LETTER OF AMENDMENT #02 TO:

MTN-014

A Phase 1 Crossover Trial Evaluating the Pharmacokinetics of Tenofovir Reduced-Glycerin 1% Gel in the Rectal and Vaginal Compartments in Women
Version 2.0 dated May 1, 2013
DAIDS Protocol ID: 11885
IND #73,382

Date of Letter of Amendment: 13 February 2014

Site Instruction

The following information impacts the MTN-014 study and must be forwarded to your Institutional Review Board (IRB)/Ethics Committee (EC) as soon as possible for their information and review. This must be approved by your IRB/EC before implementation. The following information impacts the sample informed consent. Your IRB/EC will be responsible for determining the process of informing participants of the contents of this Letter of Amendment (LoA).

Implementation

Upon receiving final IRB/EC and any other applicable Regulatory Entity (RE) approval(s) for this LoA, sites should implement the LoA immediately. Sites are still required to submit a LoA registration packet to the DAIDS Protocol Registration Office (PRO) at the Regulatory Support Center (RSC). Sites will receive a registration notification for the LoA once the DAIDS PRO verifies that all the required LoA registration documents have been received and are complete. A LoA registration notification from the DAIDS PRO is not required prior to implementing the LoA. A copy of the LoA registration notification along with this letter and any IRB/EC correspondence should be retained in the site's regulatory files.

Summary of Revisions

This LoA modifies the protocol from a multi-site trial to a single site study, reduces the sample size from approximately 28 women to approximately 14 women, revises the accrual period from approximately 10 months to 7 months, and updates other sections appropriately. This LoA does not impact the overall design or the study visit schedule for MTN-014.

With the exception of changes that are reoccurring throughout the protocol and modifications to the Protocol Team Roster, text to be deleted is noted by ~~strikethrough~~ and text to be added is noted below in **bold**.

Detailed Listing of Revisions

1. The following updates have been made to the Protocol Team Roster:
   - The title of the clinical research site has been updated: ~~Bronx-Lebanon Hospital Center Clinical Research Site~~ **Bronx Prevention Center**
   - Removed: Londiwe Luthuli
   - Removed as a Site Investigator: Gonasagrie Nair, MBChB

2. Throughout the protocol word *sites* has been revised to read *site(s)*.

3. Throughout the protocol the sample size has been revised from approximately 28 women to approximately 14 women:

Protocol Summary

Sample Size: Approximately ~~28~~**14** women

Study Regimen:

	N	Period 1 Once daily application of TFV RG 1% gel 2 Weeks	Washout ~6 weeks	Period 2 Once daily application of TFV RG 1% gel 2 Weeks
Sequence A	~~14~~**7**	Vaginal		Rectal
Sequence B	~~14~~**7**	Rectal		Vaginal

Section 4.5, *Study Groups*:

Approximately ~~28~~**14** women will be randomized equally across 2 sequences. All study participants will complete a 14 day study period each of rectal and vaginal dosing in a randomly assigned order.

Section 6.1, *Regimen*, Table 1: Study Product Regimen:

Table 1: Study Product Regimen

	N	Period 1: 2 Weeks	Washout: ~6 Weeks	Period 2: 2 Weeks	Dose and Frequency
Sequence A	~~14~~**7**	Vaginal		Rectal	Entire contents of a single applicator will be inserted daily
Sequence B	~~14~~**7**	Rectal		Vaginal	Entire contents of a single applicator will be inserted daily

Section 10.3, *Sample Size and Power*:

[…]

Based on the MTN-001 and MTN-006 data, the TFV concentrations in blood, vaginal fluid, rectal fluid, and tissue biopsy are quite variable. **In MTN-001,** ~~Take~~ vaginal tissue TFV ~~in MTN-001 for example, the~~ mean concentration **was**~~is~~ 218 ng/mg and the standard deviation **was**~~is~~ 280, **thus**~~so~~ the coefficient of variation (CV) **was** ~~is almost~~ **approximately 1.3;** ~~1.4.~~ **the** ~~The~~ CV for the active form TFV-DP **was**~~is~~ around 1.5. **Data from MTN-001 suggest that it is reasonable to assume that t**~~T~~he **CV**~~concentration~~ **for TFV** in the vaginal fluid is **approximately** ~~in the level of~~ **1.5 or less**~~more variable with CV reaching as high as 3~~. **A s**~~S~~imilar level of variability was observed for rectal dosing in MTN 006 (**t**~~T~~issue TFV-DP 1.3, rectal sponge 1.0). ~~We~~ **Based on these two studies, the** estimate**d** ~~the~~ CV for tissue TFV-DP is around 1.0-1.5~~, and with~~**. The data from MTN-006 suggest that the concentrations in rectal fluid are more variable (CV=1.0~3.0 depending on sampling time post-dose). With** improved technology for **the measurement of TFV in** fluid level**s** ~~of TFV~~, we estimate~~d~~ **the** ~~its~~ CV ~~around 2~~ **will be smaller than what were observed in MTN-006**.

To compute the power **available to** ~~for~~ compar**e**~~ing~~ the concentration~~s~~ of **TFV in** one compartment under the two routes of gel use, we assume **a**~~the~~ coefficient~~s~~ of variation **of** ~~vary at~~ 1, 1.5 or 2, and we assume the within-individual correlation **(rho)** ranges from 0.2 to 0.8. For **an** effective sample size **of** 12 or ~~2~~**1**4 participants who have complete~~d~~ data for both rectal use and vaginal use, ~~we list~~ the power to detect **a 10-fold difference in** ~~significant difference~~ **TFV** ~~between the~~ concentration~~s~~ under **the** two routes **of use is shown** in Table 13~~, when the fold change of concentration is 10~~. The coefficient~~s~~ of variation remain**s** the same for the concentrations under **the** two routes of use. Because it is expected that the same compartment delivery will result in **a** higher concentration than the cross compartment delivery, ~~we use~~ **a** one-sided type I error **is used to calculate** ~~in the~~ power ~~computation~~. The test statistic **used** is one sample test of mean being different from 0, accounting for within-subject correlation.

Table 13 shows the power **to** ~~of~~ detect~~ing~~ **a** 10-fold difference in concentration for a sample size of 12 or ~~2~~**1**4. The total effective sample size of ~~2~~**1**4 will provide the following power

~~performance~~: for ~~less variable drug concentration such as tissue TDF-DP, which will be collected only in half of total effective sample size (12 women at a single US site), suppose its CV is around 1.0-1.5, we have power~~ **drug concentrations measured in a particular compartment with a CV ranging from 1.0 to 1.5, there is an estimated power of approximately 80%** to detect a 10-fold difference or more~~; for more variable drug concentration with CV around 2, such as those in rectal or vaginal fluid, we have close to 80% power to detect a 10-fold difference.~~ **CV for rectal fluid can be as high as 3.0, therefore, the power to detect a 10-fold difference may be less than what is displayed in Table 13.**

Table 13: *The power to detect **a** significant difference for crossover design for a sample size 12 or **1**~~2~~4*

The power to detect a significant difference for crossover design for an effective sample size of 12 or 14, the coefficient variation 1.0, 1.5 or 2, within subject correlation 0.2, 0.5, or 0.8, and the actual fold difference is 10. The one sided type I error is set to 0.05.			
CV	Rho	Total sample size	
		12	~~2~~**1**4
1.0	0.2	0.94	~~1.0~~**0.96**
1.0	0.5	0.95	~~1.0~~**0.97**
1.0	0.8	0.96	~~1.0~~**0.98**
1.5	0.2	0.68	0.~~9~~1**74**
1.5	0.5	0.70	0.~~9~~3**76**
1.5	0.8	0.73	0.~~9~~4**79**
2.0	0.2	0.48	0.~~7~~3**53**
2.0	0.5	0.50	0.~~7~~5**55**
2.0	0.8	0.52	0.~~7~~8**57**

[…]To compensate ~~the~~ **for** potential data loss~~,~~ **if some participants fail to complete the cross-over periods** in the MTN-014 trial**,** ~~four more~~**additional** participants ~~will~~**may** be enrolled~~, thus a sample size of 28 is targeted~~ **at the discretion of protocol team**.

Section 10.4, *Randomization Procedures*, first sentence:

~~Randomization to the sequence of gel administration will be stratified by site, with equal number of participants recruited for each of the two sites.~~ ~~Within each site,~~ p**P**articipants will be randomly assigned with the ratio 1:1 to one of two study product use sequences:

Section 10.5, *Participant Accrual and Retention*, second sentence:

The study will enroll **approximately** ~~28~~**14** women.

The second sentence of the *Informed Consent* Section of APPENDIX III: SAMPLE INFORMED CONSENT FORM (SCREENING, ENROLLMENT, LONG-TERM STORAGE):

Approximately ~~28~~**14** females will participate in this study ~~at two sites~~.

4. Throughout the protocol the accrual period has been modified from approximately 10 months to 7 months:

Protocol Summary

Study Duration: Accrual will require approximately ~~10~~**7** months ~~per site~~. Each enrolled participant will be followed for approximately 10-13 weeks, depending upon their menses schedule.

Section 4.4, *Time to Complete Accrual*:

Accrual is expected to be complete in approximately ~~10~~**7** months ~~per site~~.

Section 10.5, *Participant Accrual and Retention,* first sentence:

The accrual period is expected to require approximately ~~10~~**7** months. […]

5. Modifications have been made to remove the specific number of sites listed within the protocol and Sample Informed Consent:

   Section 4.1, *Identification of Study Design*:

   MTN-014 is a Phase 1, ~~multi~~**-single** site, randomized two-sequence, two-period open label crossover study.

   The second sentence of the *Informed Consent* Section of APPENDIX III: SAMPLE INFORMED CONSENT FORM (SCREENING, ENROLLMENT, LONG-TERM STORAGE):

   Approximately ~~28~~**14** females will participate in this study at ~~two~~**one** site~~s~~.

6. The protocol specification noting that a subset of participants will provide vaginal and rectal biopsies has been removed throughout the protocol, as the entire cohort will provide samples:

   Section 2.8, *Other Protocol Considerations, Justification* and first sentence of the paragraph:

   Justification for the use of a Flexible Sigmoidoscope to Collect Rectal Biopsies ~~in a Subset of Participants.~~

   Rectal biopsies will be collected in ~~a subset of~~ participants using a flexible sigmoidoscope.

   Section 5.2, *Inclusion Criteria*:

   ~~Participants in the biopsy subset must also meet the following criteria at Screening to be eligible for inclusion:~~

   Section 5.3, *Exclusion Criteria*:

   1) Participant report of any of the following:
      n) History of bleeding problems ~~(Participants in the biopsy subset only)~~

   Section 6.7, *Prohibited Medications and Practices,* first sentence of the second paragraph:

   Furthermore, ~~a subset of~~ participants will also be counseled not to use NSAIDs, aspirin and/or other drugs that are associated with the increased likelihood of bleeding for 72 hours prior to and following mucosal biopsy collection.

   Section 7.14, *Intensive Pharmacokinetics and Mucosal Gene Expression Microarray Subset*, the first paragraph and second to last sentence of the second paragraph as well as Table 12:

   7.14 *Intensive Pharmacokinetics and Mucosal Gene Expression Microarray ~~Subset~~*

   Participants ~~at a single US site~~ must agree to the collection of biopsies in order to take part in MTN-014. All women ~~at the US site~~, provided that they are not found to be ineligible for other reasons, will provide a vaginal and rectal biopsy. ~~It is anticipated that all US participants (approximately 14 participants) will be enrolled into the study and take part in the biopsy subset.~~ […] Details regarding the quantity and timing of mucosal sample collection for intensive PK and mucosal gene expression microarray ~~for this subset~~ are described in Table 12.

   […]

   All heterosexually-active participants will be reminded of the importance of using male condoms with each sex act, as all ~~biopsy subset~~ participants will be at increased risk of HIV/STI transmission following biopsy collection.

   Section 10.6.1, *Primary Data Analyses on PK Measures*, seventh sentence has been modified:

   […] For the tissue concentrations collected in 14 participants ~~in the US site~~, a signed rank Wilcoxon test will be also conducted.[…]

The title of Table 12: *Intensive Pharmacokinetics and Mucosal Gene Expression Microarray Subset Sample Collection* as well as the footnote to the table have been modified:

Table 12: *Intensive Pharmacokinetics and Mucosal Gene Expression Microarray ~~Subset~~ Sample Collection*

~~■To be collected during the screening process at the US site on all women who have not already had their screening terminated due to ineligibility.~~

Section 13.4.1, *Risks*, *Vaginal and Rectal Biopsies Collection* Section:

~~For a subset of participants,~~ ~~v~~**V**aginal and rectal biopsies carry the risk of discomfort or pain during the procedure and for a few hours afterwards. […]

Rectal enema

Participants ~~enrolled in the biopsy subset~~ will also have an enema. […]

Flexible sigmoidoscopy:

Participants ~~enrolled in the biopsy subset~~ will have a flexible sigmoidoscopy.

Section 7.0, *Study Procedures*, Tables 2, 5, and 9:

Table 2: Screening Visit:

Screening Visit		
Component		Procedures
Behavioral/Counseling		• Provide counseling o Biopsy procedure ■
Laboratory	Pelvic Samples	• Collect pelvic specimens for: o Vaginal biopsy for mucosal gene expression microarray■
	Rectal Samples	o Collect rectal biopsy for mucosal gene expression microarray■

* If indicated, ~~■To be collected/performed during the screening process at the US site on all women who have not already had their screening terminated due to ineligibility (samples to be collected are for gene expression only).~~

Table 5: *Visit 16: Period 1 End (Day 14)* and Table 9: *Visit 32: Period 2 End/Final Clinic Visit (Day 70-89*):*

Visit 16: Period 1 End (Day 14) AND Visit 32: Period 2 End/Final Clinic Visit (Day 70-89*)		
Component		Procedures
Behavioral/Counseling		• Provide counseling o Biopsy procedure ■
Laboratory	Pelvic Samples	• Collect pelvic specimens for: o Vaginal biopsy for mucosal gene expression microarray■
	Rectal Samples	o Collect rectal biopsy for mucosal gene expression microarray■

* If indicated~~, ■ All participants at the US site only. See Section 7.14 for additional details.~~

Appendix I, SCHEDULE OF STUDY VISITS AND EVALUATIONS

	Visit 1 SCR	Visit 16/Period 1 End. (Day 14)	Visit 32 Period 2 End/Final Clinic (Day 70-89)*
BEHAVIORAL/COUNSELING			
Biopsy procedure counseling	~~∞~~ X	■ X	■ X
LABORATORY (vaginal and cervical swabs as required)			
Vaginal biopsies for PK and gene expression microarray	~~∞~~ X	■ X	■ X
Rectal biopsies for PK and gene expression microarray	~~∞~~ X	■ X	■ X

*= If indicated ~~∞=To be collected at the US site on all women who have not already had their screening terminated due to ineligibility (samples to be collected for gene expression only), ■= To be collected on a subset of approximately 14 US participants,~~

APPENDIX III: SAMPLE INFORMED CONSENT FORM (SCREENING, ENROLLMENT, LONG-TERM STORAGE):

Tissue Samples

~~Approximately 14 from the United States [Site participating in the Rectal and Vaginal Tissue Subset to insert the following language:, all of the participants at this site,]~~ **All** participants will provide rectal and vaginal tissue (biopsies) to help researchers better understand how the study drug enters and exits the body and what effect the drug has on the tissue, including what effect the study drug has on your genes.

~~*[Site not participating in the Rectal and Vaginal Tissue Subset please insert the following language:]*~~
~~This research site is not participating in the collection of these extra samples.~~

~~*[Sites participating in the Rectal and Vaginal Tissue Subset please insert the following language:]*~~

Other possible risks, section:

[…]

~~*[Sites participating in the Vaginal/Rectal Tissue Subset please insert the following language:]*~~
Vaginal and Rectal Tissue Samples: […]

7. The protocol allowance to permit MTN-014 participant enrollment in MTN-015 and MTN-016, has been removed from the protocol:

Section 7.10.1, *Participants Who Become Infected with HIV-1*, second paragraph:

~~Participants who become infected with HIV-1 while on study product may be offered enrollment in MTN-015, the MTN Seroconverter Study, provided their study site is taking part in MTN-015. Participants are offered enrollment in MTN-015 (www.mtnstopshiv.org) at the visit when seroconversion confirmation test results are discussed with the participant.~~

Section 7.10.2, *Participants Who Become Pregnant,* second paragraph:

~~Participants who become pregnant while on study product may be offered enrollment in MTN-016 (www.mtnstopshiv.org), provided their study site is taking part in MTN-016.~~

Section 9.7, *Pregnancy*, fifth paragraph:

~~A participant who becomes pregnant during the course of study participation may be offered participation in MTN-016, the Prevention Agent Pregnancy Exposure Registry, provided their study site is participating in MTN-016. This registry study is anticipated to capture pregnancy outcomes as well as infant health information, (including growth and development), to evaluate the safety and teratogenic risks of microbicide and oral PrEP exposure in pregnancy.~~

Section 9.6, *HIV-1 Infection*, the second to last sentence:

~~These participants are also offered participation in MTN-015, the MTN Seroconverter Study, provided their site is participating in MTN-015, which also includes provisions for the clinical management and/or referral of participants infected with HIV.~~

8. Section 13.6, *Participant Confidentiality*, has been updated to note that the Certificate of Confidentiality has been obtained:

The MTN has ~~applied~~**obtained** ~~for~~ a Certificate of Confidentiality from the US Department of Health and Human Services that is applicable for this study.

The above information will be incorporated into the next version of the protocol at a later time if it is amended.