

**SUMMARY OF CHANGES
INCLUDED IN THE FULL PROTOCOL AMENDMENT OF:**

MTN-016

DAIDS Protocol #: 10737

**HIV Prevention Agent Pregnancy Exposure Registry:
EMBRACE Study**

**THE AMENDED PROTOCOL IS IDENTIFIED AS:
Version 2.0/11 February 2014**

Information/Instructions to Study Sites

The information contained in this protocol amendment impacts the MTN-016 study and must be forwarded to your Institutional Review Board (IRB)/Ethics Committee (EC) as soon as possible for their information and review. IRB approval is required before implementation of the modifications contained in this amendment. All IRB requirements must be followed.

Please file this Summary of Changes, Version 2.0 of the protocol and all associated IRB correspondence in your essential documents files for MTN-016.

Summary of Revisions

This amendment incorporates previously issued Clarification Memos. To ease in the review process, all revisions, including Clarification Memos and new revisions are displayed below without distinction. A summary of revisions is provided below:

1. In the Protocol Summary, updates were made to the Sample Size, Study Duration, and Study Objectives and Endpoints.
2. In Section 2, INTRODUCTION, background information is updated to incorporate relevant recent research and omit product-specific language
3. In Section 3, STUDY OBJECTIVES, edits are made to study objectives to prevent redundancy in data collection and analysis across protocols.
4. In Section 4, STUDY DESIGN, minor edits are made for clarity and consistency with the modified study objectives
5. In Section 5, STUDY POPULATION, eligibility criteria are clarified
6. In Section 7, STUDY PROCEDURES, the developmental screening assessment has been omitted
7. In Section 8, ASSESSMENT OF SAFETY, minor edits are made for clarity and consistency with current policy
8. In Section 9, CLINICAL MANAGEMENT, minor edits are made for clarity and consistency with revised study procedures
9. Section 10, STATISTICAL CONSIDERATIONS, language is modified to reflect the revised study objectives and endpoints, power estimates, oversight of the Interim Study Review (ISR) Committee, and planned data analysis

10. Section 11, DATA HANDLING AND RECORDKEEPING, has minor updates for consistency with current policy
 11. Section 12, CLINICAL SITE MONITORING, has been revised to include all authorized representatives allowed to inspect study-related documentation
 12. Section 13, HUMAN SUBJECTS PROTECTION, is updated to reflect current DAIDS Protocol Registration template language
 13. Section 14, PUBLICATION POLICY, is updated to describe current DAIDS/NIAID and MTN publication policy
 14. The sample informed consent documents have been updated to reflect modified study procedures and facilitate participant understanding of the study
 15. Other minor updates, corrections, and clarifications are incorporated
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Rationale

The primary purpose of this full version amendment is to update the study objectives and prevent redundancy in data collection and analysis across protocols. The overall scientific priorities, study design, study population, and study visit schedule remain consistent with Version 1.0.

Of note, one primary outcome from Version 1.0 of this protocol (spontaneous pregnancy loss) has been omitted from the protocol, as this outcome is and will continue to be evaluated primarily within parent protocols. By design, spontaneous pregnancy loss cannot be fully investigated within MTN-016, as certain spontaneous pregnancy losses are excluded by MTN-016 eligibility criteria. The word “diagnosed” was removed from the dating of outcomes throughout the protocol and consent forms to assure that the date always used is the date of occurrence. When needed for clarity in the sentence, the word “occurred” replaces the word “diagnosed”.

Additionally, Section 10, STATISTICAL CONSIDERATIONS, was updated to include enrollments in the study under Version 1.0 and their implications for future conduct under Version 2.0.

Implementation

This amendment is now official MTN-016 protocol documentation. Prior to implementing the revisions listed below, MTN-016 study sites will submit this Summary of Changes and protocol Version 2.0 to all relevant regulatory entities and IRBs/ECs. Upon receipt of all regulatory and IRB approvals and completion of protocol registration procedures, the protocol modifications listed below will be implemented. With exceptions to modifications to the Protocol Team Roster, detailed modifications of the protocol text are indicated by strikethrough (for deletions) and **bold** (for additions). Unless otherwise stated, section numbers reflect the current version of the protocol.

Detailed Listing of Revisions New to Version 2.0

1. *In Section 2, INTRODUCTION, edits are made to update relevant background research and context. The following sections regarding parent study product-specific information are omitted in their entirety: 2.4.1 through 2.4.4 (and all subsections), inclusive.*
2. *In Section 3, STUDY OBJECTIVES, the following edits are made.*
 - 3.1 Primary Objectives
 - ~~1. To evaluate the prevalence of spontaneous pregnancy loss in mothers exposed to an active study agent during pregnancy as compared to that in mothers not exposed to an active study agent during pregnancy.~~
 - ~~2. To evaluate the prevalence of major malformations in infants of mothers exposed to an active study agent during pregnancy as compared to that in mothers not exposed to an active study agent during pregnancy.~~
 - 1. To compare adverse pregnancy and delivery outcomes between participant mothers assigned to an active agent with those of mothers assigned to placebo/control.**
 - 2. To compare the prevalence of major malformations identified in the first year of life between infants of mothers assigned to an active agent with those of infants of mothers assigned to placebo/control.**
 - 3.2 Secondary Objectives
 - ~~1. To monitor for adverse pregnancy outcomes~~
 - ~~2. To evaluate growth parameters in the first year of life among infants born to mothers exposed to an active study agent during pregnancy, as compared to those of mothers not exposed to an active study agent during pregnancy.~~
 - ~~3. To provide a cohort of infants not exposed to an active study agent representing the background incidence of major malformations among babies born to women participating in HIV prevention trials.~~
 - 1. To compare growth parameters in the first year of life between infants of mothers assigned to an active agent with those of mothers assigned to placebo/control.**
 - 2. To evaluate the prevalence and persistence of HIV drug resistance mutations in plasma among HIV-infected infants.**
 - 3.3 ~~Exploratory Objectives~~
 - ~~1. To monitor for select risks of prevention agents identified during pre-clinical reproductive toxicology studies by trimester(s) of exposure.~~

- ~~2. To evaluate the prevalence and persistence of HIV drug resistance mutations in plasma among HIV infected infants.~~
- ~~3. To compare the results of developmental screening at select time points in the first year of life among infants born to mothers exposed to an active study agent during pregnancy as compared to those of mothers not exposed to an active study agent during pregnancy.~~

3. *In Section 4, STUDY DESIGN, the following edits are made.*

4.1 Identification of Study Design

The HIV Prevention Agent Pregnancy Exposure Registry will be a prospective observational cohort investigation of women with exposures to active and non-active study agents in trials investigating **candidate HIV prevention** agents ~~intended for HIV prevention~~ and the infants resulting from those pregnancies. Parent trial participants should be offered enrollment in the Registry whenever it is determined that there has been an exposure to a study agent during pregnancy.

Optimally, participant mothers will be enrolled in MTN-016 prospectively (prior to the outcome of pregnancy being known). Participant mothers may also be enrolled in the registry after the pregnancy outcome is known, but only up until one year following the date of pregnancy outcome ~~diagnosis~~. Sites are encouraged to enroll participants as early in pregnancy as possible ~~so as to~~ maximize the validity of the data. ~~A mother-infant pair may be enrolled up to the time the baby is one year of age, but retrospective data, i.e., data obtained after pregnancy outcome is known, will be omitted from the primary analysis (see Section 10).~~ **Further information on planned analyses is available in Section 10.**

- ~~• Pregnancy loss as evidenced by the following:

 - ~~• Negative urine pregnancy test performed by study staff~~
 - ~~• Clinically confirmed intrauterine demise at a gestation > 20 weeks~~
 - ~~• Ultrasound evidence at any gestation~~~~
- ~~• Major malformation (structural abnormality with surgical, medical, or cosmetic importance) identified before one year of life~~

1. Pregnancy and delivery outcomes:

- **delivery prior to 37 completed weeks of gestation**
- **stillbirth or intrauterine fetal demise (≥ 20 weeks)**
- **spontaneous abortion (< 20 weeks)**
- **ectopic pregnancy**
- **intrapartum hemorrhage**
- **postpartum hemorrhage**
- **non-reassuring fetal status**
- **chorioamnionitis**
- **hypertensive disorders of pregnancy**
- **gestational diabetes**

- **intrauterine growth restriction**

2. **Major malformations, defined as structural abnormalities with surgical, medical, or cosmetic importance.¹**

Where pregnancy and delivery outcomes are included both in parent protocols and MTN-016, these outcomes will be assessed primarily in the parent protocol. Inclusion and exclusion criteria to be applied for the identification of major malformations will be consistent with those outlined in Holmes, ~~1999~~**2001**, **except that abnormalities may be ascertained up to one year of age.** In summary, major malformations will include structural abnormalities meeting the following criteria:

1. Having surgical, medical, or cosmetic importance
2. Ascertained up to one year of age
3. Independently confirmed according to criteria outlined in the MTN-016 SSP Manual

The following will be excluded as major malformations:

1. ~~All birth marks~~
1. ~~All m~~**Minor physical features**
2. Deformities that represent the normal response of fetal tissue to mechanical forces, i.e., atypical body part growth and/or appearance attributable to fetal position and/or pressure of surrounding maternal tissue(s). For example, molding of the skull, also known as positional plagiocephaly, would not be considered a major malformation
3. **Physical features at birth that are normally present before 37 weeks of gestation**
4. ~~Structural abnormalities detected only at autopsy or at surgery~~**Findings on prenatal ultrasonography but not on physical examination**
5. **Genetic disorders**

These inclusion/exclusion criteria will be applied to reported major malformations to determine their eligibility for inclusion as primary endpoints. ~~A subset of the MTN-016 protocol team, in collaboration with external experts if required, will review, at least annually, all reported major malformations for eligibility as inclusion as primary endpoints.~~

4. *In Section 5, STUDY POPULATION, edits are made to Inclusion Criteria.*

5.2 Inclusion Criteria: Mother

Individuals who meet the following criteria are eligible for inclusion in the study:

1. Able and willing to provide written informed consent to take part in the study
2. During participation in a parent protocol, has/had a known confirmed pregnancy, meeting at least one of the following sets of criteria in A or B:

- A: Two consecutive monthly study visits, **at least 14 days apart**, with positive pregnancy tests, **in the absence of signs/symptoms of miscarriage or participant report of pregnancy termination**.
- B: One or more of the following assessments:
 - Auscultation of fetal heart tones
 - Positive pregnancy test confirmed by clinic staff in the presence of clinically confirmed enlarged uterus
 - Positive pregnancy test confirmed by clinic staff in the presence of missed menses (**no menses occurring at least 60 days from the first day of the last menses**) by participant report
 - Clinical assessment of fetal movement
 - Demonstration of pregnancy by ultrasound

5. *In Section 7, STUDY PROCEDURES, second paragraph, second sentence, the following edits are made:*

In cases where protocol-defined visits were missed because the mother or infant had not yet enrolled, such missed visits will not be considered protocol **deviations or** violations.

In Section 7, third paragraph, the following edits are made:

It is expected that, in most cases, all required visit procedures will be completed at one visit; however, more than one visit may be ~~completed~~ if needed to complete all required procedures. If a participant is being followed in her parent trial, site staff ~~will make every effort to~~ may schedule and complete MTN-016 visits on the same day as parent protocol visits. ~~Completion of the parent MTN protocol visit should take priority if time or other factors do not allow for both study visits to be completed on the same day.~~ **MTN-016 study visits may be completed off-site with consent and an approved SOP for this purpose.**

A new fifth paragraph is added in Section 7, prior to the list of scheduled visits.

Procedures and documented results from the parent MTN study may be utilized for MTN-016, if the procedure(s) was performed/sample(s) was collected in the visit window and within the past 30 days, provided that the test kit, laboratory and/or specified clinical assessments, and method of data collection are the same in both studies. See the EMBRACE SSP Manual at <http://www.mtnstopshiv.org> for additional information.

In Section 7.3, first sentence, the following edit is made:

If the mother is still pregnant, a minimum of one **obstetrical** ultrasound exam should be performed.

In Section 7.6, first sub-bullet of first bullet, the following clarification is made:

- Written informed consent for screening and enrollment of infant, **or verbal confirmation of previous consent, if already obtained**

In Section 7.7, Months 1, 6, and 12: Infant, second bullet, Clinical, the developmental screening assessment is omitted. Corresponding edits are made to the Appendices, including APPENDIX II, SCHEDULE OF STUDY VISITS AND EVALUATIONS and the sample informed consent documents. Guidelines for photographs are also clarified.

- Clinical
 - Update medical history
 - Update medication history
 - Weight
 - Length
 - Head circumference
 - Physical exam (see Appendix III)
 - ~~Developmental screening assessment (6 and 12 months only)~~
 - If specific consent for this has been obtained, photographic documentation of suspected or confirmed anomalies as clinically indicated. Photographs should include at least the following images: Anterior-posterior (AP) and lateral of face/head, neck and upper third of thorax, standing up picture of child (front and back), hands, feet, as well as AP and lateral images of any suspected abnormal finding. **Photographs should NOT be restricted to the suspected abnormal finding.**

In Section 7.10, first sentence, the following edits are made:

If, at any point in the study participation, a major malformation is suspected in an infant or other pregnancy outcome, a Major Malformation ~~Eligibility~~ Assessment Form **Worksheet**, and, if needed, a Major Malformation Assessment Form should be completed.

In Section 7.14, the following edits are made:

Pediatric exams will be performed by an **experienced** clinician ~~who has completed some formal training in pediatrics. Developmental screening assessments will only be performed by staff members who have completed specialized training provided for the performance of developmental screening assessments.~~

6. *In Section 8, ASSESSMENT OF SAFETY, minor clarifications are made.*

In the third paragraph, first sentence, the following edit is made:

The study team will monitor for and track unanticipated problems ~~definitely, probably, or possibly~~ related to study procedures and/or to participation in the study, until participants' time of termination from the study.

In the fourth paragraph, last sentence, the following clarification is made:

Once a participant is no longer enrolled in the parent study, any unanticipated study-related injury will be reported to the site IRB/EC **according to individual IRB requirements** and DAIDS Medical Officer.

The following text is added to the very end of Section 8:

Relationship to study participation or procedures will be assessed based on the following definitions:

- 1. Related: There is a reasonable possibility that the problem may be related to study participation**
- 2. Not Related: There is not a reasonable possibility that the problem is related to study participation**

7. *In Section 9, CLINICAL MANAGEMENT, several clarifications are made.*

In Section 9.1, the following edits are made:

Participants may voluntarily withdraw from the study for any reason at any time. The principal site investigators may, ~~with the approval of the Protocol Safety Review Team (PSRT),~~ withdraw participants to protect their safety, and/or if participants are unable or unwilling to comply with study procedures. **Participants also may be withdrawn if the study sponsors, government or regulatory authorities (including the Office of Human Research Protection (OHRP)), or site IRBs/ECs terminate the study prior to its planned end date. Study staff will record the reason(s) for all withdrawals in participants' study records. In the event that participants who voluntarily withdraw from the study wish to re-join the study, they may resume study procedures and follow-up.** ~~Early (premature) termination of study participants will occur only under certain criteria.~~

~~The criteria for early termination from the study for an individual participant are:~~

- ~~• Request by participant to withdraw~~
- ~~• In the case of infants, the legal guardian declines follow up evaluations~~
- ~~• Request by the principal investigator to protect the participant's safety and/or if the participant is unable or unwilling to comply with study procedures~~

Participants will be asked to complete a final/early termination study visit.

In Section 9.2, the following edit is made:

Any infant noted to have abnormal or clinically suspicious findings on physical exam, ~~developmental screening assessment,~~ growth monitoring and/or testing will be provided with or referred to local providers of pediatric care. Note, all women, upon enrolling in the study, will receive referrals for prenatal care if they are still pregnant. In the case of HIV drug resistance mutation testing, the IoR/designee will make reasonable efforts to furnish a written copy of the results to the infant's care provider, with permission of the parent(s) or guardian, as applicable. In the case of identified structural anomalies and/or potential deviations from normal health ~~and/or development,~~ the IoR/designee will make every effort to communicate directly with the referral entity, provided that consent has been obtained for this purpose.

8. *In Section 10, STATISTICAL CONSIDERATIONS, the several edits are made. Corresponding edits for consistency (e.g., regarding sample size) are made in the sample informed consent documents and Protocol Summary.*

10.1 Overview and Summary of Design

This is a prospective observational cohort study of pregnant women and their infants identified **in microbicide in HIV prevention agent trials conducted by the MTN**. Infants will be followed for 12 months. Infants from multiple-birth pregnancies are eligible for enrollment. Participants and their infants can be enrolled for subsequent pregnancies and will need to be re-enrolled into EMBRACE.

~~A large portion of participants are expected to come from one trial: MTN-003. We expect that the MTN-003 trial population will be similar to the HPTN-035 trial population; as such, we expect that about 590 (~14%) of the 4200 enrolled women in MTN-003 will get pregnant. Given that it is difficult to anticipate the number of pregnant women generated by future and other HIV prevention trials, we will conservatively estimate the number of eligible pregnant women at 590. Based on the latest HPTN-035 data, we estimate that 350 live infants (~60% of 590) will be eligible for EMBRACE.~~

~~In an exploratory analysis, we will compare developmental endpoints across arms. The nature of this analysis cannot be specified as the protocol has not yet identified developmental assessment measures validated for African populations.~~

10.3 Sample Size

The overall number of participants to be enrolled in this protocol depends on pregnancy rates in the parent studies. At the time Version 2.0 of this protocol was approved, information was available on the number of pregnant women enrolled into MTN-016 from parent protocol MTN-003 (VOICE). Below we summarize the number of MTN-003 participants who were eligible and enrolled in MTN-016. Among 5,029 participants enrolled into MTN-003, 428 (9%) participants reported a pregnancy. Among these, 243 (57%) were eligible for MTN-016 based on two consecutive monthly visits with a positive pregnancy test (Criteria A from Section 5.2). Of these, 195 (80%) were enrolled in MTN-016 based on this eligibility criterion. An additional 17 women were enrolled based on Criteria B, resulting in a total of 212 pregnant women enrolled in MTN-016 from parent protocol MTN-003. A total of 201 infants were eligible for enrollment from MTN-003. Of these, 185 (92%) infants were enrolled into MTN-016.

Sample size estimates for the number of pregnant women and infants expected from future safety and effectiveness studies, such as MTN-020, are based on data from MTN-003, since future trial populations are likely to be similar to the MTN-003 trial population. For example, assuming 3,476 women are enrolled into MTN-020, we estimate that there will be approximately 313 (9% of 3,476) pregnant women and 147 (47% of 313) infants eligible for enrollment into MTN-016. ~~As the HPTN-035 trial population will be very similar to the MTN-003 trial population, we can, based on the latest HPTN-035 data, estimate the number of eligible pregnant women from the 4200 enrolled in MTN-003 to be about 590 (~14%), and the number of live infants to be approximately 350 (~60% of 590).~~ This is a conservative estimate as it is difficult to anticipate the number of pregnant women generated by future and other MTN trials. **Both MTN-003 and MTN-020 required use of a highly effective method of contraception as a criterion for enrollment. Future trials that do not have this eligibility requirement may have a higher pregnancy rate.**

Pregnant women may have elected to terminate participation in the parent study, and/or do not wish to meet all eligibility criteria, and/or not wish to enroll in this study, and/or have been become lost to follow-up in the parent study. Of the ~~500~~ **313** eligible women ~~that we estimate will become pregnant during MTN-020~~, we estimate that ~~15%~~ **approximately 50%** of the women eligible for this study will not enroll due to one of the above reasons. **This estimate is also based on the enrollment trends for MTN-003.** Thus, we estimate that ~~500~~ **158 (50% of 313)** pregnant women and ~~300~~ **136 (92% of 147)** live infants (i.e., ~~60%~~ **60%** of ~~500~~ **158**) will enroll into **MTN-016 from MTN-020**. ~~this study.~~ We will target no more than 5% lost to follow-up of pregnant women and of mother/infant pairs.

In the absence of a contraceptive effect for any of the HIV prevention agents ~~the active agent or the placebo~~, we expect the number of **enrolled** pregnant women to be ~~approximately~~ **essentially** balanced, ~~producing~~ **resulting in** about ~~100~~ **79** pregnant women in each of the five arms of the ~~MTN-003 trial~~ **per study arm (total n=158)**. Similarly, the number of ~~live~~ **enrolled** infants should be approximately balanced, producing about ~~60~~ **68** live infants in each of the five arms **per study arm (total n=136)** of the ~~MTN-003 trial.~~ The primary objective involves a comparison of the ~~placebo arms to each of the active arms~~ **endpoint by parent protocol study arm.** ~~Vaginal and oral placebo arms will be pooled, therefore, each comparison with an active arm will involve about 300 pregnant women (or about 180 live infants) in a 2:1 placebo to active product ratio.~~ Pregnant women are not randomized to the ~~intervention~~ **study** arms, thus it is anticipated that a certain level of imbalance will be present that may increase or decrease power. **However, study power estimates were generated assuming an equal number of participants in both study arms.** We will make the conservative assumption that each of the three comparisons will involve 180 (placebo) and 80 (active) pregnant women or 108 (placebo) and 48 (active) live infants.

The **overall** proportion of pregnancy loss in ~~HPTN-035~~ **MTN-003** among the women with ~~two consecutive monthly study visits with positive pregnancy tests~~ is about ~~20%~~ **enrolled in MTN-016 was approximately 6%.** Since it is anticipated that the additional primary outcomes for pregnant women will range in frequency from 1%-10% in the placebo arm, a plot showing minimum absolute differences of 5%-20% for a sample size of 158 (79 in each arm) with 80% and 90% power is shown below (Figure 1). For example, if a primary outcome were observed in 5% of pregnant women in the placebo arm, then the study would have at least 80% power to detect a minimum absolute difference of 16%.

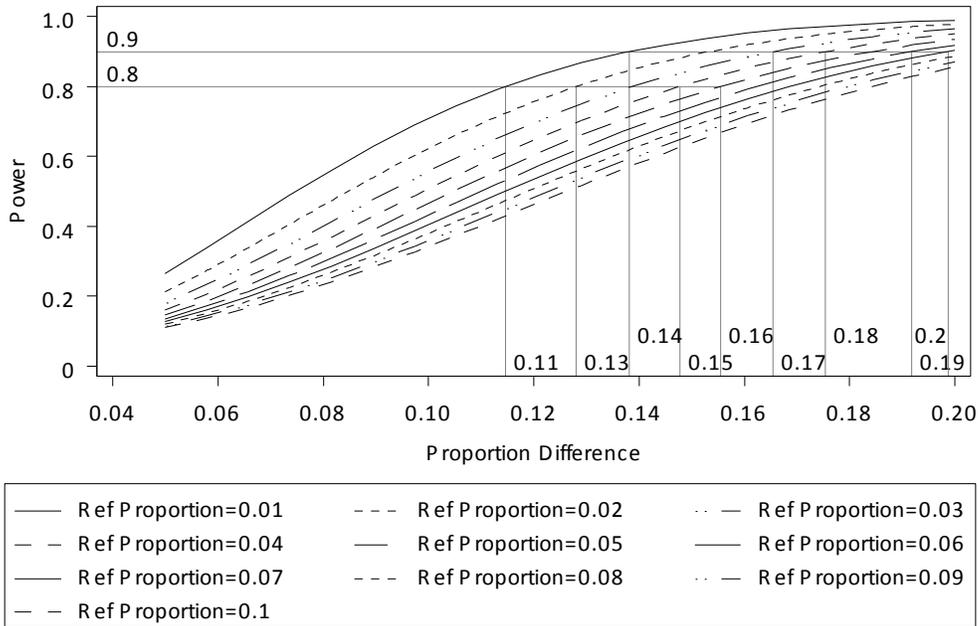


Figure 1: Power estimates for maternal outcomes

Similarly, since major malformations in infants are relatively rare, thus, for the purpose of power calculations, we will conservatively assume that the proportion of major malformations will be range in frequency in the placebo arm from 1% to 5%. A plot showing minimum absolute differences of 2% - 15% for a sample size of 136 (68 in each arm) with 80% and 90% power is shown below (Figure 2). For example, if a major malformation were observed in 1% of infants in the placebo arm, then the study would have at least 80% power to detect a minimum absolute difference of 13%.

Maternal mortality for African and Indian MTN sites ranges from 0.2% to 2%, while the infant mortality rate (i.e., death of infants below one year of age) ranges from 5 to 11 per 100 live births.⁵⁰⁻⁵⁷ The latter will impact the sample size available for the analysis of the secondary endpoints on the growth parameters of infants in the first year of life. Thus, up to 11% of the infants might not have complete growth parameter assessment over the first year. Therefore, comparison of infant growth in the first year might include 96 (placebo) and 43 (active) infants. Note that an 11% decrease in sample size translates into a 6% absolute decreases in power (i.e., from 80% to 74% power).

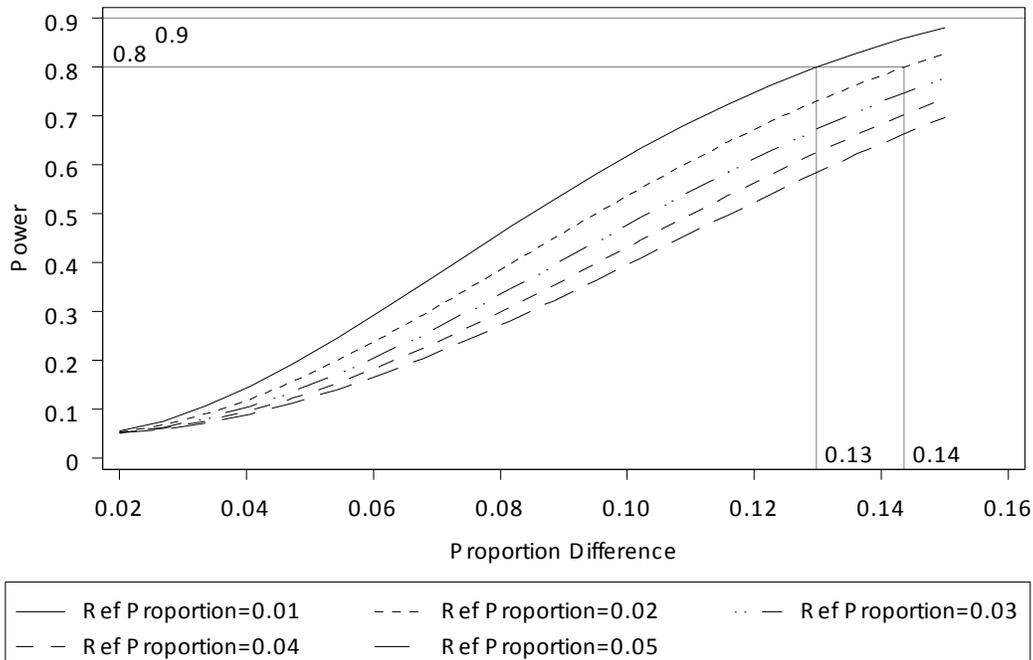


Figure 2. Power estimates for infant outcomes.

10.4 Blinding

Blinding/unblinding processes will be dictated by the parent study from which the participants come **protocols**. Both study staff and participants will be blinded to the random assignments of participants assigned to study treatment groups that include a study product. However, the assignment of participants to the mode of administration (e.g., vaginal or oral interventions in MTN 003) cannot be blinded. Randomization documentation and other pharmacy records must not be accessible to study staff members who complete other study procedures with participants.

Blinding will be maintained until all data are entered into the parent study database, all study endpoint data and other data included in the final analysis have been cleaned and verified, and the data are ready for final analysis in the parent study. Unblinding of data will only occur after the unblinding of the data in the parent study. This will be explained to participants as part of the study informed consent process.

There are no circumstances under which it is expected that unblinding will be necessary for the provision of medical treatment or to otherwise protect the safety of study participants. **If in the event that** an Investigator feels that specific product knowledge is necessary to protect participant safety **while the parent protocol is still blinded**, the Investigator will notify the Protocol Chair, Protocol Biostatistician, and DAIDS Medical Officer (or designees) to consider and jointly rule upon the request **they should follow the processes outlined in the parent protocol for early unblinding**.

10.5 Participant Accrual and Retention

All women meeting criteria outlined in Section 5, including those identified as both pregnant and HIV-infected, will be recruited into this study. Once a participant has

enrolled in the study, the study site will make every reasonable effort to retain her for the entire study period. A maximum of 5% loss-to-follow-up of enrolled pregnant women and ~~babies~~ **infants** will be targeted.

10.6 Data and Safety Monitoring Analysis

10.6.1 Interim Study Review (ISR) Committee ~~Study Monitoring Committee (SMC)~~

No Data and Safety Monitoring Board (DSMB) oversight is planned for this observational study, **since pregnancy outcome data are assessed by the DSMB in their review of the parent protocol. If treatment assignment is blinded in the parent protocol, the** ~~The MTN SMC-ISR Committee~~ will conduct interim reviews of study progress (pooled data only), including rates of participant accrual, retention, **and assessment completion of the primary and main secondary endpoint assessments, and study or lab issues** ~~outcomes while the parent protocol is blinded. These~~ **Additional** reviews will ~~take place~~ **are anticipated to occur** approximately every 4 to 6 months, ~~or as needed~~ **annually following the initial review**. At the time of these reviews, or at any other time, the ~~SMC-protocol chair in combination with an external MTN reviewer~~ may recommend that the study proceed as designed, proceed with design modifications, or be discontinued. The ~~SMC-protocol chair or external MTN reviewer~~ may consider recommending termination of this study if recruitment and retention are lower than targeted, or if study data quality is poor. **The MTN-016 protocol leadership team will routinely monitor study conduct and progress (including participant accrual, retention and data quality), see the MTN-016 SSP for additional details. Once results from a parent protocol are unblinded and analyzed, analysis of the primary and secondary outcomes by study arm can be conducted.**

10.7 Data Analysis

In most cases, the analyses will be stratified by parent protocol. However, when appropriate, analyses combining data from multiple protocols may be conducted. When the use of descriptive statistics to assess group or site characteristics or differences is required, the following methods will be used: for categorical variables, the number and percent in each category; for continuous variables, the mean, median, standard deviation, quartiles and range (minimum, maximum). Typically, within-arm assessment of the change from the baseline measurement to a follow-up measurement will be analyzed using McNemar's test (for categorical response variables) or the paired *t*-test or Wilcoxon signed-ranks test (for continuous variables). In general, when use of formal testing to assess differences between **study** arms is required, the following methods will be used: for binomial response variables, chi-square tests **(or Fisher's exact test, if appropriate)** and logistic regression; for continuous variables, *t*-tests and linear regression, or nonparametric methods if data are non-Normal. To assess baseline differences between **study** arms, participants will be compared for baseline characteristics including demographics and laboratory measurements using descriptive statistics.

~~For each of the three active interventions in MTN-003, the proportion of~~ **participants with adverse pregnancy and delivery outcomes (as specified in Section 10.2.1)** ~~less~~ will be compared **by study arm** ~~to the one observed in the pooled placebo arms (i.e., vaginal and oral placebos)~~ using a Fisher exact test **or chi-square test, depending on the prevalence,** ~~and with~~ a two-sided level of significance. ~~Using the same method,~~ **The**

proportions of major malformations observed in infants will be compared **using the same methods**.

~~For the secondary endpoints, the proportion of adverse pregnancy outcomes will be compared using similar methods as described above.~~ For each of the growth parameters (i.e., birth weight, serial length, weight, and head circumference), the mean observed in the active agent and non-active agent groups will be compared using a Student's *t*-test at selected time points: birth, ~~4~~**one**, ~~six~~**6**, and 12 months. More generally, GEE (~~g~~**Generalized e**~~Estimating e~~**Equation**) methods and robust variance estimates will be used to evaluate group differences over the first year. Incomplete data from infants that are lost to follow-up or terminate early in the study (including from death) will be included in these analyses if a growth parameter is available at one of the selected time points.

Among infants who acquire HIV-1 infection, the proportion of infants with HIV-1 drug resistance mutations will be compared by study arm using Fisher's exact test with a two-sided level of significance. ~~Finally in an exploratory analysis, we will compare developmental endpoints across arms. However, the nature of these analyses cannot be specified as the protocol has not yet identified developmental assessment measures validated for African populations.~~

Note that caution must be exercised in the interpretation of any difference (or lack of difference) found. Although **characteristics of the women in the active agent arm and placebo/control arm**~~non-active agent groups~~ should be ~~comparable~~**similar** at baseline (in the parent studies) due to randomization, women who ~~get~~**become** pregnant in the active agent group may not be ~~comparable~~**similar** to women who ~~get~~**become** pregnant in the non-active agent group (possibly due to a potential contraceptive effect of the active intervention).

9. *In Section 11, DATA HANDLING AND RECORDKEEPING, minor updates are made to web links and references to current policies.*
10. *In Section 12, CLINICAL SITE MONITORING, text now references current DAIDS policy.*
11. *In Section 13, HUMAN SUBJECTS PROTECTIONS, text is edited for consistency with current DAIDS policy.*

In Section 13.1, the following edits are made:

13.1 Institutional Review Boards/Ethics Committees

Each participating ~~institution~~**study site** is responsible for assuring that this protocol and the associated site-specific informed consent documents and study-related documents (such as participation education and recruitment materials) are reviewed by ~~an~~**its responsible IRB/EC** ~~or IRB responsible for oversight of research conducted at the study sites.~~ Any amendments to the protocol, **informed consent forms, or other study-related documents** must be approved by the responsible IRB/EC, **MTN CORE** and DAIDS prior to implementation.

~~Subsequent to the initial review and approval, the responsible IRBs/ECs must review the study at least annually. Each investigator will make safety and progress reports to the~~

~~IRBs/ECs at least annually and within three months after study termination or completion. These reports will include the total number of participants enrolled in the study, the number of participants who completed the study, all changes in the research activity, and all unanticipated problems involving risks to human subjects or others. Study sites will submit documentation of continuing review to the DAIDS Protocol Registration Office in accordance with the DAIDS Protocol Registration Policy and Procedures Manual.~~

In Section 13.2, the following edits are made:

13.2 Protocol Registration and Study Activation

Prior to implementation of this protocol, and any subsequent full version amendments, each site must have the protocol and the protocol informed consent form(s) approved, as appropriate, by their local institutional review board (IRB)/ethics committee (EC) and any other applicable regulatory entity (RE). Upon receiving final approval, sites will submit all required protocol registration documents to the DAIDS Protocol Registration Office (DAIDS PRO) at the Regulatory Support Center (RSC). The DAIDS PRO will review the submitted protocol registration packet to ensure that all of the required documents have been received.

Site-specific informed consent forms (ICFs) *WILL NOT* be reviewed and approved by the DAIDS PRO and sites will receive an Initial Registration Notification from the DAIDS PRO that indicates successful completion of the protocol registration process. A copy of the Initial Registration Notification should be retained in the site's regulatory files.

Upon receiving final IRB/EC and any other applicable RE approval(s) for an amendment, sites should implement the amendment immediately. Sites are required to submit an amendment registration packet to the DAIDS PRO at the RSC. The DAIDS PRO will review the submitted protocol registration packet to ensure that all the required documents have been received. Site-specific ICF(s) *WILL NOT* be reviewed and approved by the DAIDS PRO and sites will receive an Amendment Registration Notification when the DAIDS PRO receives a complete registration packet. A copy of the Amendment Registration Notification should be retained in the site's regulatory files.

For additional information on the protocol registration process and specific documents required for initial and amendment registrations, refer to the current version of the DAIDS Protocol Registration Manual.

~~Each study site will complete protocol registration with the DAIDS Regulatory Compliance Center (RCC) Protocol Registration Office. For additional information, refer to the protocol registration documents located at <http://rcc.tech-res.com/forms.htm>. Protocol registration must occur as a condition for site specific study activation; no participants may be screened or enrolled in this study prior to obtaining protocol registration approval and completion of all other study activation requirements. MTN CORE (FHI 360) staff will notify each study site when all activation requirements have been met by issuing a site-specific study activation notice. Study implementation may not be initiated until the activation notice is issued.~~

~~The study will be conducted in full compliance with the protocol. The protocol will not be amended without prior written approval by the Protocol Chairs and DAIDS Medical Officer. All protocol amendments must be submitted to and approved by the relevant IRBs/ECs and the RCC Protocol Registration Office prior to implementing the amendment.~~

In Section 13.3.2, the following edits are made:

Participants in this study may experience no direct benefit. Participants may benefit from **referral to early** prenatal care, and/or ~~referral to~~ PMTCT services. Participants and others may benefit in the future from information learned from this study. Specifically, information learned in this study may help prevent adverse pregnancy outcomes in the future. In addition to the benefits listed, infant participants may also have abnormalities detected as part of the evaluations in this investigation that may not have otherwise been detected. The IoR/designee will initiate referrals to local providers for ongoing evaluation and care of such infants. Infant participants may have the opportunity to access earlier ~~directed~~ care for certain abnormalities, which could improve prognosis depending on the condition.

In Section 13.5, a paragraph is added to the end of the section:

The MTN has obtained a Certificate of Confidentiality from the US Department of Health and Human Services that is applicable for this study. This Certificate protects study staff from being compelled to disclose study-related information by any US Federal, State or local civil, criminal, administrative, legislative or other proceedings. It thus serves to protect the identity and privacy of study participants. Since the Certificate cannot be enforced outside of the US, however, it will apply only to US site staff and participants.

In Section 13.6.2, minor edits are made:

Infants born to women participating in EMBRACE will be offered enrollment in this study in accordance with guidelines set forth in the US 45 CFR 46 and **DAIDS policy (<http://www.niaid.nih.gov/LabsAndResources/resources/DAIDSClinRsrch/Documents/enrollingchildrenrequirements.pdf>)**.

A new section (13.6.3) is added to the end of Section 13:

13.6.3 Prisoners

MTN-016 does not meet the criteria for prisoner participation per US 45 Code of Federal Regulations (CFR) 46.306 (a)(2)(D). MTN-016 is not suitable for further reviews by local IRBs/ECs for the inclusion of prisoners.

12. *In Section 14, PUBLICATION POLICY, minor edits are made:*

~~DAIDS/NIAID and MTN policies will govern publication of the results of this study. Any presentation, abstract, or manuscript will be submitted by the Investigator to the MTN Manuscript Review Committee, NICHD and DAIDS for review prior to submission.~~

13. In Section 15, APPENDICES, several edits are made:

In APPENDIX II, SCHEDULE OF STUDY VISITS AND EVALUATIONS (Infant), the Developmental Screening Assessment is omitted from the table for consistency with the protocol document.

In APPENDIX III, COMPONENTS OF EXAMINATIONS, minor edits are made.

Under the first bullet, Growth parameters, the first sub-bullet is deleted and the second sub-bullet is modified:

Growth parameters

- ~~• Assessment of gestational age by physical parameters~~
- Length, weight and head circumference, ~~with locally derived percentiles, if available~~

Under the third bullet, Detailed examination, the seventh sub-bullet is modified:

- Cardiovascular - heart murmurs, pulses, ~~blood pressure~~

In APPENDIX IV, SAMPLE INFORMED CONSENT – MOTHER AND INFANT (Screening and Enrollment), the edits are made for clarity and consistency.

In the fourth paragraph, the second sentence is edited for clarity:

You are being asked to take part in this study so that we can look at how HIV prevention ~~studies~~ **medications** might affect pregnancy and baby outcomes. HIV is the virus that causes AIDS.

Under What Do I Have To Do If I Am In This Study?, minor edits are made for consistency with modified protocol:

What Do I Have To Do If I Am In This Study?

~~The study is planned to continue until the year 2013, but you will only stay in the study until the outcome of your pregnancy is known. (within 1 month after your pregnancy ends).~~

Under Ultrasound Visit, two sentences are added to the end of the paragraph:

Ultrasound Visit

Have an ultrasound to check the growth of your baby if this applies to you and if you do not have complete results from an ultrasound taken by another doctor. **An ultrasound is a test that uses sound waves to check on the growth of your baby. It is done by placing a device on your belly. It does not involve any procedures or examinations inside of you.**

Under Newborn/Initial Visit, the following edits are made:

Newborn/Initial Visit

If your pregnancy results in a live birth, this visit should take place after the birth of your baby, but **ideally before** ~~when~~ your baby is 10 days old ~~or younger~~. **This visit should take about xx hours.** You will be asked questions about where you live, how to keep in touch

Under the third bullet of the first paragraph, minor edits are made for clarity:

- If it looks like something might be wrong with your baby, the study doctor might take ~~a pictures~~ of your baby and share ~~that the~~ pictures with experts who may be able to see what the problem might be.

Under Baby Follow-Up Visits (1, 6, and 12 months), minor edits are made for consistency.

Baby Follow-Up Visits (1, 6, and 12 months)

We will ask you to bring your baby in for follow-up visits to make sure your baby is healthy. You will be asked questions about where you live, how to keep in touch with you and your infant about the health of your baby and any medicines your baby might be taking. **These visits could take about xx hours.** At this visit, the study staff will also do the following to make sure your baby is healthy:

- Measure the weight, length, and head size of your baby
- Perform a physical exam
- ~~• Check how well your baby is developing (only at the 6 and 12 month visits)~~
- If it looks like something might be wrong with your baby, the study doctor might take ~~a pictures~~ of your baby and share ~~that those~~ pictures with experts who may be able to see what the problem might be. If you agree to have pictures taken of your baby, you will be asked to mark your permission at the end of this consent. We can give you a copy of any of the photographs. If you do not wish to have photographs taken of your baby, you will be able to mark at the end of this consent that no photographs may be taken of your baby.

Under APPENDIX V, SAMPLE INFORMED CONSENT – MOTHER (Screening and Enrollment), edits are made for consistency with the modified protocol.

In the fourth paragraph, the second sentence is edited for clarity:

You are being asked to take part in this study so that we can look at how HIV prevention ~~studies medications~~ might affect pregnancy and baby outcomes. HIV is the virus that causes AIDS.

Under What Do I Have To Do If I Am In This Study?, minor edits are made for consistency with modified protocol:

What Do I Have To Do If I Am In This Study?

~~The study is planned to continue until the year 2013, but Yyou will only stay in the study until the outcome of your pregnancy is known. (within 1 month after your pregnancy ends).~~

Under Ultrasound Visit, two sentences are added to the end of the paragraph:

Ultrasound Visit

Have an ultrasound to check the growth of your baby if this applies to you and if you do not have complete results from an ultrasound taken by another doctor. **An ultrasound is a test that uses sound waves to check on the growth of your baby. It is done by placing a device on your belly. It does not involve any procedures or examinations inside of you.**

A new appendix is added to the protocol (APPENDIX VIII: SAMPLE INFORMED CONSENT – OFF SITE VISIT (MOTHER AND INFANT)).

14. *The protocol title, version number and date are updated throughout the protocol document. To reflect relevant changes since Version 1.0, the Cover Page, List of Acronyms, Protocol Summary, Table of Contents and References have been updated and a Table of Figures has been added to the protocol. Minor editorial and typographical edits and updates (including edits and updates to acronyms and abbreviations) are made throughout the protocol document, including the sample informed consent forms.*