

Measuring Adverse Pregnancy and Newborn Congenital Outcomes (MANGO): An leDEA Collaboration Study



Study logo:

The MANGO Study

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1. Study Overview

Abstract	<p>Background: Few HIV treatment programs routinely collect and monitor reproductive health outcomes, especially for pregnancy and birth outcomes. Surveillance systems that are ready and responsive to future reproductive health issues arising from antiretroviral use, for instance, need be urgently established within large HIV treatment programs.</p> <p>Objective: The objective of this project is to develop a surveillance program for adverse pregnancy and birth outcomes in a resource-limited setting in order to examine the impact of HIV infection and ART exposure (which is now nearly universal for HIV+ women) at conception and during pregnancy.</p> <p>Methods: In part 1 of this protocol, we will use a mixed prospective and retrospective cohort study design to implement a PV program at two health facilities affiliated with the Academic Model Providing Access to Healthcare (AMPATH) in western Kenya. We will analyze these data to examine any associations between ART and (a) pregnancy and (b) infant/birth outcomes. In part 2, we will create standardized protocols and data exchange standards within leDEA to enable the merging and analysis of multiregional leDEA PV data. This will include establishing a Data Coordinating Center based at Indiana University (IU) to serve as a hub for collecting, disseminating and archiving data from the study in Kenya as well as data collected through a similar, ongoing study at health facilities in South Africa affiliated with the Southern Africa leDEA (leDEA-SA).</p> <p>Anticipated Results: This study will establish PV infrastructure within leDEA that can be scaled within the broader leDEA network.</p>
Study sites	<ol style="list-style-type: none"> 1. Moi Teaching and Referral Hospital 2. Additional AMPATH facility (to be named) 3. Deidentified data will also be transferred to IU from two health facilities in South Africa
Aims	<p>Aim 1 (Part 1 of this protocol, p.6): Determine event rates for adverse pregnancy outcomes, congenital abnormalities (CAs) and other abnormal conditions in infants born to HIV+ and HIV- women and determine the associations between adverse pregnancy and infant outcomes and ART exposures during conception and pregnancy. <u>Hypothesis:</u> HIV infection and ART increase the risk for adverse pregnancy and perinatal outcomes (e.g. stillbirth, preterm delivery, small-for-gestational-age) compared to HIV- women, but there will not be a significant association between HIV status or ART exposure and the incidence of CAs compared to HIV- women.</p> <p>Aim 2 (Part 2 of this protocol, p. 26): To create standardized protocols and data exchange standards within IU and leDEA. By leveraging the existing and extensive leDEA Data Exchange Standard (DES) and creating a Data Standards Task Force and a Data Coordinating Center for PV, we will add new tables and expand existing ones, as necessary, to include new concepts and fields responsive to the needs of PV among pregnant women.</p>
Number of subjects	In the first study component (C1), 1,600 subjects (800 women + 800 infants) will be contacted; In the second study component (C2), a total of 24,600 subjects (12,300 women + 12,300 infants) will have their medical record data reviewed retrospectively, and 2,460 subjects (1,230 women + 1,230 infants) will be contacted; in the third study component (C3), 706 subjects (353 women + 353 infants) will be contacted.
Enrollment schedule	Recruitment and data collection will occur over the course of 24 months.

2. List of Abbreviations

AMPATH	Academic Model Providing Access to Healthcare
ANC	Antenatal clinic
ART	Antiretroviral therapy
CA	CA
DBS	Dried blood spot
EDD	Estimated date of delivery
HIV	Human Immunodeficiency Virus
HIV+	HIV-positive
HIV-	HIV-negative
leDEA	International Epidemiology Databases to Evaluate AIDS
leDEA-EA	leDEA East Africa
leDEA-SA	leDEA Southern Africa
IRB	Institutional Review Board
IREC	Institutional Research and Ethics Committee
IU	Indiana University
L&D	Labor and delivery
MANGO	Measuring Adverse Pregnancy and Newborn Congenital Outcomes
MCH	Maternal and Child Health
MOH	Ministry of Health
MTRH	Moi Teaching and Referral Hospital
PEPFAR	President's Emergency Plan for AIDS Relief
PMTCT	Prevention of Mother-to-Child Transmission
PNC	Postnatal Care
PV	Pharmacovigilance
RA	Research Assistant
TBN	To be named
WHO	World Health Organization

PART 1: KENYA STUDY

3. Background and Significance

In May 2018, researchers from Botswana reported on a potential increased risk for infant neural tube defects among women living with HIV and exposed to dolutegravir (DTG)-containing antiretroviral treatment (ART) around the time of conception (1). This report led to HIV treatment programs, worldwide, scrambling for data on pregnancy outcomes. The across-the-board conclusion was that the current prevention of mother-to-child transmission (PMTCT) data capture tools are inadequate to address the need for more data on the impact of antiretrovirals on birth outcomes, including congenital abnormalities (CAs). Few HIV programs routinely collect and monitor reproductive health outcomes, especially for pregnancy, partially due to gaps in communication between HIV and maternal and child health (MCH) centers. The limited data that do exist often present conflicting findings (2). Nonetheless, it is essential for HIV treatment programs in resource-limited settings to institute robust monitoring tools for reproductive health outcomes; mass treatment programs result in *in-utero* exposures at scale, and invariably newer antiretrovirals with poorly understood safety profiles in pregnancy will become available for both HIV treatment and prevention. Therefore, surveillance systems that are ready and responsive to future issues arising from antiretroviral use need be urgently established within large HIV treatment programs. Furthermore, these surveillance systems can be leveraged to fill knowledge gaps regarding other therapeutic exposures during pregnancy—i.e. a broader pharmacovigilance (PV) infrastructure for pregnancy.

To address these issues, we propose creating the infrastructure necessary for PV in pregnancy for women who are HIV+ and HIV- at two pilot healthcare facilities affiliated with the AMPATH program in western Kenya. Furthermore, we propose analyzing data on pregnancy outcomes among women exposed to ART (including DTG) as a proof-of-concept of the viability of this infrastructure to generate the high-quality evidence needed to inform public health policy.

This research will leverage the infrastructure and expertise of the International Epidemiology Databases to Evaluate AIDS (IeDEA). IeDEA is collaboratively funded by the NIAID, NICHD, NCI, NIMH and NIDA Institutes of the National Institutes of Health (NIH). The mission of IeDEA is to address priority HIV research questions through analysis of large patient datasets collected by HIV programs (3). IeDEA currently follows HIV-infected individuals in six geographical regions around the world. The IeDEA consortium in East Africa (IeDEA-EA) includes HIV care and treatment programs in Kenya, Tanzania and Uganda representing 109 clinical sites that have cumulatively enrolled 318,472 adults and 64,761 children (4, 5). The Academic Model Providing Access to Healthcare (AMPATH) program in western Kenya is the largest contributor of data to the IeDEA-EA Consortium, contributing over 50% of the adult and 85% of the pediatric data. AMPATH and IeDEA-EA have extensive data management infrastructure and well curated HIV databases that help create a suitable environment for adverse pregnancy and birth outcomes surveillance.

Recent and ongoing programmatic PV activities at AMPATH: Following the World Health Organization (WHO) warning in May 2018, the Kenya Ministry of Health (MOH) released recommendations that: 1) women on DTG who may become pregnant be switched to efavirenz-containing ART, 2) pregnant women already exposed to DTG around conception or early pregnancy continue on DTG until cessation of breastfeeding to minimize risk of mother-to-child transmission, and 3) women who do not desire pregnancy and use effective contraception may continue on DTG (6). AMPATH's implementation plan for these recommendations has included: 1) dissemination of this information to providers at both HIV and ANC clinics, 2) tools for providers to counsel women about the risks and benefits of their options, and 3) a Pregnancy Monitoring Program for all women who are exposed to DTG around the time of conception or in the first trimester.

In January 2019, AMPATH began implementation of its electronic medical record (EMR) called the AMPATH Medical Records System (AMRS) in ANC clinics across its network as part of a population health strategy to improve care delivery. AMRS is an *OpenMRS*-based platform used to capture routine clinical encounter data for all HIV-positive (hereforth, 'HIV+') patients enrolled in the program and serves as the central database for AMPATH's HIV data within EA-IeDEA (7). HIV clinical data is currently collected by mid-level providers (e.g. nurses and clinical officers) at high volume HIV treatment facilities at the point-of-care (POC) using wifi-connected tablets. In 2019, AMRS is expanding the introduction of AMRS to five pilot ANC clinics at the following

facilities: Busia County Hospital, Huruma Sub-District Hospital, Kitale District Hospital, Iten District Hospital, and Moi Teaching and Referral Hospital. At these facilities, mid-level providers capture routine clinical encounter data for all HIV+ pregnant women. The expansion of routine electronic data capture via AMRS for this population, which already hosts the entirety of AMPATH's HIV clinical data, presents a unique opportunity to leverage this infrastructure to develop a PV program within AMPATH.

Ongoing PV activities within the Kenya MOH: Following the Kenya MOH recommendations cautioning the use of DTG in women of childbearing age, a combined prospective and retrospective study was initiated by the National AIDS and STI Control Programme (NASCOP) in collaboration with US Centers for Disease Control and Prevention (CDC) to assess the association between DTG and neural tube defects. Moi Teaching and Referral Hospital (MTRH) in western Kenya is one of the study sites. The data collection infrastructure developed through this study is being further leveraged by the MOH to include prospective case reporting of infant CAs in HIV+ women by facility staff at 11 public hospitals throughout Kenya using a standardized reporting form called the **Kenya MOH Surface Exam / Birth Defects Reporting Form** (Appendix 1). This countrywide surveillance study will be used to collect detailed data for infants born with neural tube defects, clubfoot and facial cleft. However, this surveillance effort has important limitations that affect its potential to draw inferences regarding ART exposures and adverse pregnancy and newborn congenital outcomes: 1) it will rely on registry data for the denominator of birth outcomes among HIV- women (which does not contain data on pregnancy exposures), 2) it will not track other major CAs that could be associated with ART and other exposures, 3) it will not track outcomes for subsequent pregnancies and exposures, and 4) it relies exclusively on trained facility staff to correctly identify and classify CAs. We seek to address these limitations in our study by 1) enhancing the data collected on this form for both cases of infants with CAs and infants without CAs, 2) tracking all major CAs, which could also be associated with ART and other pregnancy exposures and improve the sensitivity of the surveillance study, 3) maintaining a secure database that contains identifiers in order to track subsequent pregnancies, exposures and birth outcomes, and 4) using a multipronged approach to data collection that involves trained facility staff and research assistants to ensure data quality. We have also been in communication with the investigators at the MOH and CDC during the development of our proposal. Thus, the broader purpose of this study is to 1) generate data that complements and strengthens the work that NASCOP/CDC are doing in the area of birth surveillance through enhanced sentinel surveillance at two participating facilities (i.e. MTRH and one other facility TBN), and 2) provide data that is most needed and relevant according to the priorities of the MOH. The surveillance data that will be collected in our study will intentionally build on the Kenya MOH Surface Exam Form that is already being implemented at the site.

On October 24-25, 2019, a PV in PMTCT stakeholder meeting was held on the MTRH campus in Eldoret, hosted by the study team. The meeting brought together stakeholders in adverse pregnancy and birth outcomes surveillance from the MOH and Pharmacy and Poisons boards in Kenya and Uganda, the US CDC, and investigators in South Africa. The goal of the meeting was to understand the landscape, opportunities and challenges for pregnancy/birth surveillance in the region. This meeting also served to establish a collaborative foundation between the study investigators and the Kenya MOH. Following this meeting, Ms. Beatrice Ochieng, the Kenya MOH PI of the aforementioned countrywide birth surveillance study, agreed to connect our study team to MOH staff who could join our study team as co-investigators. These individuals are Dr. Laura Oyiengo and Dr. Elvis Oyugi (see p.1) This close collaboration with the Kenya MOH will be instrumental to the implementation of the MANGO study.

4. Justification/Rationale

PV programs in resource-limited settings are necessary to monitor for adverse events at scale in pregnant women – a population that is frequently not included in clinical trials or post-marketing surveillance. This project strives to establish the infrastructure for PV in pregnancy for women living with HIV in Kenya, and in doing so, to address a key data gap regarding adverse pregnancy and birth outcomes associations with ART.

5. Objective, Aims, and Hypotheses

The objective of this project is to develop a surveillance program for adverse pregnancy and birth outcomes in a resource-limited setting in order to examine the impact of HIV infection and ART exposure (which is now nearly universal for HIV+ women) at conception and during pregnancy.

Aim 1: Determine event rates for adverse pregnancy outcomes, CAs and other abnormal conditions in infants born to HIV+ and HIV- women and determine the associations between adverse pregnancy and infant outcomes and ART exposures during conception and pregnancy.

Hypothesis: HIV infection and ART exposure increase the risk for adverse pregnancy and perinatal outcomes (e.g. stillbirth, preterm delivery, small-for-gestational-age) compared to HIV- women, but there will not be a significant association between HIV status or ART exposure and the incidence of CAs.

6. Study Setting and Patient Population

Kenya is one of the priority PEPFAR countries and has been leading innovation for ART implementation for those living with HIV. After Botswana, Kenya has been the next country in sub-Saharan African to roll-out DTG, as alternative first-line ART (8). In fact, starting in October 2017, the Kenya Ministry of Health (MOH) piloted a broader rollout of DTG for first-line ART, including for women of reproductive age. AMPATH, headquartered in Eldoret, Kenya and a large HIV treatment and care partner for Kenya Ministry of Health and the leading consortia member for leDEA-EA, was selected for this broader rollout. As of August 2019, more than 9,000 women of reproductive age had been exposed to DTG-containing ART.

Additionally, unlike other settings with birth defect surveillance such as Botswana where the vast majority of women deliver at a health facility, in Kenya, approximately a third (37%) of women deliver at home rather than in a health facility (irrespective of whether or not they ever attended ANC during pregnancy) (9). These statistics carry critical implications for birth surveillance in Kenya given that delivering in a health facility may be associated with social determinants of health (e.g., home delivery may be a marker of lower socioeconomic status, poor nutritional status and inadequate access to folate-enriched foods, and folate deficiency is a risk factor for preterm birth and neural tube defects), which in turn could influence the incidence of adverse birth outcomes. Hence, capturing only in-facility deliveries could introduce bias in the denominator for estimating birth outcomes and will therefore be addressed through field outreach of women who enroll in ANC clinic at the site but do not deliver at the study site.

The initial surveillance infrastructure will be established at Moi Teaching and Referral Hospital (MTRH) in western Kenya. Located in the city of Eldoret, MTRH is the second-largest national referral hospital in Kenya and headquarters of the AMPATH program. As a referral hospital, MTRH services a catchment of 4 million people throughout the surrounding region that includes an ethnically and culturally diverse urban patient population as well as referrals from the surrounding peri-urban and rural areas. In 2017, the antenatal clinic (ANC) and maternity ward at MTRH serviced approximately 7,200 women (HIV+ and HIV-) and 12,300 deliveries, respectively. Given a county-level HIV prevalence among women of childbearing age (15-49 years) of 5.5%, this entails an estimated 677 deliveries among HIV+ women annually. MTRH has collaborated with AMPATH since its inception. The ANC clinic at MTRH has provided *integrated* HIV and MCH care since 2015, following Kenya Ministry of Health recommendations. Accordingly, all HIV+ women receive WHO guideline-based HIV care and treatment within the ANC clinic, rather than the HIV clinic from the time of pregnancy through 18-24 months postpartum.

Following the initial implementation of the study at MTRH, implementation at additional site within the AMPATH network will also be sought and the protocol/IRB amended accordingly. The additional site(s) selected for the study will be informed by the early implementation experience at MTRH. Preliminary, candidate sites include Kitale District Hospital and Busia District Hospital.

7. Study Design and Methodology

7.1 Overview of Study Design

This study will involve the three study components (C1, C2 and C3), presented in chronological order (Figure 1, next page):

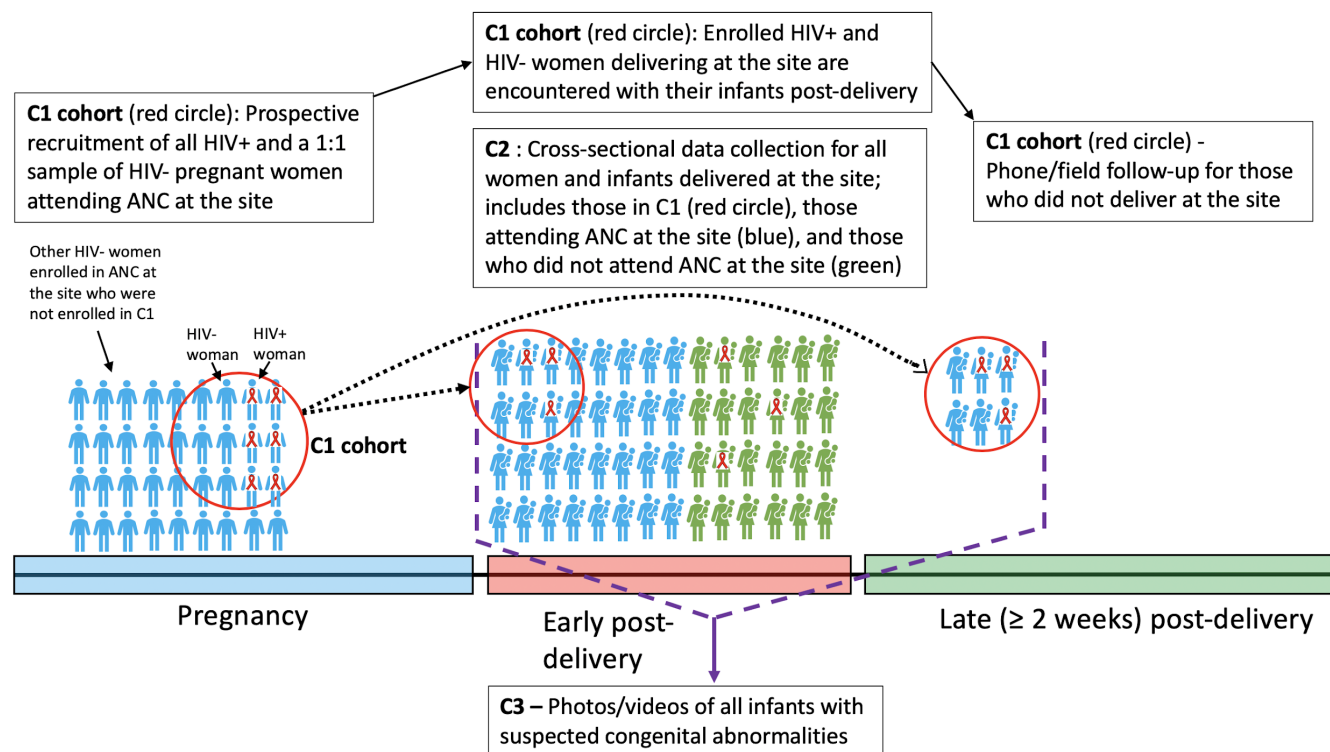
C1. Prospective recruitment of pregnant women enrolling in ANC at the site. All HIV+ pregnant women and a 1:1 systematic sample of HIV- pregnant women enrolling in antenatal clinic at the site will be prospectively enrolled in the study. In addition, medical record data for the mother and infant will be collected at the time of delivery for all women who deliver at the site and as such will contribute to a subset of C2 (below). Women

enrolled in C1 who do not deliver at the site will be contacted by phone and through field follow-up to ascertain their pregnancy and infant outcomes. Infants born to women enrolled in this component who are suspected of having CAs will be enrolled as outlined in C3 (below) if their mothers deliver at the site. If their mothers do not deliver at the site these infants will be enrolled in the field.

C2. Data collection for deliveries at the site. Data will be collected retrospectively from medical records for all women who deliver at the site (including the women enrolled in C1 above), as well from the medical records of all newborn infants and stillbirths delivered at the site. Missing or incomplete data will be clarified with

C3. Photos/videos of infants with CAs. All newborn infants and stillbirths ≥ 24 weeks gestational age delivered at the site, as well as all infants born to women enrolled in C1, will be assessed by surface exam for the presence of CAs. Video and photographs will be taken of CAs identified on surface exam. These images will be reviewed and classified by panel of experts in genetics, dysmorphology and teratology. Mothers of infants with major CAs will be contacted by phone at 1, 6, and 12 months post-delivery to ascertain their infants' vital status and care engagement status.

Figure 1. Overview of study design components (C1, C2 and C3)



7.2 Eligibility Criteria

C1. Prospective recruitment of HIV+ and HIV- pregnant women enrolling in ANC at the site:

Inclusion criteria for women

- Pregnant and enrolled in ANC at the study site;
- Understands English or Swahili.

Exclusion criteria for women

- Any physical or mental disability that prevents the woman from providing informed consent

Inclusion criteria for infants

- All infants at any gestational age who are born to enrolled women will be included

Exclusion criteria for infants

(none)

C2. Data collection for all deliveries at the site:

Inclusion criteria for women

- a. Woman delivers at the site and the delivery is registered at the site

Exclusion criteria for women

(none)

Inclusion criteria for live/stillborn infants

- a. Infant is delivered at the site and results in the infant/stillbirth being registered at the site

Exclusion criteria for infants

(none)

C3. Photos/videos of infants with CAs:

Inclusion criteria for infants

- a. The infant is live or stillborn at ≥ 24 weeks estimated gestational age
- b. The infant has a suspected CA on surface exam

Exclusion criteria for infants

(none)

Rationale for eligibility criteria: We will exclude infants born prior to arrival at the hospital in C2, with the exception of infants born to women enrolled in C1. We believe that this will help to reduce bias given that sick infants (including those with possible CAs) are more likely than healthy infants to be brought to the hospital following a home birth. All infants born to women in C1 will be included regardless of their birth location. As the results of all pregnancies in the C1 cohort will be documented, the risk for bias will be minimized. Infants with possible CAs that are < 24 weeks gestational age at delivery will be excluded from photo/video documentation in C3 due to (i) the difficulty of identifying CAs in extremely premature infants and (ii) in Kenya, non-live births < 24 weeks gestation are considered miscarriages rather than stillbirths, and (iii) pregnant women < 24 weeks in the Kenya setting may not be admitted to obstetric wards but rather to medical or surgical wards depending on their clinical presentation. The approximate age of subjects in each study component is expected to be 15-49 years (i.e. reproductive age). However, there is no age limit given that this is an observational study (see Participation of women, children, and minorities section below for further details). Finally, age will not be considered during recruitment of women in this study, including the recruitment of women in C1. However, given the vulnerabilities associated with adolescent pregnancy and the ethical importance of not excluding this group from the research on the basis of age, we will review the demographic characteristics of those enrolling in C1 on an ongoing basis to determine whether purposeful sampling of adolescents (e.g. 15-24 years of age) is necessary to obtain a meaningful sample of this group.

7.3 Study Procedures

In-service training of clinical staff on surface examination, adverse drug event reporting and referral resources for infants with congenital abnormalities: This surveillance study will rely on routinely collected clinical data and newborn surface examination by clinical nursing staff together with the study team. This structure promotes the sustainability of the study but makes it critically important that the staff are well trained in order to collect complete, reliable data in the ANC and Labor and delivery (L&D) settings, including (i) identifying and documenting adverse drug events that may have occurred during a pregnancy, and (ii) correctly classifying birth outcomes and gestational age assessment (i.e. Ballard scoring (10)) and CAs by infant surface exam, including those abnormalities that may have led to pregnancy loss (spontaneously or following termination). The staff will also be given comprehensive information summarizing the resources available for infants with major CAs, such as pediatric specialty clinics affiliated with MTRH and the surrounding area. Participants will be given with a list of these clinics that include their contact information and operating hours to facilitate appropriate referrals and follow-up care for these infants. This resource guide will be created in consultation with Dr. Julia Songok (study key investigator and Head of the Dept. of Paediatrics at Moi University). Therefore, the study team will train ANC and L&D clinical and clerical staff in these areas prior to and during the study. Standardized WHO training materials (e.g. newborn exam, atlas of congenital anomalies) will be used in these training sessions, with

additional training sessions every 4-6 weeks during the study and as indicated by regular data quality audits by the study team (11, 12) (Table 1). The training program will also include separate sessions for ANC and L&D physicians and clinical officers. These sessions will occur every 2-3 months and will be used as a forum to (i) sensitize these staff members to the study, (ii) provide continuing medical education (CME) on CAs and surface exam and present new literature in the field of adverse pregnancy and birth outcomes, and (iii) disseminate the results of the study. Finally, the research assistants will also participate in these sessions, which will strengthen their knowledge of CAs and proper surface examination, understanding of the study, and grasp of relevant medical terminologies (e.g. drug names and CA nomenclature).

The structure and timing of these sessions will conform to the MTRH Care Protocol and accommodate the routine work duties of the clinical staff. Dr. John Humphrey (co-PI), Dr. Julia Songok (co-I) and an expert in genetics/teratology at IU will lead these in-service sessions throughout the study, with input from the co-investigators. Once a second site is selected, this training schedule will be adapted according to the number of staff, training needs, and schedules.

Table 1. Training schedule for L&D and ANC staff.

Timing	Training Activity
Pre-implementation phase (3 weeks)	<ul style="list-style-type: none"> • <u>Large group knowledge sessions</u> with ANC and L&D staff (e.g. 15-25 participants per session); 1 hour per session, 6 sessions. The goal of these sessions will be to provide an overview of the study and instruct staff in adverse pregnancy and birth outcomes detection and documentation, including how to (i) counsel women on safe medications during pregnancy, (ii) obtain a comprehensive exposure history from women during pregnancy and at delivery and document it in the medical records, (iii) identify and document adverse events suspected to be drug-related during pregnancy; (iv) conduct a surface exam of a newborn infant using a standardized approach recommended by WHO. • <u>Small group skills sessions</u> with ANC and L&D staff (e.g. 5 participants per session); 30-45 min per session, 3 sessions per group. The goal of these sessions is to consolidate and test individuals' knowledge gained through the large group sessions.
Implementation phase (every 4-6 weeks during data collection)	<ul style="list-style-type: none"> • <u>Large group knowledge sessions</u> with ANC and L&D staff (e.g. 15-25 participants per session); 1 hour per session. The goal of these sessions will be to provide a refresher on the study and in pregnancy/birth outcomes detection and documentation, engage the staff in the study by providing updates on data collection progress and results, and solicit feedback from the staff on ways that the study can be improved. • <u>Small group skills sessions</u> with ANC and L&D staff (e.g. 5 participants per session); 30 min per session. The goal of these sessions is to consolidate and test individuals' knowledge gained through the large group sessions. • <u>Large group CME / study sessions</u> with physicians and clinical officers (e.g. 10-15 participants per session); 30-45 min per session every 2-3 months. The goal of these sessions is to sensitize staff to the study, provide CME on adverse pregnancy/birth outcomes, and disseminate the results of the study.

C1: Prospective recruitment of pregnant women enrolling in ANC at the site: C1 is a prospective, longitudinal cohort study that will recruit HIV+ and HIV- pregnant women enrolled in ANC at MTRH and follow them, along with their infants (with and without congenital abnormalities), through delivery. The goal of this cohort is to (i) address the selection bias associated with capturing in-hospital deliveries, and (ii) facilitate enhanced data collection for exposures and outcomes among a sentinel cohort of women and infants delivered in the Kenya setting. The specific aims are:

Sub-Aim 1. 1a) Characterize the prevalence of comorbid conditions, exposures and pregnancy outcomes for HIV+ and HIV- pregnant women, and growth/CAs in their infants/stillbirths; 1b) determine possible associations between maternal exposures during pregnancy and pregnancy/infant outcomes.

Sub-Aim 2: Compare pregnancy and newborn outcomes for women who deliver at MTRH vs. outside MTRH.

Recruitment: All HIV+ pregnant women and a 1:1 systematic sample of HIV- pregnant women enrolled in ANC at MTRH will be recruited in person by a research assistant (RA) following referral from the clinic staff after the woman's routine ANC visit (e.g. for every HIV+ woman recruited, we will also recruit every 5th HIV- woman who visits the clinic). To enhance recruitment, the ANC staff will be sensitized to refer women to the RA for enrollment, and the paper files of HIV+ women will be flagged with a notice about the study (Appendix 2). An RA will be stationed in the ANC clinic throughout the week to receive referrals and administer written informed consent. The consent will request permission for (i) questionnaire administration and medical record review (ii) phone

follow-up both prior to and after delivery, (iii) infant surface exam by the RA, (iv) photos/videos of infant CAs for expert review (see **ANC Consent**).

Study Procedures: Following informed consent, the RA will review the mother's medical file and mother-baby booklet with the mother and administer a brief questionnaire. The purpose of this interaction is to document (i) basic demographic and clinical data, including the woman's location of residence (ii) any medications or other exposures (e.g. alcohol, tobacco, herbal medications, vaccines/exposures, non-prescription medications [drug name but not dose/frequency]) during the periconception and pregnancy periods (which will include labeled pictures of common drugs used during pregnancy to aid participant recall and understanding), (iii) where the woman intends to deliver (e.g. at MTRH, home, or another facility). These data will be entered by the RA into a customized data management system (e.g. REDCap) using a tablet (see **ANC Enrollment Form**). The RA will also place a brightly colored sticker on the woman's Mother-Baby booklet and medical file to indicate that she is enrolled in the study, as well as a brightly colored notice inside the woman's paper file (see Appendix 3 and 4). The purpose of these notices is to alert the clinical staff that the woman is enrolled in the study so that they can notify the RA by phone or in person when the women delivers.

Following this initial encounter and beginning three weeks prior to the estimated date of delivery (EDD), the RA will call the woman once per week to ask whether the woman is still pregnant and if so, verify her planned delivery location (if different than initially stated at study enrollment). Women who have a planned delivery date (e.g. induction of labor or elective c-section) will also be asked to notify the RA when she presents to the hospital for delivery. The purpose of this communication, like the sticker and chart notice, is to ensure that the woman's pregnancy exposures, delivery and newborn outcomes are accurately captured (see Appendix 5 for description of phone interaction/script).

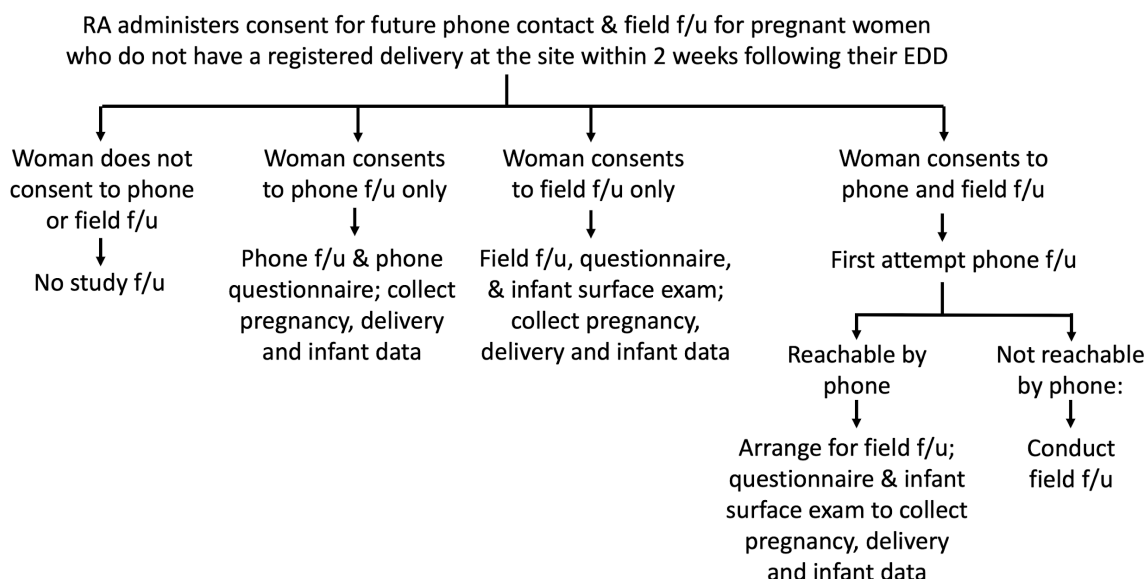
Enrolled women who deliver at MTRH will then be encountered, along with their infants, by an RA on the postpartum ward after the woman has delivered and prior to her discharge from the hospital. During this interaction, the RA will review the mother's medical file and mother-baby booklet with the mother and enter pregnancy and delivery data, as well as infant data (see **Delivery Form** and **Infant Form**). The purpose of this encounter, like the ANC encounter, is to actively ascertain any pregnancy exposures (e.g. medications, substances) with the woman, as well as to ascertain delivery and infant information that was not collected during the initial encounter. These forms will be the same forms used to collect retrospective data from the medical records of all women/infants delivered at the site in C2. (C1 involves active ascertainment of this information through medical record review and interviews with the women to clarify missing or incomplete data, while C2 involves retrospective medical record review). The RA will also conduct a surface exam of the infant to identify possible CAs, followed by photos/videos of the CAs for expert review and classification (based on the woman's initial and continued consent for these procedures; see C3 below). The RA will have a health-related degree/diploma and complete training sessions led by the study team so that he/she will be qualified to conduct the surface exam.

For women whose delivery outcomes are not documented within two weeks following their EDD (as ascertained by an incomplete Delivery Form), the data management system will be configured to alert the study team via an automated email that contains the subject's study ID. The RA will then contact the woman by phone or field follow-up in her home (contingent on the woman's prior consent for these procedures when she enrolled in the study). Contacted women will be administered the Delivery Form that will capture their pregnancy and birth outcomes. Women who are encountered by phone will also be asked whether the RA may encounter them either in their home or at the MCH clinic during a future appointment (e.g. for the 2- or 6-week postpartum visit). Women that agree to either situation will be encountered by an RA at an agreed-upon place and date. For all infants of enrolled women encountered in person by the RA, the RA will review the woman's mother-baby booklet to collect pregnancy/delivery information as done for women who delivered at the site. The RA will also conduct a surface exam, followed by photos/videos of identified CAs contingent on the mother's prior and current consent (see C3 below for details on photos/videos of CAs).

Women who consented to phone and field follow-up but who are not reachable by phone will be traced by the RA in the community using the locator information given by her to the study team during the ANC encounter. To conduct this tracing, we will leverage our extensive experience at AMPATH conducting community tracing for HIV+ patients, including pregnant and postpartum women, who are lost to follow-up (13-15). During daytime

hours throughout the week (on weekdays and weekends), the RA will take an AMPATH vehicle or public transport to the woman's home using the locator information given to her by the woman. Only unmarked vehicles (e.g. vehicles without the AMPATH logo) will be used to avoid the potential for unwanted disclosure to community members that may see the vehicle parked outside the woman's home. Community tracing can be challenging due to poor locator information, so at the time of enrollment we will elicit the woman's location in as much detail as possible using her own narrative description that includes key landmarks and detailed hand drawn maps and google mapping. Before tracing is attempted, the RA will also liaise with a community health worker who is knowledgeable of the area in order to obtain additional geographic details as necessary. Up to two days will be allocated to trace each woman. The RA will knock on the woman's home door, and once meeting her, find a private place with her to conduct the study procedures (e.g. in the woman's home or elsewhere in the community according to her preference). If the woman is not alone or if someone other than the woman first encounters the RA, the RA will not disclose the reason for the visit until she and the woman are in a private location acceptable to the woman (see Potential Risks and Protection Procedures below). (See Figure 2 for a summary of the phone and field follow-up consent algorithm for C1).

Figure 2. Consent algorithm for phone and field follow-up for women who do not deliver at the site.



Finally, the consent form will contain a re-contacting provision that will allow for future phone contact (see Informed Consent section below). This will facilitate the establishment of a longitudinal cohort of WLHIV and their HIV-exposed infants that can be leveraged for future research (see Future Research section below).

The MANGO study in the context of Covid-19 (updated May 13, 2020). Over the course of developing this protocol, a novel coronavirus named SARS-CoV-2 emerged to cause a global pandemic that has affected nearly every country on earth. The impact of SARS-CoV-2 infection (i.e., coronavirus disease 2019 [Covid-19]), on pregnancy and newborn outcomes are not well understood. Emerging evidence suggesting the possibility of in utero transmission of SARS-CoV-2 include: 1) SARS-CoV-2 has been detected in human blood samples¹; 2) elevated IgM antibodies have been detected in three neonates after birth (IgM antibodies are too large to cross the placenta so detection in a newborn could reasonably be assumed to reflect fetal production following in utero infection)^{2,3}; 3) three of 33 neonates born to mothers with Covid-19 developed early-onset symptomatic SARS-CoV-2 infection suggesting maternal-fetal transmission.⁴ Kenya is in the midst of its own Covid-19 epidemic, with 581 confirmed cases and 26 deaths as of May 10, 2020. This presents an opportunity to address the knowledge gap concerning the impact of Covid-19 on pregnancy and newborn outcomes through the MANGO study.

Currently, MTRH is planning to utilize a tablet-based REDCap database to record Covid-19 clinical data for all confirmed or suspected patients. This will be done in order to decrease the use of paper-based charts which could become contaminated and facilitate nosocomial transmission. During C1, we will also recruit pregnant women diagnosed with Covid-19 during pregnancy and follow-them through delivery to ascertain

the pregnancy and newborn outcomes in the same manner that other C1 participants are followed. Covid-19 related data present in the REDCap database that will be abstracted as part of the MANGO study will include: date of symptom onset, symptoms reported, date of Covid-19 test, Covid-19 biospecimen type (nasal/NP swab, throat swab, sputum, BAL, exotoxin A, urine, feces/rectal swab, blood, other), test method (PCR, culture, other), test result (positive, negative), admission to hospital (yes, no), date of admission and date of discharge.

The study team will take the same measures to protect the security of the Covid-19 data as those taken for other medical record data collected during the study, including patients' HIV-related data (see Protection Procedures, section 10.2). Additionally, the research team will refrain from any face-to-face contact with any patients with confirmed or suspected Covid-19 to avoid the risk of transmission given anticipated limitations in the availability of personal protective equipment (PPE). Therefore, to recruit these patients, clinical staff sensitized to the study will ask the pregnant woman whether the RA may contact her by phone to explain the study and offer enrollment. If she agrees, the RA will call the woman during daytime hours and read the ANC consent over the phone. The woman's decision to participate will be documented next to the signature line of the consent by writing "verbal consent", along with the date, which will be sufficient documentation given the minimal risk nature of the research in C1 and the unique circumstances inhibiting in-person contact. Written consent will then be documented at a later date (e.g. following delivery at MTRH or during field follow-up) ≥ 2 weeks after diagnosis and after the patient has recovered from Covid-19. The Covid-19 data in REDCap will also be collected at a later date during the study so as not to interfere with the patient's routine care.

C2. Data collection for all deliveries at the site: RAs will be stationed on the maternity wards to extract medical record data close to real time (i.e., after delivery and prior to the woman/infant discharge from the postnatal ward) for all women and their infants (including those with and without CAs) delivered at the site. RAs will extract data from various medical record sources, described below, using customized data entry system installed on tablets. The data will be entered into the aforementioned Delivery Form and Infant Form, will be based on the **Kenya MOH Surface Exam / Birth Defects Reporting Form** (see Appendix 1 – the personal identifier fields on this form will not be collected except as listed in the identifier-only database below). As described in the background, this MOH form is currently being used at 11 MOH-sponsored health facilities in Kenya, including MTRH, to collect birth defect surveillance exclusively for infants with neural tube defects, talipes equinovarus (club foot), and facial clefts. Ms. Beatrice Ochieng is the PI of this Kenya MOH project. Thus, in collaboration with the Kenya MOH, we will use this data collection tool as a foundation for the MANGO data collection tool, augmenting the MOH birth surveillance efforts at MTRH through enhanced data collection for all deliveries (not only cases of major CAs), detailed pregnancy exposure data, and rigorous training of healthcare workers in CA detection and reporting.

To complete the forms, the RA will retrospectively extract the following data, detailed in the below sources:

Mother-Baby Booklet (MOH 216): The mother-baby booklet is a patient-held medical record that is filled by clinical staff following each clinic visit and at several time points during a hospital admission for delivery (i.e. at triage, immediately following delivery, and at discharge). This booklet holds pregnancy and delivery related information for each medical visit including antenatal exposures, delivery information and child growth and immunization information. The booklet remains with the patient throughout the antepartum, intrapartum, and postpartum periods and is standardized country-wide by the Kenya MOH. As such, this document is the primary record of events during pregnancy for women attending public health facilities in Kenya. An RA will be stationed at the triage and discharge areas of the maternity wards to extract information from the mother-baby booklet into an electronic database when this booklet is routinely updated by the facility staff in real time during the woman/infant delivery hospitalization. The information that will be collected includes: maternal demographics (i.e. name, date of birth, township of residence), pregnancy-related data (i.e. last menstrual period, estimated date of delivery, ANC clinic, number and dates of ANC visits), maternal medical history (including diabetes, hypertension and other common conditions), maternal medications used during pregnancy (including folate and vitamin supplements, anti-tuberculosis drugs), delivery data (including intrapartum antiretroviral prophylaxis), as well as select maternal laboratory results (e.g. hemoglobin, RPR, HIV status), and maternal weight (prior and

during pregnancy). HIV-related data will also be collected from this document, including dates of HIV diagnosis and ART initiation, ART regimens and dates prescribed, WHO stage, CD4 count/nadir and viral load(s) during pregnancy, and any opportunistic infections and associated treatments.

Maternal medical record file: The paper medical record files of all women who deliver at the study site will be reviewed to collect obstetric data from the “Maternity Unit Inpatient Record” (i.e. gravity/parity, outcome of delivery, delivery complications, referral reasons from any outside facility) and infant data (i.e. apgar score, infant vital status [liveborn, stillbirth], birth weight, head circumference, length, any antiretroviral prophylaxis prescribed, and vital status at discharge). Laboratory data that may represent adverse drug events (e.g. renal or hepatotoxicity) during pregnancy will also be collected if present. Additionally, the obstetric record in the medical file also contains a section to describe the infant exam and the presence (yes/no) of any CAs that is filled by nursing staff following the initial assessment of the newborn. The nursing staff trained to conduct surface exams will use this section to document whether any possible CA is identified on surface exam for each live and stillborn infant ≥ 24 weeks gestational age. An RA will then review these completed medical record sections in real time during data extraction to identify infants documented to have a possible CA and approach the mother for consent for clinical photographs. HIV-related data is also available in the medical record file (e.g. in the MOH 257, a.k.a. ‘Blue Card’) and will be used to cross-check and verify the data collected through the mother-baby booklet. The findings from fetal ultrasound, which is being conducted routinely in an estimated two-thirds of pregnant women enrolled in ANC at MTRH, will also be recorded in the study electronic database as a free text field.

Infant medical record file: In most cases, the infant’s medical record file is co-located with the mother’s file at MTRH with the exception of infants hospitalized in the newborn unit (i.e. an intensive care setting), who have their own unique file. The infant’s medical record file will be reviewed to collect infant data that is missing from the maternal medical record file (listed above). Newborn ultrasound reports that are available in the file prior to the infant’s discharge from the hospital will also be recorded in the study electronic database as a free text field. These reports may document the presence of internal abnormalities that may support syndromic diagnoses in infants. For women who enroll in C1 who did not deliver at the site and cannot be contacted through phone and/or field follow-up, we will also review the infant files in the postnatal clinic to attempt to ascertain their delivery outcomes.

Maternity Register (MOH 333): The Maternity Register will be reviewed on a daily basis by the RA to cross-check data from the Mother-baby booklets and paper files for each woman (e.g. number of ANC visits, date of delivery, condition after delivery [i.e. Live birth, fresh still birth, macerated still birth]) and ensure that all deliveries are captured in the study.

Antenatal Care Register (MOH 405): The Antenatal Care Register is located in the ANC clinic at the study site. This register will be reviewed on an as-needed basis to extract data that is missing from the Mother-baby booklet, paper medical record file and maternity register (e.g. date of last menstrual period) for women who attended ANC at the site.

AMPATH Electronic Medical Record System (AMRS): Clinical data will be periodically extracted from AMRS by an AMPATH data manager for all women who were enrolled in HIV care at AMPATH prior to and during pregnancy, as well as pregnant women for whom AMRS is being used at the point-of-care in the ANC clinics at MTRH. These data will be cross-linked in the electronic database using participants medical record numbers and will include clinical appointment dates and HIV-related data (e.g. ART regimens and dates, dates of enrollment in HIV care and ART initiation, WHO stages, CD4 counts and viral loads immediately prior to and during pregnancy). For HIV+ women enrolled in C1 who do not deliver at the site and cannot be contacted through phone and/or field follow-up, we will also review AMRS to attempt to ascertain their delivery outcomes and care engagement status (either at MTRH or transferred out).

To reduce the prevalence of missing or incomplete data given the study’s reliance on routine facility data, such data entries will be clarified with the woman following verbal consent at the time of data capture and/or cross-checked with the medical chart and provider as needed to enhance the completeness of the surveillance data. The RA will be targeted and practical when discussing missing data with these individuals, clarifying key exposures (e.g. approximate dates of ART or other medication names or categories during pregnancy), being sensitive to the potential for recall bias and the time burden on patients and providers. These procedures will

also help to improve clinical documentation and clinical data quality as a service to the program and are good clinical practice. Certain data (e.g. last menstrual period, estimated date of delivery, medications during pregnancy) will also be collected in duplicate from multiple above sources (e.g. mother-baby booklet and maternal file), including AMRS, and we will associate the source document for each variable in the electronic study database. This will also help ensure data quality and completeness by mitigating the potential for missing data in any particular source. We will develop a hierarchy protocol to reconcile differences between data sources according to the variable type between different sources. For example, clinic visit dates and ART prescriptions documented in AMRS will have higher priority than these data documented in the Mother-Baby Booklet given that these are required fields during AMRS data entry, while other medications documented in the Mother-Baby Booklet (e.g. anti-seizure or blood pressure medications) will have higher priority than AMRS data given that these are not required fields in AMRS and may be more subject to missingness.

Identifiable data (i.e. name, date of birth, medical record number) will be collected from these sources; these data are also collected on the Kenya MOH Surface Exam / Birth Defects Reporting Form. The purpose of collecting identifiable data will be to link these data to the AMRS data, subsequent pregnancies for women, and the Kenya MOH birth defects database. Each woman and infant will be assigned a unique study ID that will link the mother-infant dyad and each subject's identifying information in the electronic database. All identifiable data will then be entered into a REDCap database hosted by AMPATH in Kenya. Non-identifying demographic and clinical data for each subject will then be entered into a separate, secure electronic database hosted by Indiana University (IU) using each subject's study ID so that no identifying information is stored on IU servers. Separate, random study IDs that maintain the mother-child link will then be generated to code the dataset when it is cleaned and prepared for any data transfers or analyses (see Data Management).

Verbal consent will be sought for all women approached by the research team to clarify missing or incomplete medical record data as part of C2 (see **C2 Consent**). The rationale for verbal rather than written consent is that (i) the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context; (ii) the only data that will be collected from women are those which relate to *routine and existing medical record data* which is either missing or incomplete upon review of the medical records by the RA, and as such, clarifying these routine data can enhance the quality of the care system for pregnant women and infants; (iii) the study is an extension of an ongoing Kenya MOH birth surveillance registry which does not require consent.

As part of the study, we will develop standard operating procedures (SOP) for the RAs to ensure that no new data are collected beyond what is or should be present in the patient's medical records. Furthermore, we will also develop an SOP to guide the research assistants on when to inform the clinical staff of relevant clinical information after contacting a woman enrolled in C2. An example of this would be if the woman states that she tested positive for syphilis but did not receive treatment (and there is no documentation of treatment in the patient's file). The research assistant would then 1) document the relevant clinical data in a study-specific form (see **Missing Clinical Information Form**); 2) inform a healthcare worker (e.g. nurse or clinical officer) on the ward of the finding. A copy of the form would be placed in the patient's file, and a duplicate copy would be kept by the study team in a locked cabinet in the study office.

C3. Photos/videos of infants with CAs: Structured photos/videos of suspected abnormalities in infants will be taken by the RA for all infants ≥ 24 weeks gestation (born alive or stillborn) prior to the infant's discharge from the hospital. The purpose of this procedure is to confirm and properly classify these abnormalities. Infants with suspected CAs will be identified by the clinical staff during the routine newborn surface exam following delivery and documented in the obstetric record in the mother's paper medical record file. Nursing staff in the L&D and postpartum wards will be trained to recognize CAs as part of the in-service training detailed above. The staff member identifying the abnormality will notify the RA who will then seek written consent from the postpartum woman to photograph and video the abnormalities using a study-dedicated tablet (see Figure 2, next page, and *Photo consent*). Consent will also be requested for (i) clarification of pregnancy exposures or complications during pregnancy (e.g. hospitalization for a febrile illness) directly with the woman, and (ii) phone follow-up of all mothers of infants with possible CAs at 1, 6 and 12 months post-delivery to ascertain the infant's vital status, engagement in care, and subsequent hospitalizations and surgical procedures. The RA conducting the phone calls will also provide information for pediatric clinic follow-up for women and infants who are not engaged in care.

In addition to active reporting to the RAs by the nursing staff, the RAs will also review the obstetric records for all deliveries during the data collection in C2 to identify infants documented by the nursing staff to have a possible CA (see **CA Form**). Prior to the study, we will work with the MTRH OB/GYN Protocol Committee (headed by Dr. Bett Kipchumba, study co-I) to supplement the obstetric record to facilitate routine capture of infant surface exam data (see Appendix 6 for example of obstetric record form for CAs used in South Africa). The obstetric record supplement may contain a simple drawing of the infant which can be marked on as well as a list of checkboxes with major CAs that can be checked off to reduce the documentation burden on the clinical staff and mitigate documentation errors.

Photo/video documentation process: The photo/video protocol will follow the standardized approach used in the National Children's Study (NCS) (16). The protocol will consist of 15 standardized photographic images and three 10-second videos. The 15 photos will include the following views: (1) frontal face; (2) nares; (3) top of head; (4) back of head; (5) 3/4 view of face from the left; (6) 3/4 view of face from the right; (7) right lateral head; (8) left lateral head; (9) top of right hand; (10) top of left hand; (11) right palm; (12) left palm; (13) top of right foot; (14) top of left foot; and (15) bottom of both feet. The three 10-second videos will include a full body dorsal view, a full body ventral view, and a frontal view of the face. The photos and videos will also be accompanied by a separate audio file in which the RA capturing the images will describe the CA(s) that were identified by the nurse and/or RA during the surface exam. The audio recording, which will not contain identifying information, will be used to contextualize the images for the experts who will later be reviewing the images and classifying the CAs that are present.

We will hire RAs with healthcare degrees (e.g. nurse, clinical officer), who will be further trained in surface examination by the study team, to take the photos/videos and provide an additional assessment of infant to identify if there are other CAs missed by the clinical staff. Whenever possible, the RA will inform the nursing staff whenever these additional CAs are noted so that it may be documented in the medical record. In the event of inconsistent findings between the nurse and RA, who will both be trained in surface exam, a third person (e.g. another RA or nurse) will be called upon to adjudicate whether an exam finding is normal or abnormal. Consent for photos/videos will be sought for all infants in whom CAs are present or *possibly* present on exam. Dr. Songok and Dr. Humphrey are pediatricians trained in surface exam and will also be available in real time to discuss these cases with the RAs. Although the primary outcome of the study will be the prevalence of major abnormalities (i.e., abnormalities that have medical, surgical, or cosmetic significance – see Table 2) the staff will be instructed to document all CAs, including those minor abnormalities that do not qualify as a major abnormality (e.g., epicanthal folds, 5th finger clinodactyly, single transverse palmar crease). The clinical and research staff will be trained to identify and document both major and minor abnormalities, which will all be eligible for photo/video by the RA as part of C3. This will be performed in order to ensure that all CAs are being captured during the study. Additionally, the documentation of minor CAs may increase the chance of detecting major CAs, given that the presence of three or more minor CAs is associated with an increased risk for a major CA (17). Once the data collection quality is established to be adequate for the detection of major CAs (e.g. by the RA not detecting CAs that were missed by the nursing staff) the nursing staff and RA will focus only on the detection and documentation of major CAs. This will decrease the workload for the clinical and research staff, and CA review panel, and improve the overall efficiency of the study. During the study, we will communicate with the clinical and research staff to understand the burden of reporting minor and major CAs. Although the goal of recording major and minor abnormalities is to ensure that no major CAs are missed, we will be alert to the possibility that the burden of reporting minor abnormalities may dilute the detection of a major malformation.

Table 2. Summary of major congenital abnormalities (CAs) identifiable on surface exam.

Skull <ul style="list-style-type: none"> • Anencephaly • Encephalocele • Holoprosencephaly • Hydrocephaly Eyes <ul style="list-style-type: none"> • Microphthalmia • Anophthalmia • Colobomas (iris) Ears <ul style="list-style-type: none"> • Microtia Mouth / throat <ul style="list-style-type: none"> • Cleft lip/palate • Micrognathia • Macro or macroglossia Neck <ul style="list-style-type: none"> • Cystic hygroma 	Chest <ul style="list-style-type: none"> • Pectus excavatum • Absent, hypoplastic clavicles Back <ul style="list-style-type: none"> • Meningomyelocele • Spina bifida Abdomen <ul style="list-style-type: none"> • Omphalocele • Gastroschisis Genitalia <ul style="list-style-type: none"> • Ambiguous genitalia Extremities <ul style="list-style-type: none"> • Arms (absent or limb deficiencies) • Hands and feet (polydactyly, syndactyly, polysyndactyly, absent digits, ectrodactyly)
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Digital media data management and CA panel: The photos, videos and audio recordings will be stored in encrypted files on study-dedicated computers and a secure online cloud storage account hosted by IU, linked to the infant's electronic database data using only the infant's study ID. Mothers who refuse to consent for photographs will be asked to describe to the RA, who will hand draw if possible, any defect that has been noted on the infant. The mother's description and photos of the drawings will then be entered by the RA into the study database for review by a multidisciplinary team of clinical experts (see CA panel below). The photos/videos will obscure identifiers (e.g. the hospital wrist or ankle band) as much as possible so the reviewers will not have access to other identifying information. We will form a panel of experts during the study to review and classify all CAs that are present. The panel will include a pediatrician from IU (TBN) and Kenya (TBN), a geneticist/dysmorphologist from IU (Dr. Molly McPheron) and an OB/GYN from MTRH (TBN). Each member of the panel will be sent the photo/video documentation to review and classify each CA by assigning a diagnosis to each CA using ICD-10 codes (to maintain consistency in reporting along with other birth surveillance studies in Africa including the Tsepamo study in Botswana and B positive study in South Africa). The presence of multiple CAs in an infant suggestive of a particular syndrome (e.g. trisomy 18 or 21, fetal alcohol syndrome) are to be classified as the syndrome rather than classifying each CA individually. Relevant pregnancy, delivery, and infant clinical data collected in the electronic study database that does not contain identifiers will also be made available for the panel to review. This may include fetal or neonatal ultrasound findings that may indicate the presence of internal defects (e.g. heart, brain, kidney). However, the members of the panel will be blinded to all pregnancy exposures. Each panel member will sign a confidentiality agreement prior to participating on the panel.

The full panel will meet every 2-3 months to discuss and finalize the diagnoses for each case. The expert in genetics/dysmorphology at IU will lead the panel and his/her diagnosis will have the highest weight in the consensus diagnosis. In this manner, the panel will also provide a platform to strengthen Kenyan professional capacity in the field of genetics/dysmorphology through close collaboration with a trained geneticist at IU. The review panel will also develop and apply exclusion criteria on the categories of CAs which are not relevant to analyses involving the teratogenic potential of medicines. Following each panel meetings, the diagnoses will be returned to the RA on a paper study form, which will be placed in the infant's paper file (or mother's file if the infant does not have a file) by the RA.

Management of infants with congenital abnormalities (CAs): There is currently a lack of dedicated clinical resources and referral centers for children with CAs. Therefore, the study team will work with Dr. Julia Songok (co-I and Head of Dept. Paediatrics at Moi Univ.) to catalyze the establishment of a CA clinical management committee for this vulnerable patient population. The committee will optimally include Drs. Songok, Humphrey, a pediatric surgeon, and Dr. McPheron (co-I and expert in pediatric genetics at IU), as well as relevant nursing and social work staff representatives. This committee will thus serve as a multidisciplinary referral resource that the clinical and study staff may use to refer infants with CAs, who will then determine the appropriate longitudinal management in a multidisciplinary team-based setting. Such committees have been used effectively in the U.S. and Indiana University for the management of children with complex genetic and CAs and are likely to be a feasible way to improve patient outcomes at MTRH. The committee will meet periodically (e.g. monthly) to review referred cases.

Concerning the study team's role, in most cases, the RA will become aware of newborns in the hospital infant with a CA because the clinical staff have already documented it in the paper medical record. In these cases, the RA will confirm with the nurse that the clinical team is aware of the CA and that it is being addressed. However, the study team will not intervene in the specific inpatient clinical management by the clinical team given the observational nature of the study, except to refer the child's name to Dr. Songok as part of the CA clinical management committee. The RA will however carry a list of pediatric referral information which will be offered to the clinical staff to use for discharge planning (see In-service training of clinical staff on surface examination, adverse drug event reporting and resources for infants with congenital abnormalities on p.9). No action will be taken by the study team for infants with minor CAs, which by definition are structural changes that pose no significant health problem in the neonatal period and tend to have limited social or cosmetic consequences for the affected individual (e.g. single palmar crease, clinodactyly).

In the event that a CA in an infant born in the facility is first identified by the RA (e.g. for an infant with multiple CAs that were not fully documented by the clinical staff), the RA will bring this finding to the attention of the clinical staff, who will then be responsible for confirming the finding, updating the medical record, and

implementing the appropriate clinical management at that time. As described in C3, photos/videos of the infant will be taken by the RA with the consent of the mother, and following review of the images and proper classification of the CA by the expert panel, the diagnosis will be returned to the RA on a paper study form which will be placed in the infant's paper file (or mother's file if the infant does not have a file) by the RA. In the event that a CA in an infant born in the community is first identified by the RA during community tracing (i.e. for women enrolled in C1 who do not deliver at the study facility), the RA will triage the infant according to whether the CA is classified as major or minor (see Table 2). For example, a neural tube defect first identified in an infant in the community will be referred immediately to the facility for management, as this lesions poses a more acute health risk of hydrocephalus or meningitis. Additionally, if the parent or caregiver cites insufficient funds as the reason why the infant has not been taken to the facility, the RA will offer to cover the cost of transport to the facility so that the infant can be evaluated. If the defect is minor, the RA will inform the parent of the potential finding and provide information for routine pediatric clinic follow-up in the future (e.g. at the time of the next infant's immunization visit).

7.4 Data Management

A data manager at Indiana University will develop the data collection instruments in REDCap which is a secure web platform for building and managing online databases and surveys. REDCap is installed on IU servers as well as AMPATH servers. The data collection instruments which contain fields with personal identifiers (i.e. name, date of birth, medical record number) will be stored in the AMPATH REDCap database (i.e. an identifier-only database) (Appendix 7). These identifiers will be used to link the research data to the AMPATH electronic medical record system (AMRS) data. All other research data that does not contain identifiers will be housed in the IU REDCap database using a unique study ID for each participant. The data manager will create dedicated summary reports in REDCap that will be used to track surveillance data for purposes of data safety and quality and real-time assessments of adverse events that could be related to ART exposures during pregnancy. These reports will also be used by the study team to coordinate data collection for prospective follow-up of women who enroll in ANC but do not deliver at the study site. Data will be extracted from AMRS by an AMPATH data manager, who will be responsible for linking these data to the main REDCap database. This combined database will be de-identified and securely transferred to Indiana University for the analyses. (See Protection Procedures section below for data security details).

7.5 Study Population, Sample Size, Data Analysis and Interpretation

Sample size: For C1, there are approximately 400 HIV+ pregnant women enrolling in ANC during a one year period. A 1:1 systematic sample of 400 HIV- pregnant women will also be prospectively enrolled (i.e. enrolling n=1 HIV- pregnant woman for every n=1 HIV+ woman enrolled), thus totaling n=800 pregnant women who will be prospectively recruited (i.e. contacted) in C1 during year one. After delivery, their infants will also be recruited. Assuming a 1:1 mother:infant ratio, there will also be n=800 infants prospectively recruited during year one. Hence, for C1 there will be **800 women + 800 infants** (Table 3).

For C2, there are approximately 12,300 annual deliveries at MTRH, the maternal and infant data for these deliveries will be collected retrospectively (i.e. these subjects' data, but not the subjects themselves, will participate in the study). Thus, the total number of patients whose data will be collected retrospectively as part of C2 is **12,300 women + 12,300 infants** (Table 3). We estimate that 1 in 10 of these women will be contacted for verbal consent to clarify missing or incomplete medical record data in their own or their infant's medical records. Hence, the total number of subjects who will be verbally consented is **1,230 women + 1,230 infants**.

For C3, the total prevalence of major CAs among all infants is estimated to be approximately 3% (18-20). All infants with CAs and their mothers enrolled in C1 and C2 will be prospectively recruited for photos/videos of the CAs and infant follow-up as described in C3. Thus, the total number of women (and infants) who will be prospectively recruited in C3 is: $[12,300 + 800] \times 0.03 = \mathbf{393 \text{ women, and } 393 \text{ infants}}$ (Table 3).

Table 3. Summary of annual study population in each of the study components (C1, C2 and C3).*

Study component	Study population		
	Women	Infants	Total
C1. Subjects <u>contacted</u> by the study team and enrolled prospectively	800	800	1,600
C2a. Subjects whose data will be reviewed <u>retrospectively</u>	12,300	12,300	24,600
C2b. Subjects <u>contacted</u> by the study team for verbal consent	1,230	1,230	2,460
C3. Subjects <u>contacted</u> by the study team and enrolled prospectively	353	353	706

* Note that the current study design entails that there will be some overlap in the study populations between C1 and C2, in that the women enrolled in C1 who deliver at the study site will also have their data collected retrospectively as performed for all other women/infants delivered in C2. For conceptual reasons, and because we do not know precisely how many women enrolled in C1 will deliver at the study site (and thus contribute to C2), we have not considered this overlap in the above sample size estimates.

Data analysis and interpretation: The goal of this study is to review the records for (i) all women who deliver at the surveillance site, and (ii) all infants delivered at the site. This is estimated to include 12,300 deliveries annually at MTRH (and the subsequent site(s) when identified) over the two-year course of the study according to facility and DHIS estimates (i.e., up to 24,600 deliveries total during the study). Among these individuals, we estimate that up to 8% (n=1000) will be consented for anonymous photographs of CAs annually, (i.e., 2000 total during the study). Taking the example of DTG as outlined in the Background, if we assume the rate of neural tube defects in the DTG-exposed group is 0.9% and the non-DTG group is 0.1% (based on the Botswana data) over the course of one year, assuming a two-sided alpha of 0.05, in order to reach a power of 80%, 529 observations would be required in the DTG group and 2,645 observations would be required in the non-DTG or HIV uninfected groups each (which is a 1:5 ratio for DTG to non-DTG/HIV uninfected observations). Given the approximations involved in the sample size calculations are based on very low proportions, we performed a simulation study without these approximations, and find that a sample size of 560 observations in the DTG group and 2,800 observations in the non-DTG group will provide adequate power. Given the inclusion of all congenital anomalies, we will have >80% power to detect a significant difference between the DTG and non-DTG/HIV uninfected groups, assuming we are able to observe approximately 560 pregnancies in the DTG and 2,800 pregnancies in the non-DTG-exposed groups. The outcomes we will evaluate include:

- Pregnancy outcomes: Live Birth, Still Birth, Miscarriage, Termination of pregnancy, Ectopic Pregnancy, Molar Pregnancy, Not Pregnant, Other); also including pre-term delivery (<37 weeks gestational age) or very pre-term delivery (<32 weeks gestational age).
- Infant/birth outcomes: CAs on surface exam (e.g., extra digit, hydrocephalus, skull defects, eyes, face, mouth/lip/palate, chest, abdomen, anus, limbs, spine (including neural tube defects), hips, genitalia, skin, etc.); also including low birth weight, small for gestational age (<10th percentile) or very small for gestational age (<3rd percentile).

For the analysis, we will first describe the risk for each type of congenital anomaly by the exposure groups, reporting our findings with incidence rates (events per 1000 deliveries) and rate ratios (in parallel to the Tsepamo Birth Defect Surveillance study in Botswana). Our primary outcome is the incidence of all congenital anomalies, with secondary outcomes considering the risk of neural tube defects specifically and additional outcomes listed above. We will use univariate and multivariate Poisson regression models, adjusting for repeated measures for the same woman (again, parallel to the Botswana study). Covariates we will consider adjusting for in our models may include various demographic (e.g. age, education status, marital status, occupation, ethnicity, and nationality), clinical (e.g. WHO clinical stage, CD4 count, HIV viral suppression status, duration of current ART use, past ART exposures, stage of pregnancy at first ART exposure), and pregnancy-related (e.g. parity, gestational age at ANC enrollment, number of ANC visits, maternal complications such as pre/eclampsia, and other medication exposures including prenatal vitamins or folic acid supplementation), and treatment program/site. For the primary analysis, we will aim to define exposure to ART periconception granularly (i.e. within the first 6 weeks of conception), and will ascertain this definition rigorously and broaden our definition if needed.

8. Dissemination

We anticipate at least two manuscripts prepared by the end of the award period, one describing the process of enhancing infrastructure for PV in pregnancy at our pilot sites, and the second describing the findings of DTG and other ART exposures and birth outcomes analysis. We will disseminate results to key stakeholders through in-person presentations at country-level venues, WHO, leDEA investigator meetings, UNAIDS/Spectrum meetings, and international HIV conferences.

9. Team of Collaborators

This proposal leverages pre-existing, strong collaborations within the larger leDEA network, including in-country academic institutions, US-based academic institutions, and large HIV treatment programs in resource-limited settings. Within AMPATH, the Infectious Disease, Pediatrics, Reproductive Health, and Informatics teams will be involved.

10. Ethics Considerations

The study will be conducted in accordance with legal and regulatory requirements, as well as the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human participants and the Declaration of Helsinki. In addition, the study will be conducted in accordance with the protocol, GCP guidelines, and applicable local regulatory requirements and laws.

10.1 Informed consent

C1: Prospective recruitment of pregnant women enrolling in ANC at the site: Written consent will be obtained for all pregnant women enrolled in C1. Consent will take place with the pregnant woman following her routine ANC appointment during regular clinic hours. A private room in the ANC clinic will be used to protect subjects' privacy. The RA will be stationed in this room to receive referrals from the facility staff following the pregnant woman's routine clinical appointment. Only the pregnant woman, and not her male partner, will be required for consent given the minimal risk nature of the research, to avoid the risk of unwanted disclosure of the woman's HIV status if she is HIV+. Pregnant women will be given the option of consenting for future phone contact and/or field follow-up separately in order to respect their privacy, as well as consent for future phone contact and use of their deidentified data for future analyses after the study is complete. Pregnant women will also be asked to consent for surface exam of the infant by a member of the study team and photos/videos of any CAs that are identified.

C2: Data collection for all deliveries at the site. Written informed consent will not be required from the women and infants whose data will be collected retrospectively as part of C2 (see Study Procedures, C2 description above for rationale).

C3: Photos/videos of infants with CAs. For women enrolled in C1, consent will take place as described in C1 above. During the post-delivery encounter with the woman (whether she is encountered at the facility post-delivery or in the community through tracing), the RA will confirm with the woman her continued consent for the RA to conduct the infant surface exam and take photos/videos of any identified CAs. For women whose infants are identified with possible CAs in C2, consent will take place in person with the RA prior to the woman and infant's discharge from the hospital following delivery. A private area in or near the postpartum ward will be used to administer the consent to protect subjects' privacy. Consent may take place during daytime or evening hours depending on the timing of delivery, anticipated discharge, and when the woman is awake and not occupied with routine care activities. Both parents will be requested to consent if they are reasonably available. However, only one parent's consent will be required given the minimal risk nature of the procedure.

Consent forms will be available in English or Swahili and administered according to the subjects' preference. Consents will be read to subjects by the RA using simple language, allowing adequate time for subjects to ask questions and consider their participation. The consent will clearly explain the purpose of the study procedures and that the choice to participate or not participate without affecting the woman's care she receives. Consents will also contain a recontacting provision for future research (see Future Research, below).

Training in conducting informed consent: The PIs are board-certified physicians who have experience in human subject research, CITI training certification, and completed an Institutional Course in Responsible Conduct of

Research that met NIH Requirements for instruction in responsible conduct of research. The Kenyan PI, Dr. Edwin Were, is the Chair of the Institutional Research and Ethics Committee (IREC) at Moi Teaching and Referral Hospital in Kenya. The RAs who will be obtaining consent will be trained in person by the PIs in the procedure of the informed consent process. They will be trained by the PIs to protect participants' privacy during the consent process and to present the consent document in a manner understandable to the subject. This training will take place prior to beginning the recruitment period and the RA's competence in presenting the informed consent document will be verified by the PI prior to beginning the study. The PIs will provide ongoing feedback to the RAs during the study.

Steps to minimize coercion or undue influence: There will not be any threat of harm or adverse consequences if the subject does not agree to participate in the study. The information provided during the consent will be presented in a balanced way with equal emphasis on all elements of consent (e.g. there will not be over-emphasis of benefits and under-emphasis of risks). Subjects will be informed that they do not have to decide immediately whether they should participate in the study. The contact information of the principal investigator and Kenyan co-investigator will be given to women who wish to further consider participating, ask additional questions, or enroll at a later time within the recruitment window. After enrollment, subjects will be informed that they may withdraw from the research at any time and ask any questions that may arise. Adequate time will be provided for subjects to consider their participation in the study and ask questions.

10.2 Potential risks and protection procedures

Risks

- a. Loss of confidentiality or privacy: There is potential risk of loss of confidentiality due to exposing names, dates, or other identifiable medical information, including unwanted disclosure of women's HIV status or presence of CAs in her infant that may lead to social or economic harm (e.g. experienced or perceived risk of sexual, gender-based and domestic violence, or loss of employment if the woman's HIV status is accidentally disclosed to others, stigmatization by the community). Women who are encountered by the RA, either at the hospital or at their home, may feel uncomfortable about potential invasion of their privacy.
- b. Loss of time: Women may feel inconvenienced due to the time spent participating in the study.
- c. Psychological/emotional stress or discomfort: Women may feel uncomfortable discussing some aspects of their medical history with interviewers. Women who deliver a child with one or more CAs may also experience emotional pain that may be exacerbated by the request to take photos of the abnormalities.

Protection Procedures

- a. Loss of confidentiality or privacy: (1) *Subjects:* We will maintain subject confidentiality by enrolling and conducting the interviews in a private setting whenever possible (e.g. a private room adjacent or within the antenatal clinic or wards). Study staff will be trained in confidential procedures by the PIs. All study team members will sign a confidentiality agreement prior to the study. These procedures will help to minimize the risk of unwanted HIV disclosure. In addition, for field follow-up of women who are encountered at their home, the RA (and outreach worker, if present) will simply identify herself as an individual working on a maternal and child health project until privacy can be assured. The RA will ensure that all interactions with the subjects are undertaken in a confidential environment, which may require that the study team and subject move to a more private location after the initial contact. Such practices have been used effectively in our prior outreach and tracing studies to protect subject confidentiality and limit risk of accidental disclosure of participants' HIV status (13-15). The RAs will undergo extensive training to ensure that patients' privacy and confidentiality are protected.

For women that do experience social or economic harm (e.g. experienced or perceived risk of sexual, gender-based and domestic violence, or loss of employment due to unwanted HIV status disclosure), the following resources will be available: 1) referral to the Legal Aide Center for Eldoret (LACE), a charitable organization located at the AMPATH Headquarters, that provides pro bono legal aid and access to justice for people affected by and living with HIV; 2) individual counselling with a Mentor Mother (an HIV+ mother who acts as a counsellor and peer navigator to guide HIV+ patients to appropriate healthcare and legal services); 3) referral to a social worker at each facility who can facilitate access to appropriate resources (e.g. shelter, food, medical assistance). (See Data Safety and Monitoring below).

To minimize the loss of privacy for women encountered through field follow-up, we will seek to hire RAs that 1) are familiar with the communities served by the study facility, 2) have experience conducting community tracing in prior research or as part of a facility's tracing team, and 3) have human subjects research experience that includes working with HIV+ women. Hence, these assistants will be familiar with their community's culture and customs and competent to respect their community's notions of what constitutes privacy. Only one to two RAs will trace the woman in the community, which will ensure a small party that will be less intimidating and uncomfortable for the woman. Toward this end, we will seek to hire female RAs for the study to facilitate the study interactions with the participating women. During the consent process, women will be notified that they do not have to answer any question during the questionnaire or interview that they do not want to answer for any reason, including those questions they may feel are too invasive or private.

(2) *Data Security*: During all study components (C1-C3), subject demographic and clinical data will be abstracted directly from the various clinical sources into electronic databases (i.e. REDCap) by the RAs using tablets (Samsung Galaxy Tab A). A dedicated identifier-only REDCap database (server hosted by AMPATH in Kenya) will be used to store all identifying information; a separate REDCap database hosted by IU will be used to store data that does not contain identifiers (linking to the identifier-only database using a unique study ID assigned to each subject). The tablets will be password-protected and configured to erase the device after 10 failed passcode attempts. The REDCap app will also be password-protected with a password unique from the tablet's main password; the app will also lock out for 15 minutes after five failed login attempts. The identifier-only database will only be accessible by the PIs and research coordinator, while the identifier-free database will only be accessible by the study team. Only the identifier-free database will be used for generating progress reports and analyses. These reports will remain confidentially stored in encrypted files on study-dedicated computers and a secure online cloud storage account hosted by IU.

The photos/videos and audio files will also be recorded using the same tablets used to collect data in REDCap. However, these files will be saved to removable SD cards on the tablets. After an infant with CAs is enrolled, the SD card containing the multimedia will be immediately transferred to a study-dedicated laptop where it will be uploaded by the research coordinator to a web-based secure cloud storage account that is compliant with IU's HIPAA policies and procedures for protected health information. Soft copies of the images will also be transferred to a USB drive that will be stored in a locked file. As previously stated, the media will be anonymized as much as possible by ensuring that identifiable information (e.g. hospital numbers or wrist bands) are not imaged or recorded; the digital files will be labeled only with the subject's study ID.

All confidential paper forms (i.e. consent forms) will be maintained in a locked file cabinet behind locked doors in the leDEA office in the Chandaria Building (MTRH campus) where the study team is situated. Access to these materials will only be allowed by the minimum key investigators and study personnel.

The master dataset generated from the identifier-free REDCap database will be deidentified prior to analysis by creating new study IDs that maintain the link between mothers and their infants but cannot be decoded to identify subjects' personal information. The master dataset will be cleaned and stored on a secure Box server hosted by IU. This data file will be limited to that clinical information which is necessary for the analysis. See Part 2: Developing an leDEA multi-regional infrastructure for further details on data safety and transfer.

- b. Loss of time: We will inform potential participants of the time required to complete the study activities during the consent process.
- c. Psychological/emotional stress or discomfort: The study staff will be trained in this area and will work closely with trained nurses and counsellors on the wards who will assess each woman's ability to participate in the study. Women who are not considered psychologically/emotionally able to participate in the study will not be requested to consent for photographs. Given that congenital anomalies are fixed and unchanging over time, we will seek to approach the woman at a later time (e.g. following a routine postnatal visit at the site). Counselling and support services will be made available to women as needed (i.e. the same resources described above for women who experience social/economic harm, as well as pediatric referral services).

Benefits

There are no direct benefits to the women and infants who will participate in the study. However, infants will be examined by a trained healthcare worker who is working on behalf of the study. This individual will be trained to

assess whether the infant may have a CA. If a CA is detected, the healthcare worker will refer the mother and infant to the study team. The RA working on behalf of the study will be knowledgeable about existing clinical resources and support and will be able to share this information with the mother. For HIV+ women who are disengaged from care and encountered in the community, the study team will encourage them to engage in HIV and maternal and child health care. In addition, this study will improve the quality of care delivered at the study sites by providing training to staff on CA surveillance and examination of newborns as well as enhancing the quality of routine program data. Finally, the results of this study will provide generalizable knowledge about the epidemiology of adverse pregnancy and infant outcomes in sub-Saharan Africa for women with and without HIV and their infants which is intended to benefit this population.

10.3 Participation of women, children, and minorities

This study will involve recruitment of pregnant women in C1, and postpartum women and their newborn infants in C3. The justification for this is that (i) this research will directly impact our understanding of the health and wellbeing of this population, and (ii) the research involves no more than minimal risk to participants. We will also include pregnant and postpartum women who are < 18 years of age along with their infants. The justification for including adolescents in this research includes (i) in Kenya, pregnant adolescents are considered emancipated and do not require the consent of a parent or guardian for medical care under the National Commission for Science, Technology and Innovation, (ii) these women are of reproductive age, and as such, the public health issues surrounding adverse pregnancy and birth outcomes are directly relevant to them, (iii) the research involves no more than minimal risk to participants, (iv) excluding this population from this research as we would be depriving this population of the opportunity to benefit from the findings of the research, particularly given the epidemiology relevance of adolescent pregnancy in the Kenya setting. These adolescent women will be asked to consent for their own and their infant's participation in the study. Moreover, requiring permission of the adolescent's parent could risk unwanted disclosure of the adolescent's HIV or pregnancy status, outweighing the minimal risk nature of the adolescent's and their infant's participation in the research.

11. Data Safety and Monitoring

Only the PIs, research coordinator, co-investigators, and RAs who conduct the informed consent will have access to individually identifiable data. Paper-based facility records will not leave the facilities where they are housed. It is necessary to retain identifiable data rather than de-identified data for the study because 1) it is necessary to link the study data to routine program data; 2) it will be important to be able to link subsequent pregnancies for women (e.g. for women who have been initiated on ART during one pregnancy and continue on it for a subsequent pregnancy, thereby constituting a first trimester exposure for the second but not the first pregnancy); 3) the project aims to also identify serious and fatal adverse events that may have occurred during the course of a pregnancy.

The REDCap database used to store de-identified Kenya study data will be hosted by IU. This database will be password protected and only accessible by the study team. Computers and tablets used to access the REDCap database and enter data will be password protected. The data will be coded and processed for specific analysis. The data files will be limited to the clinical information which is necessary for the analyses.

This study is of minimal risk and does not involve interventions, so an external data safety and monitoring board will not be established. However, an internal data safety and monitoring committee will be established and will include the study PIs and statistician. Interim analyses will be conducted at least annually to assess the safety of ART for HIV+ women during pregnancy. Signals of possible adverse outcomes, such as prematurity (>25%)(21, 22), stillbirths (>5%)(23), neural tube defects (>0.08% in HIV+ or HIV- women)(24), virologic failure in mothers (>10%)(25), or other unanticipated negative outcomes, will be shared with the AMPATH program and Ministry of Health to facilitate appropriate system response according to the nature of the outcome. Analyses will also assess for significant differences in outcomes between HIV+ and HIV- women, and these differences will be reported to the program as well.

The RA will encourage all women encountered through field follow-up engage in care at the facility for themselves and to bring their infants for care. The names of HIV+ mothers and their HIV-exposed infants found to be disengaged from care will be referred to the facility staff who will communicate with the Mentor Mother at the

facility and AMPATH defaulter tracing team to re-engage the woman and infant in care. Caregivers of infants identified to have a major CA on surface exam (e.g. encephalocele, neural tube defect – a list of conditions will be established for the RAs to use), will also be encouraged to seek care at the facility for the infant. The RA will inform the clinical staff if the major CA is not documented in the medical records so that the staff can document this finding and arrange for appropriate follow-up.

There will also be continuous monitoring of serious and unexpected adverse events including social and economic harm by the investigators. These events will be ascertained through weekly discussions with the nursing staff and phone-based follow-up of women enrolled in C1 and C3. Serious and unexpected adverse event will be reported to the IREC and IRBs as per standard procedures. The internal advisory committee will also discuss all serious and unexpected adverse events and determine causality.

The PIs will also review the provisions to protect the confidentiality and privacy of participants with the RAs on a weekly basis during the first month of the study and monthly thereafter during recruitment. During the study, the RA will ascertain whether any adverse events (e.g. loss of confidentiality, or social or economic harm resulting from accidental disclosure of a participant's HIV status) have occurred through weekly meetings with the maternal and child health clinic staff and facility outreach team during recruitment. The RA will also be available to receive reports of adverse events through direct communication from participants, who will be instructed to use the RA's contact information in the consent form in such cases. Clinicians, social workers, peer counsellors, and staff at the Legal Aide Center for Eldoret will also be given the contact information of the RA in the event that they encounter a subject who has experienced an adverse event (see Protection of Human Subjects). The RA will report all adverse events to an internal committee that includes the members of the study team. Publication or presentation of the results of this study will be agreed upon in collaboration with the study investigators. The funding agency has no input in the decision to present or publish study data or the nature of the data that are presented or published. The data will be retained for a minimum of 7 years prior to disposal.

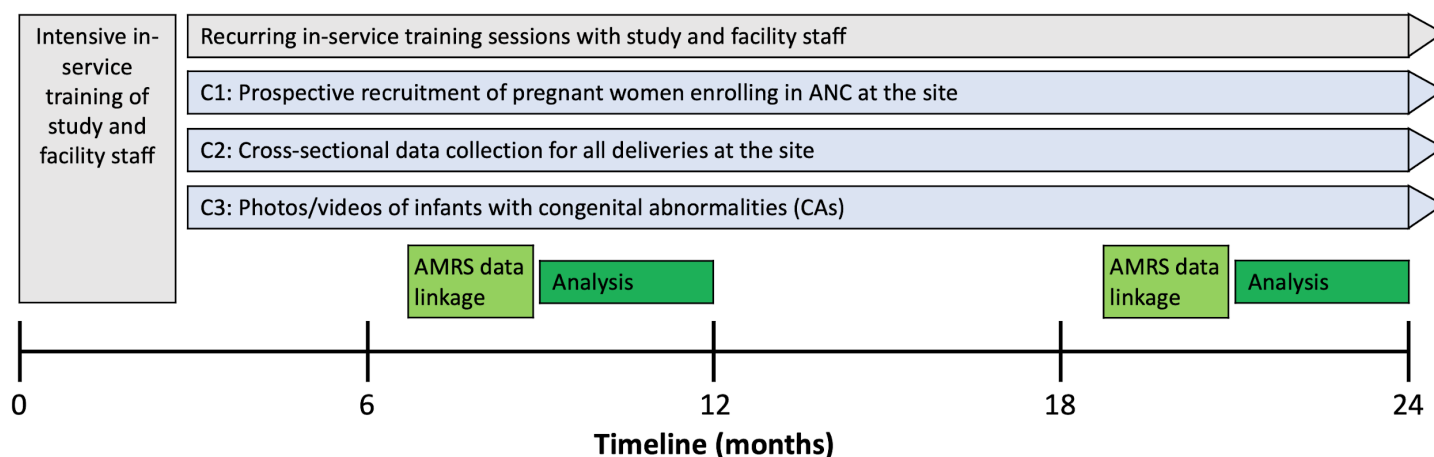
12. Institutional Review Board (Kenya study)

The investigators from Kenya and Indiana will obtain regulatory approval from the Institutional Research and Ethics Committee (IREC) at Moi Teaching and Referral Hospital, which will cover all Kenya study sites, as well as approval from the IRB at Indiana University. Investigators in Kenya will also obtain a research permit from the National Commission for Science, Technology and Innovation (NACOSTI) in accordance with Kenya research regulations. All correspondence with the IRBs will be retained in the regulatory or trial master file. Copies of IRB approvals will be filed with other study documents.

13. Timeline (MANGO study)

The duration of the study will be two years (Figure 4). We will develop the protocol, prepare data collection tools, and obtain IRB approvals in the pre-awared period, hire and train study personnel prior to and during enrollment, and conduct AMRS data extractions and analyses on an annual basis during the study.

Figure 4. Timeline of study activities and analyses.



We plan to achieve the following milestones during the study:

Milestone 1: We will complete staff training and pre-implementation preparations by month 2;

Milestone 2: We will begin data collection and enrollment of postpartum women by month 4;

Milestone 3: We will begin preliminary analysis by month 12;

Milestone 4: We will complete the second analysis by month 24.

14. Anticipated Problems

- a. Accrual: We will monitor accrual on an ongoing basis during the study. If >10% of women do not consent for C1 or C3, we will explore the reasons for refusal to participate through discussions with subject at the time of consent and review of the study procedures and consent document. Any alterations in the protocol or consent resulting from these assessments will be submitted to the Kenya IRB (IREC) and other regulatory boards as required. If >10% of HIV+ and HIV- women who do not deliver in the facility refuse to be encountered in the community (and therefore do not have delivery outcomes ascertained by the study), we will explore whether these women can be encountered by the RA at the maternal and child health clinic of the study site during their next appointment. We will record basic demographic and clinical data (deidentified) for subjects who refuse to participate in the study in order to compare their characteristics to those who participate to assess for bias.
- b. Woman has died or abandoned the infant after childbirth: If during tracing the study team learns that the woman has died or abandoned the baby after childbirth (either through communication with a relative or caregiver at the home or a neighbor), and the infant is alive, the study team will approach the father or other legal guardian to provide consent for a surface exam by the RA. The RA will not disclose the woman's HIV status to anyone in the woman's home or community, as the father/guardian may not be aware that the mother was HIV-infected or that the infant is at risk of HIV infection. Given that there is 50% mortality in untreated, HIV-infected infants and high morbidity/mortality among infants whose mother is deceased, it is ethical to ensure that the infant receives appropriate care. The RA will emphasize the need to bring the infant to a health center for care and evaluation and communicate the subjects' contact information to the facility staff. If the RA or community health worker has any imminent concern about the infant's health, he/she will request to bring the infant to the facility during that encounter for urgent evaluation.
- c. Missing variables in chart: If medical record review reveals substantial missing data for a given field, we will bring this to the attention of the facility staff to remind/train staff to collect this data appropriately as a way to improve data quality, and we will document this issue as a potential implementation barrier. We will also note this issue in the electronic database and use imputation for missing values or disregard variable in the analysis, as appropriate. We will also compare the completeness of the data collected in the C1 cohort (which has active ascertainment of pregnancy exposures by the RAs for all women) to the data collected in C2 (which relies primarily on retrospective chart review) to gain insight into the impact of missing data in the study.
- d. Suboptimal engagement by the clinical staff and staff turnover: Prior to beginning data collection, we will identify a member of the nursing staff to serve as a champion for the study. She/he will serve as a liaison between the RAs and nursing staff, assess the study processes on the nursing staff side, identify barriers to implementation and bring them to the attention of the investigators, identify staff turnover events and training needs for new staff. We will incentivize the clinical staff to participate in the training sessions and data collection efforts by providing refreshments at the sessions and supplies to enhance the comfort of their work environment (e.g. new refrigerator or microwave for staff break room).
- e. Identification of stillbirths: Surface examination of stillborn infants is not currently conducted routinely at MTRH and will require a change in routine practice and documentation for the clinical staff. However, identifying and documenting CAs in stillborn infants is important because major CAs have a higher prevalence in stillborn compared to liveborn infants, and up to 20% of stillborn infants have major CAs (1, 2).
- f. Language barriers: If more than 20% of patients approached are excluded from enrollment due to language barriers (e.g. unable to understand English or Kiswahili), we will seek an amendment of the IRB protocol to include consent / questionnaires in additional language(s) (e.g. Luyha or Luo) in order to ensure that we obtain a representative sample of the population.

15. Funding

This supplement is funded through the NIH-NICHD grant # 5U01AI069911-14 (14 is the 14th year of the grant). The title of the overall grant is “East Africa International epidemiology Databases to Evaluate AIDS (leDEA) Regional Consortium.”

16. Future research

The C1 cohort of mother-infant dyads offers a variety of opportunities for future research such as (i) establishing a longitudinal HIV-exposed uninfected (HEU) infant and HIV-unexposed uninfected (HUU) infant cohorts to track neurodevelopment and other clinical outcomes, (ii) longitudinal follow-up of infants with major CAs to determine survival and care engagement status, and (iii) laboratory investigations to assess potential exposures in greater depth (e.g. red blood cell folate levels in women to assess for potential associations with neural tube defects in infants, urine ethyl glucuronide (EtG) levels to assess for alcohol exposure, serum cotinine levels to assess for tobacco exposure, and serologies in the mother and infant to assess for infections associated with CAs and growth restriction in infants– e.g., Zika virus, TORCH infections). Additional research can also include qualitative work to more deeply explore the experiences and perceptions of parents of infants with major CAs in the Kenya setting and care systems to link and engage this vulnerable population in care.

17. Project Management (Table 3)

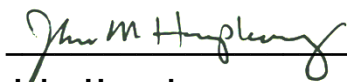
Table 3. Investigators, roles and responsibilities.

Investigator	Role	Responsibilities
John Humphrey	Project co-PI, USA	Responsible for study conceptualization, implementation, data collection, analysis, and manuscript writing; participate in internal data safety and monitoring committee
Edwin Were	Project co-PI, Kenya	Assist with study conceptualization, implementation, analysis and manuscript writing; participate in internal data safety and monitoring committee
Rena Patel	Project co-PI, USA	Responsible for study conceptualization, implementation, data management and harmonization with S. Africa data, and manuscript writing; participate in internal data safety and monitoring committee
Bett Kipchumba	Co-I, Kenya	Assist with study implementation and manuscript writing
Wycliffe Kosgei	Co-I, Kenya	Assist with study implementation and manuscript writing
Julia Songok	Co-I, Kenya	Assist with study implementation and manuscript writing
Marsha Alera	Study Coordinator, Kenya	Facilitate staff hiring and training, study implementation, and participate in manuscript writing
Megan McHenry	Indiana University	Assist with C1 and C3 cohort follow-up; manuscript writing
Molly McPheron	Indiana University	Expert in genetics, teratology and dysmorphology; will lead CA panel
Edward Leichty	Indiana University	Assist with study implementation and manuscript writing
Beverly Musick	Data Manager	Assist with development of data collection instruments and analysis
Laura Oyiengo	Kenya MOH	Assist with study conceptualization and results dissemination
Elvis Oyugi	Kenya MOH	Assist with study conceptualization and results dissemination
Caitlin Bernard	co-investigator	Assist with study implementation and manuscript writing
Ushma Mehta	U of Cape Town	Responsible for study implementation in South Africa
Emma Kalk	U of Cape Town	Responsible for study implementation in South Africa
Amy Slogrove	U of Cape Town	Responsible for study implementation in South Africa
Andrew Boule	U of Cape Town	Overall project oversight, analysis and manuscript writing
Mary-Ann Davies	U of Cape Town	Overall project oversight, analysis and manuscript writing
Kara Woolls-Kaloustian	leDEA co-PI, USA	Provide overall project oversight and primary mentorship to JH and RP throughout the project, including study conceptualization, enrollment, data collection, analysis, and manuscript writing; participate in internal data safety and monitoring committee.
Constantin Yiannoutsos	leDEA co-PI, USA	Lead statistical analysis; assist with manuscript writing and review

18. Signatures of Principal Investigators



Edwin Were



John Humphrey



Rena Patel

PART 2: Developing an leDEA multi-regional infrastructure

19. Background

The MANGO study in Kenya is part of a larger grant to create and test a multiregional infrastructure for PV in pregnancy within the International Epidemiology Databases to Evaluate (leDEA) consortium. The deidentified data collected through this study in Kenya will be combined with deidentified data collected through a similar, ongoing PV study (called “B positive”) led by the Centre for Infectious Disease Epidemiology & Research (CIDER) at the University of Cape Town in South Africa. CIDER is a collaborating institution with the Southern Africa leDEA (leDEA-SA), the largest leDEA region in terms of HIV+ patient numbers. Dr. Mary-Ann Davies is the co-principal investigator of the leDEA-SA.

The data for our study in Kenya will be collected in a way that can be harmonized with the South Africa study data in a deidentified manner. It is our intention that that this effort will lead directly to expansion of PV in pregnancy efforts at additional regional sites affiliated with the global leDEA network (e.g., West and Central Africa leDEA), ultimately creating a robust multiregional infrastructure prepared to respond to future issues around PV in pregnancy in resource-limited settings. The following sections describe how the data from these sites will be managed by the investigators and infrastructure at IU and leDEA.

20. Approach

20.1 Specific Aim

(Aim 1 is described in Part 1: Kenya study)

Aim 2: To create standardized protocols and data exchange standards within IU and leDEA.

Goal: By leveraging the existing and extensive leDEA Data Exchange Standard (DES) and creating a Data Standards Task Force and a Data Coordinating Center for PV, we will add new tables and expand existing ones, as necessary, to include new concepts and fields responsive to the needs of PV among pregnant women.

20.2 Study Settings and Patient populations

Sentinel sites at AMPATH, Kenya. (described in Part 1 above)

Sentinel sites at CIDER, South Africa. South Africa, a priority PEPFAR country, is one of the countries hardest hit by HIV. CIDER at the University of Cape Town was established in 2000 and conducts multi-disciplinary research on priority infectious diseases in Southern Africa, in order to improve disease prevention and management. CIDER has strong links to service providers at provincial and national level, and a long track record of conducting operations research around program effectiveness and service delivery challenges.

CIDER initiated the “B positive” project in 2016 which has overseen the establishment of a Pregnancy Exposure Registry (PER) at two sentinel sites in the Western Cape (one urban, one rural). As related to this proposal, our ART exposure ascertainment is robust, but additional enhancement efforts need to continue to bolster pregnancy and birth outcomes within PER. Once further developed, our infrastructure can likely easily be applied to numerous exposures and settings in the region. Of note, we have enrolled some women who started DTG after 28 weeks gestation as part of an unrelated study. It is anticipated that DTG as part of first-line ART will rolled-out in South Africa in the coming months.

Sentinel sites CIDER’s “B positive” PER initiated enrolment at the urban site (Gugulethu Midwife Obstetric Unit [GMOU]) in September 2016 and the second, rural site (Worcester MOU [WMOU]) in February 2018. Routine clinical data as recorded by clinicians in the clinical stationary are collected into a module in the provincial EMR platform called Provincial Health Data Centre (PHDC), which in use in primary care throughout the Western Cape. The PHDC is a province-wide population-based database which links individual health records via a unique patient identifier. Data for the PER cohort at the two sites is available via the PHDC. This allows validation of the PER exposure data via electronic pharmacy records in the PHDC and improve outcome ascertainment beyond the sentinel facilities. We are able to further validate ART exposure via a well-validated electronic HIV

database. Thus, ART exposure ascertainment is robust, but additional enhancement efforts need to continue to bolster pregnancy and birth outcomes within PER. Up to 50% of the women attending antenatal care at these sites deliver at the same sites, with another significant proportion delivering at the relevant secondary and tertiary referral hospitals. We collect selected antenatal data for the women who register for and then deliver at our two study sites. We also collect data for all deliveries occurring at our sites and ascertain exposure history during antenatal care retrospectively. Exposures collected include ward stock dispensing (supplements, vaccines, antibiotic treatment for syphilis and other STIs & UTIs, infant PEP) and treatment for HIV and other chronic medical conditions. We engage with the clinical staff regularly to improve clinical record keeping to include use of chronic, OTC and traditional medications; and smoking, alcohol and recreational drug exposures. We have now collected data on ~11,000 women over the last two years.

The Western Cape PER currently enrolls women at their first ANC visit to the primary care facility. Data are entered at this visit and updated from the clinical records at delivery. The initial PER was launched in 2016 at Gugulethu Midwife Obstetric Unit (GMOU), a primary health care facility in Cape Town. Women who attend antenatal care (ANC) at GMOU are enrolled and followed-up regardless of delivery site within Cape Town. GMOU provides for approximately 5000 ANC bookings and 2,500 deliveries annually (Table 1). Women with ante- and perinatal complications are referred to secondary (Mowbray Maternity Hospital) and tertiary (Groote Schuur Hospital) services as appropriate. The HIV prevalence is 30%. We have further enrolled 900 HIV-infected and uninfected women at this site to follow more closely for a related study monitoring outcomes for HIV exposed and uninfected and HIV unexposed children of these women. The Worcester sentinel site began enrolment in 2018. Approximately 3500 women attend ANC at Worcester MOU (WMOU) and related primary care antenatal sites annually, with delivery at WMOU, Worcester Hospital and Tygerberg Hospital. WMOU serves a rural population with an HIV prevalence 14%. At the MOUs obstetric care is provided by midwives with referral to hospital if a higher level of care is indicated. The Maternity Case Record (MCR) is a patient held document in which all clinical information is recorded from first ANC to postnatal discharge (similar to the Mother-Baby Booklet in Kenya). It is retained at the site of delivery. Most women in South Africa deliver at a health care facility; a minority who delivery out of facility will attend with the infant soon afterwards to obtain the necessary clinic cards and registration. Once we have optimized the existing PER we hope to scale up surveillance efforts with the Klipfontein-Mitchell's Plain subdistrict of Cape Town (potentially an additional two primary care sites and one district hospital. The PER is situated within the PHDC.

The total HIV+ and HIV- patient populations included in the Kenya and South Africa datasets on an annual basis are shown below (Table 4):

Table 4. CIDER and AMPATH study sites, county HIV prevalence, and annual patient volumes.

Study site	County HIV prevalence	Antenatal Clinic	Total Deliveries	Estimated HIV+ Deliveries
CIDER, South Africa				
Gugulethu Midwife Obstetric Unit	30%	5,000	2,500*	750
Worcester Midwife Obstetric Unit	14%	3500	1500*	490
AMPATH, Kenya				
Moi Teaching and Referral Hospital	5.5%	7,200	12,300	677
Busia District Hospital	9.4%	2,600	4,000	376
Total	--	18300	18300	2293

20.3 Enhancement of Infrastructure at CIDER, South Africa

Funding through this grant will be leveraged to enhance the current surveillance infrastructure in SA-leDEA as follows:

1. Data manager and epidemiologist support. Despite successful implementation of the PER, both initiative of the “B positive” project, analysis of the substantial data being collected is slowed due to limited data manager and epidemiologist support. Pregnancy outcome ascertainment requires further optimization. The additional staff will work to enhance gestational age and outcome ascertainment (inference engine approach) within the

PHDC. S/he will be dedicated to PER activities and liaise directly with operational team to optimize reports, data cleaning and validation.

2. Staff training in birth surveillance reporting. The current structure of the PER program relies on routinely collected clinical data and newborn surface examination by clinical nursing staff. This structure promotes the sustainability of the PER program but makes it critically important that the staff are well trained in order to collect reliable data and correctly classify birth outcomes and congenital abnormalities by examination, including those abnormalities that result in pregnancy loss (spontaneously or following termination). This supplement will support training of clinical and clerical staff in complete and accurate documentation, surface examination and photographing suspected congenital malformations at each site. Standardized WHO training materials (e.g. newborn exam, atlas of congenital anomalies) will be used to in these training sessions, with additional training provided during the study as indicated by regular data audits by the study team (7, 8).
3. Enhanced training and support for adverse drug event reporting. The project also aims to also identify serious and fatal adverse drug events that may have occurred during a pregnancy or in the postpartum period. This supplement will support training of clinical staff to enhance their capacity to assess and report adverse drug reactions including serious adverse events in pregnant and postpartum women and reporting of these events in our study database. We will enhance our data collection forms to capture additional adverse drug event data according to the leDEA-DES including laboratory-based adverse events such as renal and hepatotoxicity. This will enable triangulation of this data with the South Africa Medicines Information Centre Database, an independent organization situated at the University of Cape Town's Faculty of Health Sciences.

20.4 Creation of Standardized Protocols and Data Exchange Standards within leDEA

Both Indiana University and the University of Cape Town have developed comprehensive analytics and informatics processes to assemble, harmonize, validate, and analyze epidemiological cohort data of diverse provenance. Both teams have led development of statistical methods to design and perform studies which target specific data gaps and use these additional data to correct and minimize biases arising from the analysis of observational data. East Africa leDEA (EA-leDEA) has had a leading role in chairing several leDEA Global Working Groups including Pediatrics, the newly constituted PMTCT, Data Harmonization, and Strategic Data. Dr. Kara Wools-Kaloustian has mentored the development of the leDEA Mother-Infant Working Group. In addition, Dr. Constantin Yiannoutsos leads the leDEA Pediatric Methods and Modeling (IPM2) Consortium. We propose to house the data center for this initiative within the Indiana University with extensive support and involvement from investigators at the University of Cape Town and other institutions.

leDEA has achieved extensive cross-regional data interconnectivity through a common data model established by the leDEA Data Harmonization Working Group, the leDEA Data Exchange Standard (leDEA-DES) (www.leDEADES.org). The DES was designed to meet the needs of research organizations in resource-limited settings and enable leDEA regions to share basic data such as patient demographics, visit data, and HIV-related laboratory measurements and medications. These activities simplify global analyses that merge leDEA and other global cohort data as proposed in this study. Through a Big Data to Knowledge (BD2K) supplement, the leDEA-DES was recently expanded to address more extensive needs for a wider variety of multi-regional analyses within the leDEA Collaboration. We will use the same successful process which resulted in virtually doubling the size of the leDEA DES, to add new tables and expand existing ones, as necessary, to include new concepts and fields related to the needs of pharmacovigilance among pregnant women with HIV on antiretroviral therapy. As a proof-of-concept study, we will first review and expand, if necessary, the existing tables which hold

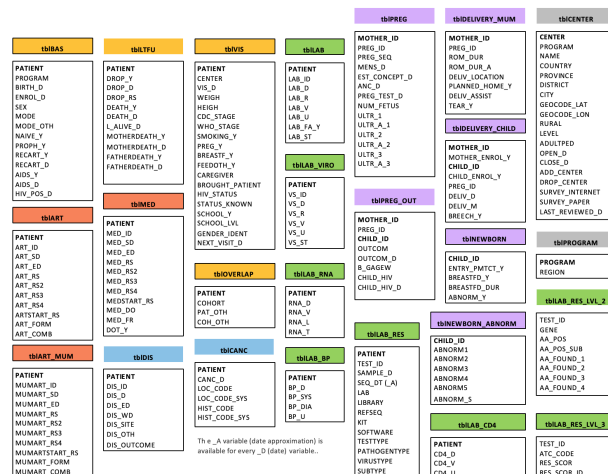
pregnancy outcomes and newborn characteristics including observed CAs (Figure 5). Importantly, the DES will be expanded to accommodate PV by capturing side effects of medications.

An leDEA Data Standards Task Force will be assembled to discuss the addition of new concepts and tables and the work will be completed in a manner similar to the process that was used through the BD2K-supported expansion of the DES. Briefly, each of the participating leDEA regions (i.e., Kenya in East Africa and South Africa in Southern Africa) will contribute at least two representatives to the task force: a clinical data specialist (usually a clinician) and a data manager. The clinical data specialist will inform the *semantics* of variables in the extended data model, bridging the point-of-care setting with the data which are generated from the sites in their region. The regional data managers will inform the *syntax*, translating information obtained from the clinicians to data concepts and queries that extend the DES. As care delivery differs across regions, the expanded leDEA-DES will allow differences in regional definitions of care to be captured and documented to avoid inadvertent bias in subsequent analyses. The participating clinical data specialists and data managers from each region will assess if the translation makes sense in terms of clinical practice and data definitions.

Figure 5. Example of leDEA-DES reference variable codes.

leDEA-DES Quick Reference Diagram

See ledeades.org for full variable specifications and code lists.



20.5 Data Transfer, Sharing and Security

We will create a Data Coordinating Center that will be based at IU. This Center will serve as a hub for collecting and disseminating all inputs and outputs involved in this project. The Data Coordinating Center will collect, disseminate and archive data for all relevant projects arising from this work, based on standard operating procedures developed within leDEA and, in particular, the leDEA East Africa and Southern Africa regional data centers. A Data Sharing Agreement will be established between parties prior to sharing or merging leDEA data across regions (see Appendix 8). This agreement will be signed by an leDEA-EA Regional Data Manager and the person receiving the data. A copy of the signed agreement will be provided to all parties.

Transfer of data from S. Africa and Kenya to the Data Coordinating Center at IU will adhere to the leDEA Data Transfer Protocol (Appendix 9) and Standard Operating Procedures for Data Sharing and Archiving (Appendix 10). These activities will be coordinated by Ms. Beverly Musick (Mango study co-investigator and leDEA-EA data manager). Prior to sending data to the data coordinating center, data managers upload data to check formatting, completeness and quality. Once data are confirmed to be in compliance with the leDEA-DES, the dataset will then be submitted to secure cloud storage to be retrieved by the authorized investigators. For security purposes, data files will be encrypted and compressed with WinZip 12 using the AES encryption algorithm. The encryption password (minimum of 10 characters long) will be communicated to the data center via fax or by telephone and not through e-mail. All personal identifying information such as names will be removed from the databases prior to transfer. No patient identifying information (e.g., names, medical identification numbers, etc.) will be transmitted within unencrypted e-mail communications. Datasets will automatically be erased from the cloud after 30 days.

20.6 leDEA Concept Sheets for Multiregional Analysis

Joint analyses for this project will be driven by leDEA concept sheets submitted by either the Cape Town or AMPATH based research groups, following standard leDEA protocol. These concepts will utilize the standard leDEA multiregional analysis concept sheet template, which requires PIs to explicitly state the requested data and planned analyses for the study (Appendix 11). Once an analysis concept sheet is developed it will be submitted to the leDEA Mother-Infant Working for comment and approval. This working group meets monthly

and is made up of leDEA investigators with expertise in maternal and child health from various leDEA regions. Once approved by the leDEA mother-infant working group, the concept sheet will be uploaded to the leDEA Hub at the following link: <http://bit.ly/iedeasubmit> for review by the leDEA Executive Committee (leDEA-EC). The leDEA-EC consists of Principal Investigators from all leDEA regions and their key Co-Investigators, as well as program staff from DAIDS, NCI and NICHD. This working group guides the multiregional research conducted by leDEA Investigators and involved Collaborators. The leDEA-EC will provide final review and approval of the concept to ensure that it complies with leDEA standards. Once the concept is reviewed, the data exchange protocol will be implemented. This approach allows for tracking of all analyses using the multiregional

20.7 Statistical Analysis (Multiregional)

The preliminary analysis plan for the combined data will follow the same approach described above for the Kenya study. The significantly larger sample size of the combined dataset will greatly enhance statistical power to detect associations between various exposures and pregnancy and infant outcomes.

We anticipate that the first two analyses to be undertaken will address:

- a. Pregnancy outcomes: Live Birth, Still Birth, Miscarriage, Termination of pregnancy, Ectopic Pregnancy, Molar Pregnancy, Not Pregnant, Other); also including pre-term delivery (<37 weeks gestational age) or very pre-term delivery (<32 weeks gestational age).
- b. Infant/birth outcomes: CAs on surface exam (e.g., extra digit, hydrocephalus, skull defects, eyes, face, mouth/lip/palate, chest, abdomen, anus, limbs, spine (including neural tube defects), hips, genitalia, skin, etc.); also including low birth weight, small for gestational age (<10th percentile) or very small for gestational age (<3rd percentile).

For the multiregional analyses, we will first describe the risk for each type of congenital anomaly by the exposure groups stratified by region, reporting our findings with incidence rates (events per 1000 deliveries) and rate ratios. Our primary outcome is the incidence of all congenital anomalies, with secondary outcomes considering the risk of neural tube defects specifically and additional outcomes listed above, all of which will be stratified by region. We will use univariate and multivariate Poisson regression models, adjusting for repeated measures for the same woman. Covariates we will consider adjusting for in our models may include various demographic (e.g. age, education status, marital status, occupation, ethnicity, and nationality), clinical (e.g. WHO clinical stage, CD4 count, HIV viral suppression status, duration of current ART use, past ART exposures, stage of pregnancy at first ART exposure), and pregnancy-related (e.g. parity, gestational age at ANC enrollment, number of ANC visits, maternal complications such as pre/eclampsia, and other medication exposures including prenatal vitamins or folic acid supplementation), and treatment program/site/region. For the primary analysis, we will aim to define exposure to ART periconception granularly (i.e. within the first 6 weeks of conception), and will broaden our definition if needed.

21. Ethics Considerations (South Africa)

In South Africa, a waiver of informed consent has been granted for the PER. Only routinely collected operational data are collected and there are no interventions. This part of the research is purely observational and part of standard of care. The waiver does not adversely affect the rights and welfare of the participants but is likely to improve the quality of care of pregnant women and their infants through strengthened health information systems and surveillance, and through knowledge gained. Data are digitized using the routine EMR platform already at use in the sentinel facilities and are subject to the provincial protections. Study staff who work with identifiable data receive Provincial and Human Subjects Protection training and are subject to the same confidentiality obligations of all staff at health facilities. Women who attend the MOUs are provided with documentation explaining that their information will be used and that they may opt out if they choose. The PHDC operates under the direct management of the WCG Department of Health and subject to national legislation regarding the handling and storage of health information. There is strict governance on the release of data for research purposes. Identified data is held indefinitely by the Provincial government on secure password-protected enterprise SQL Servers with access only to provincial staff whose role within the provincial Department of Health normally includes access to this data and those appointed as part of this study to be part

of the PHDC team. Once linkage has been completed and datasets enhanced based on linkage, all identifiers will be removed from any data to be used for the proposed research.

22. Institutional Review Board (South Africa)

In South Africa regulatory approval will be obtained from the Faculty of Health Sciences Human Research Ethics Committee (HREC) at UCT and the Health Research Ethics Committee at Stellenbosch University as an amendment to the existing 'B-positive' protocol. In addition, approval will be sought from the Western Cape Government Department of Health Research Committee. (See Part 1 for Kenya IRB protocol).

23. Team of Collaborators

This proposal leverages pre-existing, strong collaborations within the larger leDEA network, including in-country academic institutions, US-based academic institutions, and large HIV treatment programs in resource-limited settings. Within AMPATH, the Infectious Disease, Pediatrics, Reproductive Health, and Informatics teams will be involved. Within CIDER, the Pharmacovigilance and Pregnancy Exposure Registry Team, Provincial Health Data Centre, Data Management and Analysis Teams will be involved. CIDER operates closely with the Provincial Government of the Western Cape and The South African Health Products Regulatory Agency as well as local stakeholders at the facilities.

24. Timeline (multiregional)

Table 5: Timeline by aim, activities, and quarters

Aim	Activities	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
Aim 1, Preparation	DES/Task Force/Hub development								
	Protocol development, ethics approvals								
	Stakeholder meetings								
	Staff trainings								
Aim 2, Implementation	Data collection in Kenya								
	Data collection in S. Africa (ongoing)								
Aim 3, Analysis	Data analysis of combined data								
	Dissemination, manuscripts								
	Planning leDEA expansion sites								

References

1. WHO. Statement on dolutegravir: Potential safety issue affecting women living with HIV using dolutegravir at the time of conception. Geneva, Switzerland: World Health Organization (WHO); May 18, 2018.
2. Hill A, Clayden P, Thorne C, Christie R, Zash R. Safety and pharmacokinetics of dolutegravir in HIV-positive pregnant women: a systematic review. *J Virus Erad.* 2018;4(2):66-71.
3. Duda SN, Farr AM, Lindegren ML, Blevins M, Wester CW, Wools-Kaloustian K, et al. Characteristics and comprehensiveness of adult HIV care and treatment programmes in Asia-Pacific, sub-Saharan Africa and the Americas: results of a site assessment conducted by the International epidemiologic Databases to Evaluate AIDS (IeDEA) Collaboration. *Journal of the International AIDS Society.* 2014;17:19045.
4. IeDea, Collaborations ARTC, Avila D, Althoff KN, Mugglin C, Wools-Kaloustian K, et al. Immunodeficiency at the start of combination antiretroviral therapy in low-, middle-, and high-income countries. *Journal of acquired immune deficiency syndromes (1999).* 2014;65(1):e8-16.
5. International Epidemiologic Database to Evaluate AIDS (IeDEA): NICHD Maternal and Pediatric Infectious Disease Branch; [updated 12/30/2017. Available from: <https://www.nichd.nih.gov/research/supported/iedea>.
6. MOH K. Statement on the use of dolutegravir in women of child bearing age (15-49 years). Nairobi, Kenya: Office of the Director of Medical Services; June 22, 2018.
7. Tierney WM, Rotich JK, Hannan TJ, Siika AM, Biondich PG, Mamlin BW, et al. The AMPATH medical record system: creating, implementing, and sustaining an electronic medical record system to support HIV/AIDS care in western Kenya. *Studies in health technology and informatics.* 2007;129(Pt 1):372-6.
8. New high-quality antiretroviral therapy to be launched in South Africa, Kenya and over 90 low- and middle-income countries at reduced price [press release]. September 21, 2017.
9. Statistics. KNBo. Kenya Demographic and Health Survey 2014 Nairobi; 2014.
10. Lee AC, Panchal P, Folger L, Whelan H, Whelan R, Rosner B, et al. Diagnostic Accuracy of Neonatal Assessment for Gestational Age Determination: A Systematic Review. *Pediatrics.* 2017;140(6).
11. WHO. Birth defects surveillance training: facilitator's guide. Geneva; 2015.
12. WHO. Birth defects surveillance: atlas of selected congenital anomalies. Geneva; 2014.
13. Geng EH, Odeny TA, Lyamuya R, Nakiwogga-Muwanga A, Diero L, Bwana M, et al. Retention in Care and Patient-Reported Reasons for Undocumented Transfer or Stopping Care Among HIV-Infected Patients on Antiretroviral Therapy in Eastern Africa: Application of a Sampling-Based Approach. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2016;62(7):935-44.
14. Geng EH, Odeny TA, Lyamuya RE, Nakiwogga-Muwanga A, Diero L, Bwana M, et al. Estimation of mortality among HIV-infected people on antiretroviral treatment in East Africa: a sampling based approach in an observational, multisite, cohort study. *The lancet HIV.* 2015;2(3):e107-16.
15. Braitstein P, Songok J, Vreeman RC, Wools-Kaloustian KK, Koskei P, Walusuna L, et al. "Wamepotea" (they have become lost): outcomes of HIV-positive and HIV-exposed children lost to follow-up from a large HIV treatment program in western Kenya. *Journal of acquired immune deficiency syndromes (1999).* 2011;57(3):e40-6.
16. Rasmussen SA, Hernandez-Diaz S, Abdul-Rahman OA, Sahin L, Petrie CR, Keppler-Noreuil KM, et al. Assessment of congenital anomalies in infants born to pregnant women enrolled in clinical trials. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2014;59 Suppl 7:S428-36.
17. Leppig KA, Werler MM, Cann CI, Cook CA, Holmes LB. Predictive value of minor anomalies. I. Association with major malformations. *J Pediatr.* 1987;110(4):531-7.
18. Dolk H, Loane M, Garne E. The prevalence of congenital anomalies in Europe. *Adv Exp Med Biol.* 2010;686:349-64.
19. Taye M, Afework M, Fantaye W, Diro E, Worku A. Congenital anomalies prevalence in Addis Ababa and the Amhara region, Ethiopia: a descriptive cross-sectional study. *BMC Pediatr.* 2019;19(1):234.
20. Onyambu CK, Tharamba NM. Screening for congenital fetal anomalies in low risk pregnancy: the Kenyatta National Hospital experience. *BMC Pregnancy Childbirth.* 2018;18(1):180.

21. Wagura P, Wasunna A, Laving A, Wamalwa D, Ng'ang'a P. Prevalence and factors associated with preterm birth at kenyatta national hospital. *BMC Pregnancy Childbirth*. 2018;18(1):107.
22. Zash R, Souda S, Chen JY, Binda K, Dryden-Peterson S, Lockman S, et al. Reassuring Birth Outcomes With Tenofovir/Emtricitabine/Efavirenz Used for Prevention of Mother-to-Child Transmission of HIV in Botswana. *Journal of acquired immune deficiency syndromes (1999)*. 2016;71(4):428-36.
23. Zash R, Jacobson DL, Diseko M, Mayondi G, Mmalane M, Essex M, et al. Comparative safety of dolutegravir-based or efavirenz-based antiretroviral treatment started during pregnancy in Botswana: an observational study. *Lancet Glob Health*. 2018;6(7):e804-e10.
24. Zash R, Holmes L, Diseko M, Jacobson DL, Brummel S, Mayondi G, et al. Neural-Tube Defects and Antiretroviral Treatment Regimens in Botswana. *The New England journal of medicine*. 2019;381(9):827-40.
25. Ministry of Health NAaSCP. Guidelines on Use of Antiretroviral Drugs for Treating and Preventing HIV in Kenya. Nairobi, Kenya 2018.
26. Fretts RC, Boyd ME, Usher RH, Usher HA. The changing pattern of fetal death, 1961-1988. *Obstet Gynecol*. 1992;79(1):35-9.