtricitabine" and "tenofovir" that are commonly used to treat HIV infection and sometimes to treat Hepatitis. We know that Truvada® is safe and effective in reducing the risk of HIV infection when taken by men and non-pregnant women who are not infected with HIV, but more information is needed to learn about how well PrEP can prevent HIV among women while they are pregnant and just after delivery.

Truvada® is approved for PrEP in many countries around the world. In *[insert site country]*, PrEP is approved for *[insert details as relevant, e.g. of population in which PrEP is approved]*.

3. The PrEP Comparison Component will assess what woman like and do not like about taking PrEP and how safe it is during pregnancy and breastfeeding.

The PrEP Comparison Component will be done to help understand why women may choose to take PrEP if it is offered to them, and what they like and do not like about taking PrEP. The PrEP Comparison Component will also assess whether women who choose to take PrEP take the medicine every day, as directed. We also want to learn how safe it is for women to take PrEP while pregnant and just after delivery. It is important to understand this because the body changes during pregnancy, so the amount of PrEP drugs found in the blood of pregnant women may be different from non-pregnant women, as we found during the first part of the study. Finally, the PrEP Comparison Component will help us learn more about how well protected women who take PrEP are from becoming infected with HIV, especially during pregnancy when the amount of PrEP in the body could be lower than the level we know protects against HIV infection.

4. Only mothers who qualify can participate in the study.

If you decide to join the study with your baby, we will first do some tests to see if you qualify. More information about the tests is given in #5 (see below). If you qualify, you and your baby will enter the study. If you do not qualify, you and your baby cannot enter the study.

You can choose if you want to join the study or not. You are free to join or not join. If you join, you can change your mind later and leave the study. Your decisions will have no effect on the medical care that you and your baby would normally receive from your usual clinic. Your access to services, and the benefits and rights you normally have, will not be affected.

Take your time and consider your decision carefully. If you wish, you can talk to other people about the study. You can also bring other people here to learn about the study with you.

Finding out if You and Your Baby Qualify—Visit 1

5. We will ask questions, examine you, and discuss the study requirements with you.

To find out if you and your baby qualify for the study, we will:

- Review your medical records
- Ask about your health and any medicines you or your baby take
- Talk with you about the study requirements and if you meet them
- Give you a physical examination
- Have you talk with a counselor about your health behaviors
- Collect urine to check how well your kidneys are working and if there is sugar is in your urine
- Collect your blood (up to 15 mL or about 3 teaspoons) for tests. The tests will:
  - Check your blood cells
  - Check if you have Hepatitis B (a disease of the liver)
  - Check how well your liver and kidneys are working
  - Check your blood sugar levels
  - Check your blood to confirm that you are not infected with HIV

These procedures will take about 2 hours *[here and throughout this form, sites may modify the expected visit duration as needed]*.

6. You will receive SMS messages as part of this study.

As part of finding out if you qualify for this study, we will confirm that you have access to a mobile phone that is able to receive SMS messages, and confirm that you agree to receive SMS messages. For mothers who decide to take PrEP, we will also ask whether you are able to send SMS messages.

Whether you decide to take PrEP or not, all mothers will receive routine SMS messages, starting the week you enter the study. These messages will give you information about pregnancy and infant care.

Mothers who take PrEP will also receive weekly messages. The weekly message will ask how you are doing; these messages will not ask specifically about HIV or PrEP. You will be asked to respond to each weekly message within 48 hours and if you do not respond you may receive an additional text message or someone from the study team may try to call you. Someone from the study team may also call you if you respond to the message and ask for help.

7. We will also do an ultrasound scan.

We need to know how long you have been pregnant when you enter the study and whether you may be pregnant with more than one baby. To do this, you will have an ultrasound scan and we will ask you the date of your last menstrual period *[sites may use locally appropriate terminology to refer to the scan]*. The scan uses sound waves to check the size of your baby, which shows how long you have been pregnant. Ultrasound scans are commonly done among pregnant women.

To do the scan, gel is placed on your belly. Then a small device is moved back and forth on your belly to send and receive sound waves. The scan will show a picture of your baby and measure the size.

[Sites to modify first three sentences as needed:] The scan will be done at *[insert clinic name and/or location]*. It will take less than 30 minutes. We will arrange for you to have the scan at a scheduled time before entering the study. There is no cost for having the scan done.

If you have already had an ultrasound and have the report, this may not be needed.

8. We will tell you if and your baby qualify.

We will give you the results of all procedures and explain them to you.

If you or your baby have any medical problems, or you do not qualify for the study for any reason, we will tell you this. You will not be entered in the study.

If you do qualify for the study and you agree to join with your baby, you will enter the study.

Choosing to be in the Study

9. You can decide to take PrEP or not to take PrEP.

If you qualify to join the study, you have a choice. You can choose to begin taking PrEP or you can choose to not take PrEP at all. You can still be in the study if you choose not to take PrEP. If you choose to start PrEP, you will take one pill every day up to 26 weeks after your baby is born. We found in the first part of the study that women who were pregnant had less PrEP drug in their body compared to women who had recently given birth, even when they took the same amount of PrEP. Therefore, it is especially important that women who are pregnant take their PrEP once a day every day.

You can change your mind about PrEP while you are in the study. Those who start off taking it can stop; those who initially decided not to take it, may start if they so wish. Even if you change your mind, we would continue to follow you until 26 weeks after the birth of your child.

To test PrEP, the mothers and babies in each group will be compared to the mothers and babies in the other group.

Being in the Study

10. If you and your baby qualify, you will enter the study.

Different procedures will be done at your first visit depending on if you chose to take PrEP. Some procedures will be done for both groups of mothers. Other procedures will be done for only the group of mothers taking PrEP.

This visit will take about 4 hours.

For all mothers, on the day you enter the study, we will:

- Ask about your health and the medicines you take
- Give you a physical exam
- Have you talk with a counselor about your health behaviors
- Have you complete a survey on the computer, where you will answer questions about yourself, your health, and your health behaviors

- Ask about how COVID-19 may be affecting you
- Collect your blood (12 mL or about 2.5 teaspoons) for tests.
  These tests will:
  - Confirm you are HIV-uninfected
  - Check if you have syphilis or herpes
  - Check if any PrEP drug is in your blood
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests.
- Collect vaginal swabs or urine to check if you have sexually transmitted infections such as chlamydia or gonorrhea and to check the type of bacteria present that are normally found in the vagina. For these specimens, a small swab is inserted into the vagina to collect some mucus.
- Collect rectal swabs, by inserting a small swab into the rectum to get some of the fluid. If you prefer, the study staff can give you instructions so that you can collect this specimen yourself. You do not have to agree to this collection. We will ask you later if you agree to have rectal swabs collected. This testing will be done later in the study, so you and your doctor won't get the results of these tests.

For mothers taking PrEP, we also will:

- Give you a supply of PrEP
- Teach you about using PrEP and talk to you about your plans for taking your pills. **It is very important that you take the PrEP pill once a day, every day.** We will take as much time as needed for you to understand the instructions and will answer any questions you have about taking PrEP.

11. You will return to the study clinic for 3 or more visits while you are pregnant.

You will have visits when you have been in the study for about 4 weeks, 8 weeks, and 12 weeks. After that, you will have visits once every 12 weeks until delivery. The number of visits to the clinic you will have during pregnancy will depend on when you enter the study and when your baby is born. Most mothers will have about 3 visits while pregnant. Some may have up to 7 visits.

Different procedures will be done at your visits depending on if you chose to take PrEP. Some procedures will be done for both groups of mothers. Other procedures will be done for only the group of mothers taking PrEP.

Each visit will take about 1-2 hours.

For all mothers, at these visits we will:

- Review your medical records
- Ask about your health and the medicines you take, and your relationships
- Give you a physical exam
- Have you talk with a counselor about your health behaviors
- Have you complete a survey on the computer, where you will answer questions about yourself, your health, and your health behaviors
- Ask about how COVID-19 may be affecting you
- Collect your blood (up to 14 mL or about 3 teaspoons) for tests. At different visits, the tests will:
  - Check how well your liver and kidneys are working
  - Confirm that you are HIV-uninfected
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests

At three visits, we will:

- Collect two vaginal swabs to check the type of bacteria present.
- Collect a rectal swab to check the type of bacteria present. If you agreed to have this specimen collected.

For mothers taking PrEP, we also will:

- Give you supplies of PrEP
- Answer questions you have about taking your PrEP and talk to you about what makes it easier and harder to take your PrEP pills every day.
- Provide counseling based on how much PrEP is found in your blood. We will use the test results from your past visit to talk to you about how well protected you are from becoming infected with HIV.
- Have you complete a survey on the computer, where you will answer questions about taking PrEP.
- Use blood already collected for additional tests. The tests will:
  - Confirm how much of the PrEP drugs are in your blood. We will let you know once the results are available. We may invite you for an extra visit.
- At three visits, collect a small amount of fluid from your vagina to check the levels of PrEP drugs in your genital tract. This specimen can be collected by you or the clinic staff. This testing will be done later in the study, so you and your doctor won't get the results of these tests.

12. You and your baby will have a visit soon after delivery. Then you will have 3 more visits over 26 weeks.

You should contact us when your labor begins to arrange for a study visit as soon as possible after delivery. It is important for this visit to take place close to the baby's birth. This visit will take up to 4 hours.

Your next study visits for you and your baby will be when your baby is about 6, 14 and 26 weeks of age.

Each visit will take about 2 hours.

When you come to the clinic with your baby for the visit soon after delivery and when your baby is 6, 14, and 26 weeks of age we will:

- Review your and your baby's medical records
- Ask about your and your baby's health and the medicines you take, and your relationships
- Ask about how you are feeding your baby
- Give you and your baby a physical exam
- Have you talk with a counselor about your health behaviors
- Have you complete a survey on the computer, where you will answer questions about yourself, your health, and your health behaviors
- Ask about how COVID-19 may be affecting you
- Collect a vaginal swab or urine at your final visit to check for infections such as chlamydia or gonorrhea *(at the Week 26 Postpartum visit only*)
- Collect vaginal swabs to check the type of bacteria present *(at the Week 6 and Week 26 Postpartum visits only)*
- Collect rectal swabs to check the type of bacteria present *(at the Week 6 and Week 26 Postpartum visits only)*
- Take blood from you (up to 20 mL or 4 teaspoons) for tests that will:
  - Check your cells
  - Check how well your liver and kidneys are working

  - Check if you have Hepatitis B (a disease of the liver) - *at the Week 26 Postpartum visit only*
  - Check if you have syphilis or herpes - *at the Week 26 Postpartum visit only*
  - Confirm you remain HIV uninfected
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests
- Take blood from your baby (up to 1.5 mL or about ½ teaspoon) for tests that will:
  - Confirm your baby's kidneys are working well (at the first and final visit only)
- Collect rectal swabs or stool from your baby to check the type of bacteria present
- You and your baby will also have DXA scans. DXA stands for a kind of x-ray called dual energy x-ray absorptiometry. DXA scans check how hard and strong the bones are. When having the scan, you lie on a table while a machine passes over their body. Babies are held on a table while the machine passes over their body. The machine does not touch the body and the scan does not hurt. The scan may take up to 15 minutes. Scans will be done for you and your baby shortly after delivery and 26 weeks after birth or delivery. For safety reasons, you must have a pregnancy test before the scan done 26 weeks after delivery to confirm you are not pregnant before having the scan. The results of the scans will not be available while the study is ongoing.

For mothers taking PrEP, we also will:

- Give you supplies of PrEP. At your final visit, we will collect any remaining PrEP from you.
- Provide counseling based on how much PrEP drug is found in your blood. We will use the test results from your past visit to talk to you about how well protected you are from becoming infected with HIV.
- Have you complete a survey on the computer, where you will answer questions about taking PrEP.
- Use blood already collected for tests to confirm how much PrEP drug is in your blood. We will let you know once the results are available. We may invite you for an extra visit.
- Collect a small amount of fluid from your vagina to check the levels of PrEP drugs in your genital tract.

While you are in the study, we will give you the results of the tests for you and your baby. We may need to repeat a blood test for you or your baby. If you become infected with HIV during the study, we still want you and your baby to complete all your study visits, but it is your choice. We will discuss with you how to get care and treatment for HIV outside the study and arrange it with the staff there *[site to revise as needed]*.

13. You will have extra visits if you choose to change from not taking PrEP to taking PrEP.

If you choose to not take PrEP at the start of the study, you may change your mind during the study and choose to start taking PrEP. If you choose to start taking PrEP, you will be required to have two extra visits. These extra visits will take about 1 hour and Visit 1 may be combined with a regularly scheduled study visit.

At Visit 1 we will:

- Review your medical records
- Ask about your health and the medicines you take, and your relationships
- Give you a physical exam
- Teach you about using PrEP and talk to you about your plans for taking your pills
- Have you complete a survey on the computer, where you will answer questions about yourself, your health, and your health behaviors

- Ask about how COVID-19 may be affecting you
- Have you talk with a counselor about your health behaviors
- Collect urine to check how well your kidneys are working and if there is sugar is in your urine
- Collect your blood (17 mL or about 3.5 teaspoons) for tests. The tests will:
  - Check your cells
  - Check how well your liver and kidneys are working
  - Check if you have Hepatitis B
  - Check your blood sugar levels
  - Confirm you are HIV-uninfected
- Save some of the blood in case you become infected with the HIV-virus during the study for extra HIV tests

At Visit 2 we will:

- Give you a supply of PrEP
- Teach you about using PrEP and talk to you about your plans for taking your pills
- Ask about your relationships
- Have you complete a survey on the computer, where you will answer questions about yourself, your health, and your health behaviors
- Ask about how COVID-19 may be affecting you
- Have you talk with a counselor about your health behaviors
- Collect your blood (4 mL or about 1 teaspoon) for tests. The tests will:
  - Confirm you are HIV-uninfected
  - Check if PrEP drug is in your blood
- Collect vaginal swabs to check the type of bacteria present
- Collect rectal swabs to check the type of bacteria present

14. If you become infected with HIV during the study, you will have extra visits.

We will test you for HIV throughout the study using a rapid test in the clinic and another type of HIV test in the laboratory (RNA PCR). If your HIV test results are positive, we will take more blood to confirm the HIV infection and measure the HIV viral load (how much HIV is in the blood). It usually takes about a week for these results to come back.

These extra visits will take about 1 hour. At these visits we will:

- Refer you for HIV care and treatment
- Talk with a counselor about your health behaviors
- Collect any remaining PrEP from you
- Take your blood (15-25 mL or about 3.5 to 5 teaspoons) for tests.

  The tests will:
  - Confirm your HIV viral load
  - Check your cells
  - If you are found to be HIV-infected, check if the HIV in the blood is resistant to the medications in the PrEP pill
- Save some of the blood for future HIV testing

For babies, we also will:

- Take blood (up to 6 mL or about 1 teaspoon) for tests. The tests will:
  - Check if your baby is HIV-infected. If the test is positive, a second test will be done to confirm if your baby is HIV-infected.
  - If the HIV infection is confirmed, check if the HIV in your baby's blood is resistant to the medications in the PrEP pill.

15. Tests will be done at different laboratories.

We will do tests of you and your baby's specimens at our laboratory. Most of the tests will be done right away at a local facility. We will give you the results of these tests and explain the results to you. However, some specimens will be kept and tested together at the end of the study. If the results show that you or your baby may need medical care or treatment that cannot be provided by the study, we will tell you where you can go for this care. Some tests are special and must be sent to specialized laboratories outside the country, including the United States. This includes:

- Some tests for HIV viral load
- Tests for resistance to PrEP
- Tests of the amount of PrEP that can be found in blood
- Test to determine the levels of normal bacteria in your vagina and gut and your baby's gut

The results of most of these tests are not expected to be available while the study is ongoing. If any of the results may be important for your or your baby's health, we will tell you about them. Otherwise, the results will not be given to you.

If there are any specimens left-over after all the tests for this study have been done for you and your baby, we would like to keep those specimens because they may be useful for studies in the future. We will talk to you about what you want to happen to these left-over specimens in another informed consent form.

16. We may take you and your baby off the study early.

We expect you to stay in the study until you and your baby have completed all the study visits; until the baby is about 26 weeks of age. However, we may take you and your baby off the study early if:

- The study is stopped for any reason.
- We determine that you and your baby cannot meet the study requirements (for example, if you move away and cannot come to the clinic).
- We determine that staying in the study might harm you or your baby.

We will ask you and your baby to come to the clinic for one last visit. At this visit, we will do the same types of procedures we do at the weekly study visits (see #10 and #11), we may also give you a pregnancy test (see #18). We will answer any questions you may have and tell you how to contact us in the future, if you wish.

17. Please tell us if you want to leave the study.

You and your baby are free to leave the study at any time for any reason – you just need to tell us. The care that you and your baby would normally receive will not be affected, but it is important that we know your decision. We will ask you and your baby to come to the clinic for one last visit. At this visit, we will do the same types of procedures we do at the weekly study visits (see #10 and #11 above), we may also give you a pregnancy test (see #18). We will answer any questions you may have and tell you how to contact us in the future, if you wish.

18. How to stay HIV uninfected after leaving the study.

We can't give you PrEP after you finish your study visits. Before you leave the study, we will talk again about how to protect yourself against HIV infection and give you information about other clinics that offer PrEP to women. If you want to continue taking PrEP, we will refer you there. *[Sites to modify as necessary and insert information about referral letters and sharing study laboratory results with the non-study PrEP provider, as appropriate]*

19. After delivery, we will test for pregnancy again during the study.

We will test for pregnancy again during the study. If you become pregnant, you will continue in the study. When the new pregnancy is identified, if you were taking PrEP, you may continue to do so, and if you chose not to take PrEP you will be offered to start PrEP. If you choose to continue taking PrEP after you leave the study, we will refer you to clinics that offer PrEP. We will contact you after your last study visit to find out the outcome of your pregnancy.

Risks of the Study

20. There is little risk from the study procedures.

Most procedures done in this study are routine medical procedures, with little risk to you and your baby.

Blood Collection: Collecting blood can cause pain, swelling, bruising, or bleeding where the needle is inserted. Rarely, collecting blood can cause fainting or infection.

Urine collection: There is no risk from collecting your urine.

Vaginal and rectal swab collection: You may feel a little discomfort when the swabs are inserted into the vagina or the rectum. The study staff will show you the swab and you can ask any questions about this procedure before it is done.

Vaginal fluid collection: You may feel a little discomfort when the sponge in inserted into the vagina. The study staff will show you the sponge and you can ask any questions about this procedure before it is done.

Ultrasound scan: There is no risk from ultrasound.

DXA Scans: For mothers and babies who have DXA scans, there is a small risk from x-rays, also known as radiation. Radiation is energy in the form of waves. All people are exposed to a low level of natural radiation from the sun. This is called background radiation. High levels of radiation can cause cancer. However, the level of radiation from a DXA scan is much lower than the level that causes cancer. The level from DXA scans for this study is also about the same as the background radiation every person normally has over 5 days. The study staff have been trained to do DXA scans using the smallest amount of radiation possible.

HIV Testing: We will perform an HIV test at every visit, which is routinely done to make sure that you are/your child is HIV uninfected before starting or continuing PrEP. You will be counseled before and after test is done. *[Sites to insert reporting responsibilities in the state the site is located in. Also include whether if a participant tests positive, the results will become part of public health records, or any other record (medical file, etc.)]* You can come to the clinic and ask for an HIV test anytime you are worried.

Acquiring HIV Infection and Drug Resistance: You must understand this information about what PrEP can and can't do:

- PrEP cannot give you HIV.
- PrEP is most effective when taken once a day, every day.
- Taking PrEP prevents HIV in most people who take it every day, but
- **You still may become infected with HIV while you are taking PrEP**.
- It is possible that PrEP may not protect a woman against HIV infection as much during pregnancy as it does at other times because of the lower amount of PrEP drug measured in the body during pregnancy.
- **PrEP does not prevent pregnancy or STIs.**
- You still need use other methods to avoid HIV infection and other STIs while you are taking PrEP, like using condoms every time you have sex and keeping your number of sexual partners low. Study staff will discuss all the different ways you can keep yourself from getting these infections.

If you become infected with HIV while you are taking PrEP, the strain of the HIV virus in your blood might become "resistant" to Truvada® and some other medications used for HIV treatment (ART). "Resistance" means the virus can't be killed by that medication, which makes treating the HIV more difficult. If you become infected with HIV, we will test for HIV drug resistance. You will be able to discuss ART and resistance with the study staff.

If you have any questions about anything we have said here, please talk to us.

Sensitive Questions: The questions we will ask you about your sexual behavior, drug and alcohol use may make you feel uneasy. However, you do not have to answer any question that you do not want to and you can stop answering the questions at any time.

Mobile Phones: Information that you send us from your mobile phone programs will be kept confidential. We will not be able to access any private information on your mobile phone. However, it may be possible for your information to be viewed by others who have access to your phone. We will do everything possible to make sure that your information is protected. *[Sites to modify as needed: We will not mention the research study when texting you, but only text reminders about keeping up your health.]*

STI Testing: You will be tested for STIs. *[Insert here any reporting responsibilities for your state or local jurisdictions or reporting of these infections to public health authorities]*. If you have any of these infections, we will provide you with treatment.

Other Risks: Also, there may be uncommon or previously unknown risks that might occur. You should report any problems to the study staff immediately. There may also be some social risks to participating in this study. You may feel embarrassed or uncomfortable with some of the questions we will ask you, some of the procedures that will be done, or some of the test results that you will receive. You may also experience stigma as a result of being involved in a study about HIV because people may assume that you have HIV. This could mean that you are treated badly in the community or by your family and friends. Being in the study could cause problems with your relationships with your partner if they do not support your choice to participate or use PrEP. Please talk to study staff if this happens. If you have HIV or other infections, knowing this could make you worried. Study staff are trained to help you deal with any feelings or questions you have.

21. There are risks from PrEP.

You or your baby may feel sick while participating in the study. These problems may be due to taking PrEP or due to an illness that has no relation to the study, like a cold or flu. You should tell us about any symptoms that you feel while you are participating in the study. You will be given a telephone number to contact the clinic.

You should contact us if you experience any symptoms at any time.

In PrEP research studies, nausea and diarrhea were the most common side effects, but happened in only about 10% or one in ten people. Nausea and diarrhea mainly happened in the first month and then went away. A small number (<1% or one in one hundred people) in PrEP studies showed a small decrease in how their kidneys work, but this stopped when the people stopped taking the drug.

Other side effects, such as changes in bone mineral density (how much calcium and other minerals are in your bone which keeps them strong) were very rare in people taking the drug who did not have HIV and have always gotten better when the drug was stopped.

You could have these side effects or other side effects that we do not know about. Please tell the staff here if you have any side effect that bothers you or does not go away.

Other Medications: Some medications should not be taken while you are taking Truvada®. When you visit the study clinic, we will ask you about other medications that you are taking.

Truvada ® may have side effects that no one knows about yet. We will let you know if we learn anything that might make you change your mind about continuing to participate in the study.

Risks to your baby: The medicines in Truvada® are often used to treat women with HIV infection. Many HIV-infected women have taken these medicines while pregnant and breastfeeding. We know that these medicines pass through to the baby in the womb and are found in breastmilk in small amounts. The information we have so far suggests that these medicines do not cause serious problems in the baby. However, it is still possible that taking PrEP may cause some problems in the baby soon after birth or when they are older. The study staff will check for side effects in babies during study visits and tell you what to do if your baby has any side effects. You should contact the study staff at any time if you are worried about the baby's health.

22. There could be risks of disclosure of your or your baby's information.

We will make every effort to keep you and your baby's information private and confidential. Study records and specimens will be kept in secure locations. All specimens and most records will be labeled

only with a code number. However, you and your baby's names will be written on some records that are kept in the clinic. Your name and telephone number will also be stored in the computer system used to send and track SMS messages. Despite our best efforts to keep this information private, it is possible that the information could be obtained by someone who should not have it. If this were to happen, you or your baby could be treated badly or unfairly. You could feel stress or embarrassment. You should contact the study staff at any time if you are worried about your and your baby's privacy.

Information collected for this study may be used for other research in the future. For example, researchers may use information from this study to try to answer different questions about using PrEP during pregnancy. Any future research done with the information from this study must be approved by the IMPAACT Network. If any future research is done, information about you or your baby may be used; this information will be labeled with a code number, and the only link between the code number and your or your baby's name will be kept here at [*site name*]. Your or your baby's name will not be given to other researchers.

Benefits of the Study

23. There may be no benefit from being in the study.

We will test you for HIV and other STIs throughout this study. If you choose to take Truvada®, and you take it every day, it may help you to avoid HIV. Participating in this study may give you knowledge and skills that may help you to avoid HIV and other STIs. The SMS messages you receive may help ensure that you keep up with routine medical care for you and your baby. What we learn about PrEP use in young women may help women in the future decide whether to take PrEP while they are pregnant and breastfeeding.

Other information about the study

24. There are no costs to you for being in the study.

There are no costs to you for study visits or procedures or the PrEP that is given by the study, and you will not receive payment for participating in the study.

[Insert information about compensation/reimbursement here, e.g., You will be reimbursed for the cost of transport to study visits. For each visit, you will be given (specify amount).*]*

25. Study records may be reviewed by study staff and groups that oversee the study.

Groups that oversee the study include:

- *[insert name of site IRB/EC]*
- *[insert name of site drug regulatory authority]*
- *[insert name of other site regulatory entities]*
- The United States National Institutes of Health and its study monitors
- The United States Food and Drug Administration (FDA)
- The Office for Human Research Protections (OHRP)
- Other U.S., local, and international regulatory entities
- The IMPAACT Network that is coordinating the study
- Gilead Sciences (the company that makes Truvada®)

The study staff and these groups are required to make every effort to keep study records private and confidential.

The results of the study may be presented publicly or published. However, no presentation or publication will use your name or your baby's name or identify you or your baby personally.

A description of this study will be available on ClinicalTrials.gov. This website will not include information that can identify you or your baby. At most, the website will include a summary of the results of the study. You can search this website at any time.

Your and your baby's study information may be disclosed to other authorities if required by law.

26. If you or your baby gets sick or injured, contact us immediately.

Your and your baby's health is important to us. We will make every effort to protect your well-being and minimize risks. It is possible, however, that you or your baby could have an illness or injury that is study-related. This means the illness or injury occurred as a direct result of being in the study.

[Sites may modify this paragraph to reflect local institutional policies; information regarding coverage available through clinical trial insurance obtained by the site should be included if applicable; the statement regarding no program for compensation through the NIH may not be removed.] If a study-related illness or injury occurs, we will treat you or your baby or tell you where you can get the treatment you or your baby need. The cost for this treatment may be charged to you or your health insurance. There is no program to pay money or give other forms of compensation for study-related illness or injury through *[site name or]* the United States National Institutes of Health.

Who to Contact

27. If you have questions, concerns, or problems at any time, use these contacts.

- If you have questions about the study:
  [insert name and telephone number of investigator or other study staff]

- If you have questions about your rights or your baby's rights as research participants or concerns about how you or your baby are being treated in the study:
  [insert name and telephone number of IRB/EC contact person or other appropriate person/organization]

- If you or your baby have any health or other problems that may be related to study participation:
  [insert name and telephone number of investigator or other study staff]

- If you want to leave the study:
  [insert name and telephone number of investigator or other study staff]

Signature Page for Maternal Participants

Screening and Enrollment Consent for Maternal Participants of Legal Age to Provide Independent Consent

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

If you decide to join this study with your baby, sign or make your mark below.

Before deciding whether to join this study with your baby, make sure you have read this form, or had it read to you, and that all of your questions have been answered. You should feel that you understand the study, its risks and benefits, and what is expected of you and your baby if you decide to join.

We will tell you any new information from this study or other studies that may affect your willingness for you and your baby to stay in the study. You can ask questions or request more information at any time.

You do not give up any rights by signing this form.

For the optional rectal swab collection in mothers, write a 'X' to indicate your decision.

_____ Yes, I agree to provide rectal swab samples

_____ No, I don't want to provide rectal swab samples

[Insert signature blocks as required by site IRB/EC policies.]

Name of Participant (print)	Signature of Participant	Date
Name of Study Staff Conducting Consent Process Name (print)	Signature of Study Staff	Date
Name of Witness (as appropriate; print)	Signature of Witness	Date

Signature Page for Maternal Participants

Assent for Maternal Participants of Not of Legal Age to Provide Independent Consent

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

If you decide to join this study with your baby, sign or make your mark below.

Before deciding whether to join this study with your baby, make sure you have read this form, or had it read to you, and that all of your questions have been answered. You should feel that you understand the study, its risks and benefits, and what is expected of you and your baby if you decide to join.

We will tell you any new information from this study or other studies that may affect your willingness for you and your baby to stay in the study. You can ask questions or request more information at any time.

You do not give up any rights by signing this form.

For the optional rectal swab collection in mothers, write a 'X' to indicate your decision.

_____ Yes, I agree to provide rectal swab samples

_____ No, I don't want to provide rectal swab samples

[Insert signature blocks as required by site IRB/EC policies.]

Name of Participant (print)	Signature of Participant	Date
Name of Study Staff Conducting Consent Process Name (print)	Signature of Study Staff	Date
Name of Witness (as appropriate; print)	Signature of Witness	Date

Appendix VIII:
Sample Informed Consent Form for Specimen Storage and Future Use

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

You have decided to join the study named above with your baby. As part of the study, you will have blood and urine collected, in addition to vaginal and rectal swabs and vaginal fluid collection. Your baby will have blood collected in addition to rectal swabs or stool samples. After these samples are tested for the study, some samples may be left-over. The IMPAACT Network would like to keep these left-over samples and use them for other research in the future.

This form gives information about use of left-over samples. Please read it, or have it read to you, and ask any questions you may have. After we discuss the information with you, you will record your decisions on use of left-over samples at the end of the form.

1. It is your decision whether or not to allow the left-over samples to be used.

You are free to say yes or no, and to change your mind at any time. Your decision will not affect your or your baby's participation in the study. If you say no, all left-over samples will be destroyed.

2. If you agree, your and your baby's left-over samples will be kept in a repository.

[Sites should insert one of the two options shown below. Choose/adapt the second option if local regulations do not permit storage of samples for future research use in the United States.]

A repository is a secure facility that is used to store samples. The IMPAACT Network repository is in the United States. If you agree to have left-over samples stored, the samples will be kept in this repository. There is no limit on how long the samples will be kept *[sites may insert time limits or additional site-specific requirements here if required by local authorities]*.

A repository is a secure facility that is used to store samples. The IMPAACT Network has a repository in the United States. However, our local regulations require that left-over samples be stored in our country. Therefore, we will keep the samples here at our laboratory. There is no limit on how long the samples will be kept *[sites may insert time limits or additional site-specific requirements here if required by local authorities]*.

3. Left-over samples could be used for different types of research.

Left-over samples may be used for research on pregnancy, the immune system, and other diseases. The research may be done in the United States or in other locations.

If you agree, the left-over samples could also be used for research that looks at your or your baby's genes. Genes are passed to children from their birth parents. They affect how people look and how their bodies work. Differences in people's genes can help explain why some people get a disease while others do not. Your and your baby's samples would only be used to look at genes related to how the body responds to PrEP, pregnancy, and the immune system. Testing all of your and/or your child's genes, which is sometimes called whole genome sequencing, will not be done

Any research done with the left-over samples must be reviewed and approved by the IMPAACT Network. The research must also be approved by an ethics committee. The role of an ethics committee is to review the research plan and protect the rights and well-being of the people whose samples will be used.

The research done with left-over samples is not expected to give any information relevant to your or your baby's health. Therefore, the results will not be given to the study staff or to you. The results also will not be placed in your or your baby's study records.

4. There is little risk to you or your baby.

When left-over samples are used for research, they are labeled with a code number only. To protect your and your baby's privacy, no names are used. However, information such as age, gender, HIV status, and other health information may be linked to the samples. The only link between the code number and your and your baby's name is kept here at [*site name*]. Your or your baby's name will not be given to other researchers.

There may be some risks from tests of your or your baby's genes. If others found out the results of these tests, they could treat you or your baby badly or unfairly. However, this is almost impossible because the results will not be given to the study staff or to you, and will not be in your or your baby's study records.

5. There may be no benefit to you or your baby.

By allowing your left-over samples to be used for research, you and your baby will be part of the search for new information that may benefit people in the future. However, the research done with the left-over samples is not expected to directly benefit you or your baby in any way.

6. You will not be paid for use of your or your baby's samples.

There is no cost to you for use of your or your baby's left-over samples. The samples will not be sold, and you will not be paid for use of the samples. It is possible that research done with the samples could lead to a new discovery or a new product. If this happens, there is no plan to share any money with you or your baby.

7. Information from research using left-over samples may be reviewed by groups that oversee the research.

These groups include:

- The IMPAACT Network
- The ethics committees that review and approve the research
- Government and other agencies that pay for the research
- Government and other agencies that monitor the research

- Other U.S., local, and international regulatory entities

The people who do research with the left-over samples and the groups listed above are required to make efforts to keep information private and confidential.

The results of research done with the left-over samples may be presented publicly or published. However, no presentation or publication will use your or your baby's name or identify you or your baby personally.

8. If you have questions, concerns, or problems at any time, use these contacts.

- If you have questions about use of your or your baby's left-over samples:
  [insert name and telephone number of investigator or other study staff].

- If you later change your mind about use of your or your baby's left-over samples:
  [insert name and telephone number of investigator or other study staff].

- If you have questions about your or your baby's rights as a research participant or concerns about how you are being treated in the study:
  [insert name and telephone number of IRB/EC contact person or other appropriate person/organization].

Signatures

Before deciding whether to allow your and your baby's left-over samples to be used for research, make sure you have read this form, or had it read to you. Make sure all of your questions have been answered. You should feel that you understand your options and the possible risks and benefits before making your decision.

You do not give up any rights by signing this form.

[Insert initial and signature blocks as required by site IRB/EC policies and the IRB/EC determination if the level of risk to children in the categories specified in 45 CFR 46.404-407. Separate consent decisions must be documented for genetic testing].

For YOUR left-over samples, write your initials or make your mark next to your choice.

_____ I allow my left-over samples to be used for research on pregnancy, the immune system, and other diseases. I also allow my samples to be used for tests of my genes.

_____ I allow my left-over samples to be used for research on pregnancy, the immune system, and other diseases. I do <u>not</u> allow my samples to be used for tests of my genes.

_____ I do <u>not</u> allow my left-over samples to be used for any research.

For YOUR BABY's left-over samples, write your initials or make your mark next to your choice.

_____ I allow my baby's left-over samples to be used for research on PrEP, pregnancy, the immune system, and other diseases. I also allow my baby's samples to be used for tests of his or her genes.

_____ I allow my baby's left-over samples to be used for research on PrEP, pregnancy, the immune system, and other diseases. I do <u>not</u> allow my baby's samples to be used for tests of his or her genes.

_____ I do <u>not</u> allow my baby's left-over samples to be used for any research.

Name of Mother (print)	Signature of Mother	Date
Name of Study Staff Conducting Consent Process Name (print)	Signature of Study Staff	Date
Name of Witness (as appropriate; print)	Signature of Witness	Date

Appendix IX: Sample In-depth Interview Informed Consent Form

IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

Introduction

You are being asked to take part in a qualitative evaluation of the IMPAACT 2009 research study. By joining this evaluation, you will participate in one in-depth interview with study staff. Participation is voluntary. You may refuse to join, or you may withdraw your consent to participate in this interview for any reason. The qualitative evaluations are for young women who are participating in the PrEP Comparison Component of IMPAACT 2009.

Before you decide whether to participate, we would like to explain the purpose of the qualitative evaluations, the risks and benefits to you and what is expected of you.

Participation is voluntary

This consent form gives information about the qualitative evaluations that will be discussed with you. We will help you to understand the form and answer your questions before you sign this form. Once you understand this evaluation, and if you agree to take part, you will be asked to sign your name or make your mark on this form. You will be offered a copy of this form to keep.

Before you learn about the qualitative evaluations, it is important that you know the following:

- Your participation is voluntary. You do not have to take part if you do not want to.
- You may decide not to take part or you may decide to leave at any time without losing your regular medical care.
- You are not not required to participate in these interviews in order to remain in the rest of the study (IMPAACT 2009).

About the qualitative evaluation

The purpose of the IMPAACT 2009 study is to find out whether young women in sub-Saharan Africa at risk of becoming infected with HIV are interested and would be willing to take a PrEP pill once a day while they are pregnant and breastfeeding. The in-depth interview will ask young women what they like and do not like about taking PrEP. We will also ask questions to find out what makes some young women more or less interested in starting PrEP. Finally, if you are taking PrEP we will ask about difficulties you had taking PrEP and things that made taking PrEP easier.

Entering the qualitative evaluation

To understand better what makes it easier or harder for young women in this study to take their PrEP pills every day, we will be doing interviews with up to 60 young women at participating sites. You have been

selected to take part in one interview sometime after your Week 12 study visit.
These interviews will ask you questions about:

- How and when you decided to take PrEP,
- Whether you feel that you personally are at risk of HIV,
- If you decided not to take PrEP, some of the reasons why you made that decision,
- How you made daily pill-taking part of your/her routine if you decided to take PrEP,
- Where you kept your PrEP pills if you decided to take PrEP,
- Whether you talked to your family members, peers, and partner(s) about being in this study or taking PrEP pills,
- If you decided to take PrEP, if you had any symptoms or side effects from the pills, and how this might have influenced your decision to take the pills, and
- Other related topics will include, for example, whether the COVID-19 pandemic has impacted your study participation, including your decision whether or not to take PrEP, and your adherence to PrEP if you chose to take it.

1. Procedure

The interview will be led by a member of the research team that you do not work with during the study. If any of the questions make you upset, either you or the interviewer may stop the interview at any time. You will also be provided with contact and referral information if any of the questions raise issues that you would like to talk about later.

[*To be modified to reflect site practices: The interview will take place in a location that the study staff have determined will provide you with privacy and confidentiality such as the clinic, or another appropriate place. The study team will talk with you about this so you know where to go for the interview*].

The interview may be conducted remotely; for example, by cell phone or video chat, if we are not able to conduct a face-to-face study visit at the clinic or at an earlier agreed location due to COVID-19-related restrictions.

The interview should take about 1 hour. There will be no cost to you for participation. You will receive [*insert local amount*] for your time and effort.

Benefits of the qualitative evaluations

2. There may be no benefit from being in the qualitative evaluation

You may not receive any other direct benefit from being in this part of the qualitative evaluations; however, you or others in your community may benefit from this study later.

Risk of the qualitative evaluation

3. There is little risk from the qualitative evaluation procedures

To minimize any discomfort and to protect your privacy the interview will be conducted in a private area that will allow you to speak comfortably without being overheard. Although we hope that you will be comfortable answering all of the questions openly and honestly, please keep in mind that you may refuse to answer any of the questions, or stop the interview completely, at any time. The greatest risk may

involve your privacy and confidentiality. The steps that the study team have taken to protect your privacy are described below.

Other information about the qualitative evaluation

4. Privacy

Every effort will be made to keep your personal information confidential. Your personal information (name, address, phone number) will be protected by the research clinic. Your name, or anything else that might identify you personally, will not be used in any publication of information about this study.

To help assure that we get the best understanding possible from your answers during the interview, the entire interview will be audio-recorded. After the interview is finished, the recording will be typed (called a transcript) by people who know how to do this. All identifying information will be removed from the transcript. Your name will not be included on the transcript. These recordings will be destroyed after all analysis is completed.

Your records may be reviewed by the sponsor of the study (U.S. National Institutes of Health (NIH), U.S. NIMH) and their representatives, U.S. FDA, The Office for Human Research Protections (OHRP), other U.S., local, and international regulatory entities, [*insert name of site*] IRB/EC, study staff, study monitors and [*insert applicable local regulatory authorities*].

People who may review your records include: [*insert name of site*] IRB, National Institutes of Health (NIH), study staff, study monitors, and the drug company (Gilead) supporting this study.

If the study staff learns of possible child abuse and/or neglect or a risk of harm to you/your child or others, we will tell the proper authorities as we are required to do by the law.

5. New Information

You will be told any new information learned during the qualitative evaluation that might affect your willingness to stay in the study. You will also be told when the results of the qualitative evaluation may be available, and how to learn about them.

6. Why you may be withdrawn from the qualitative evaluation without your consent

You may be withdrawn from the qualitative evaluations without your/her consent if any of the following occur:

- The study is stopped or canceled.
- The study staff feels that staying in the study would be harmful to you.
- Other reasons, as decided by the study staff.

7. There are no costs to you for being in the qualitative evaluation

There will be no cost to you for participating in these in-depth interviews, and you will not receive payment for participating in the in-depth interviews.

[Insert information about compensation/reimbursement here, e.g., You will be reimbursed for the cost of transport to the clinic for these interviews. You will be given (specify amount).*]*

8. Who to contact

If you have questions, concerns, or problems at any time, use these contacts.

- If you have questions about the qualitative evaluation:
  [insert name and telephone number of investigator or other study staff]

- If you have questions about your rights or your baby's rights as research participants or concerns about how you or your baby are being treated in the qualitative evaluation:
  [insert name and telephone number of IRB/EC contact person or other appropriate person/organization]

- If you or your baby have any health or other problems that may be related to qualitative evaluation participation:
  [insert name and telephone number of investigator or other study staff]

- If you want to leave the qualitative evaluation:
  [insert name and telephone number of investigator or other study staff]

Signature page for Maternal Participants

In-depth Interview Informed Consent Form for Maternal Participants of Legal Age to Provide Independent Informed Consent
IMPAACT 2009
Pharmacokinetics, Feasibility, Acceptability, and Safety of Oral Pre-Exposure Prophylaxis for Primary HIV Prevention during Pregnancy and Postpartum in Adolescents and Young Women and their Infants

Version 3.0, dated 19 November 2020

If you decide to join the qualitative evaluation with your baby, sign or make your mark below.

Before deciding whether to join the qualitative evaluation with your baby, make sure you have read this form, or had it read to you, and that all of your questions have been answered. You should feel that you understand the study, its risks and benefits, and what is expected of you and your baby if you decide to join.

We will tell you any new information from the qualitative evaluations or other studies that may affect your willingness for you and your baby to stay in the qualitative evaluations. You can ask questions or request more information at any time.

You do not give up any rights by signing this form.

[Insert signature blocks as required by site IRB/EC policies.]

Name of Participant (print)	Signature of Participant	Date
Name of Study Staff Conducting Consent Process Name (print)	Signature of Study Staff	Date
Name of Witness (as appropriate; print)	Signature of Witness	Date

Appendix X: Guidance for Study Implementation at Sites Experiencing Operational Disruptions Due to COVID-19

To safeguard the health and well-being of study participants in the context of circulating SARS-CoV-2 and the associated coronavirus disease 2019 (COVID-19), the guidance provided in this appendix may be implemented at sites experiencing operational disruptions due to COVID-19.

The extent to which site operations may be disrupted by COVID-19 may vary across sites and over time. **All sites should follow applicable government, health authority, and institutional policies with respect to conduct of study visits and procedures, with utmost importance placed on the health and well-being of study participants and study staff.** All sites must also comply with any directives received from the study sponsor, the IMPAACT Network, and/or the IMPAACT 2009 Protocol Team. Should a determination be made in the future that the guidance provided in this appendix is no longer applicable, sites will be formally notified and instructed to inform their IRBs/ECs and other applicable regulatory entities.

Visit Scheduling and Procedural Considerations

- The allowable visit windows for all maternal and infant follow-up visits are extended by ±7 days, as follows:
  - Maternal antepartum visits at Weeks 4, 8, and 12: allowable visit window is expanded to ±14 days.
  - Maternal antepartum visits at Weeks 24 and 36: allowable window is expanded to ±21 days.
  - Maternal Labor and Delivery Visit and Infant Birth Visit: allowable window is expanded to up to 21 days after delivery/birth.
  - Maternal and Infant PP Weeks 6, 14, and 26: allowable visit window is expanded to ±21 days.
- DXA scans are required at the Maternal Labor and Delivery Visit, Infant Birth Visit, and Maternal and Infant Week 26 visits. It is understood that sites may not be able to conduct the maternal and/or infant DXA scans on the same date as the other evaluations for these visits, and may not be able to arrange a scan within the corresponding visit windows. Sites should make every effort to schedule the DXA scan to take place within these windows, but up to 28 days is allowed.
- Sites may make use of these expanded allowable visit windows as needed, however, the visit windows specified in protocol Section 6 remain the target and sites are encouraged to continue scheduling within these target windows whenever possible. Sites should consider current or anticipated operational disruptions or closures when scheduling visits and should schedule visits early or late in the allowable window, as appropriate. Visits conducted outside of allowable windows are also preferred to missed visits
- If it is not possible to conduct study visits in-person at the study site, visit procedures may be performed off-site or remotely (e.g., by telephone) as described below. Site investigators must ensure that standard operating procedures (SOPs) are in place for off-site and remote procedures.
- Sites may conduct study visits — in full or in part — off-site if permitted by applicable government, health authority, and institutional policies. Where this option is permitted, site staff should communicate with participant families to determine in advance where and when such visits will take place, with adequate protections for safety, privacy, and confidentiality. Off-site visit procedures should be conducted by site staff who are adequately qualified and trained to conduct the procedures, as determined by the site investigator, with attention paid to occupational health, biohazard containment, and specimen and data chain of custody. These staff should also be adequately qualified and trained to immediately assess and/or manage any adverse events or social impacts that may occur during the visits. If adverse events requiring further evaluation or management are identified during an off-site visit, staff conducting the visit should arrange for appropriate clinical management, in consultation with the site investigator as needed.

Study Drug Supply

- Sites are encouraged to consider current or anticipated operational disruptions or closures, and to dispense study drug in sufficient quantities to avoid gaps in study drug supply.
- Sites are encouraged to implement study drug dispensing and delivery options involving outdoor pick-up or drop-off. Sites are also advised that, when other options are not feasible, the *Pharmacy Guidelines and Instructions for DAIDS Clinical Trials Networks* permit shipment or courier of study drug from the site directly to participants. This method should only be used in the short-term and if permissible per local institutional and IRB/EC policies. Refer to the *Guidelines* for additional details on this method.

Documentation

- Site-specific contingency plans, and the implementation thereof, should be documented in essential document files for IMPAACT 2009.
- Documentation should be entered in participant study charts in real-time (or close to real-time) if any of the following occur:
  - Missed visits
  - Out-of-window visits
  - Off-site visits (document the location of the visit)
  - Incomplete or partial visits (document which procedures were performed and which were not)
  - Remote contacts performed in lieu of in-person visits (document method used to complete the contact and which procedures were performed)
  - Any other participant contacts
  - Use of alternate laboratories or alternate laboratory assays
  - Alternate provision of study drug
- In consultation with the Division of AIDS, the IMPAACT Network has developed and disseminated guidance for documenting and/or reporting protocol deviations that may occur due to limited site capacity to conduct study visits or procedures due to COVID-19. Please contact the IMPAACT Operations Center Clinical Trials Specialists with any questions related to documentation and reporting requirements.