

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

021752Orig1s030

MICROBIOLOGY REVIEW(S)

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

NDA Number: 021752

Applicant: Gilead Sciences, LLC

Stamp Date: 12/15/2011

Drug Name: Truvada

NDA Type: sNDA SLR 30

PDUFA Goal Date: 06/15/2012

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the virology information (nonclinical and clinical) provided and described in different sections of the NDA organized in a manner to allow substantive review to begin?	x		
2	Is the virology information (nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	x		
3	Is the virology information (nonclinical and clinical) legible so that substantive review can begin?	x		
4	On its face, has the applicant <u>submitted</u> cell culture data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?			Not Applicable
5	Has the applicant <u>submitted</u> any required animal model studies necessary for approvability of the product based on the submitted draft labeling?			Not Applicable
6	Has the applicant <u>submitted</u> all special/critical studies/data requested by the Division during pre-submission discussions?			Not Applicable
7	Has the applicant <u>submitted</u> the clinical virology datasets in the appropriate format as described in the relevant guidance documents and are the datasets complete?		x	Reformatting of the data may be required
8	Has the applicant used standardized or nonstandardized methods for virologic outcome measures? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?	x		Standardized: seroconversion
9	Has the applicant <u>submitted</u> draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	x		
10	Has the applicant <u>submitted</u> annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	x		
11	Have all the study reports, published articles, and other references been included and cross-referenced in the	x		

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

	Content Parameter	Yes	No	Comments
	annotated draft labeling or summary section of the submission?			
12	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?		x	

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the NDA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. Please submit a line-item virology dataset that consolidates pharmacokinetic and virologic data. Specifically, the file should allow for the comparison of drug concentrations, virus titers, and genotypic data of samples collected at, or near, the same time point(s). We request that the file include the following data:
 - Subject ID
 - Trial (i.e., iPrEx or Partners PrEP)
 - Cohort
 - Time of seroconversion (Days from Baseline)
 - HIV-1 subtype
 - Time of PK Sample Collection (Days from Baseline)
 - TFV concentration
 - TFV-DP concentration
 - FTC concentration
 - FTC-TP concentration
 - Time of virology sample collection (Days from Baseline)
 - HIV-1 RNA load
 - Genotypic data (complete HIV-1 RT amino acid sequence: if deep sequencing was used, provide a consensus amino acid sequence, identify variants that occurred in more than 1 individual and their percent within an individual's population); blank cells should be used for positions matching reference RT sequence (see guidance on submission of HIV-1 resistance data). Provide a separate dataset for allele-specific RT-PCR.
 - Genotypic data of the Index Subject if resistance-associated substitutions are identified in the Partner Subject (if available from Partners PrEP). Include an identifier for matching with the appropriate partner and a column with "Y" or "N" as to whether a phylogenetic comparison of the index and partner viruses indicates that the partner was the probable source of the subject's infection.

Multiple rows may be included for the same subject if samples collected at multiple time points were evaluated. Also, please identify the assays used for RNA load determination and genotypic data analysis (e.g., allele-specific, ultra-deep, or population-based nucleotide sequencing assay).

VIROLOGY FILING CHECKLIST FOR NDA or Supplement

2. Please provide study reports for the phenotypic and genotypic studies that were conducted for each trial, including detailed methodologies and a description of the performance parameters of assays that have not been approved. The assay descriptions should include primer and probe sequences (when applicable), a description of the sensitivity limits for minority populations, and—in the case of allele-specific RT-PCR—the detection limits for each of the degenerate bases within a codon for each resistance-associated substitution that was evaluated).
3. Please conduct an expanded HIV-1 resistance analysis for subjects who failed prophylaxis and had detectable drug levels or who were missing those pharmacokinetic data. The analysis should include a genotypic characterization of reverse transcriptase using an assay that is sensitive to minority species (e.g., 454 sequencing) and a phenotypic characterization for emtricitabine and tenofovir susceptibility if no known resistance-associated substitutions are identified.

Damon J. Deming, Ph.D.
Clinical Virology Reviewer

Date

Jules O'Rear, Ph.D.
Clinical Virology Team Leader

Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

DAMON J DEMING
06/04/2012

JULIAN J O REAR
06/04/2012

**DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW**

NDA: 021752 SN: SLR 30 DATE REVIEWED: 4/30/2012
Virology Reviewer: Damon J. Deming, Ph.D.

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Additional Submissions Reviewed

SDN 710 (eCTD [0396](#))

SDN 711 (eCTD [0397](#))

SDN 725 (eCTD [0407](#))

SDN 731 (eCTD [0412](#))

SDN 732 (eCTD [0413](#))

SDN 733 (eCTD [0414](#))

SDN 735 (eCTD [0418](#))

SDN 739 (eCTD [0419](#))

SDN 742 (eCTD [0422](#))

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Related Supporting documents: NDA 021356 (Viread®); NDA 021500 (Emtriva®); NDA 021752 (Truvada®); IND 052849 (Viread®); IND 053791 (Emtriva®); IND 067671 (Truvada®); IND 075356; IND 071859 (iPrEx); IND 108930 (Truvada® as PrEP for MSM)

Product Names:

Proprietary: Truvada® (Emtriva® + Viread®)

Non-Proprietary/USAN: Emtricitabine (FTC) and Tenofovir Disoproxil Fumarate (TDF)

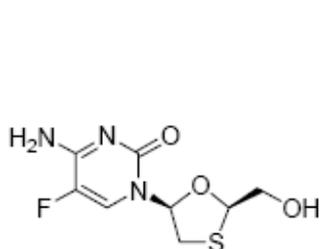
Code Name/Number:

Chemical Names:

FTC: 5-fluoro-1-(2*R*,5*S*)-[2-(hydroxymethyl)-1,3-oxathiolan-5-yl]cytosine

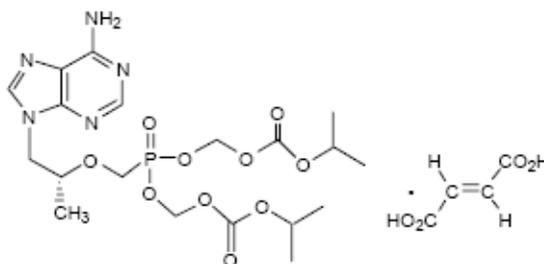
TDF: 9-[(*R*)-2-[[bis[[[isopropoxycarbonyl]oxy]methoxy]phosphinyl] methoxy]propyl] adenine fumarate (1:1)

Structural Formula:



EMTRICITABINE (FTC)

Molecular Formulas:



TENOFOVIR DISOPROXIL FUMARATE (TDF)

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FTC, $C_8H_{10}FN_3O_3S$;
TDF, $C_{19}H_{30}N_5O_{10}P \cdot C_4H_4O_4$

Molecular weight:

FTC: 247.24

TDF: 635.52

Drug category: Antiviral

Indication: Prophylaxis of sexually transmitted HIV-1 infection

Dosage Form/Route of administration: 200 mg FTC and 300 mg TDF capsule/Oral

Abbreviations: ARV, anti-retroviral drugs; BLQ, below LLOQ; EoT, end of treatment; FTC, emtricitabine; FTC-TP, emtricitabine triphosphate; HIV-1, human immunodeficiency virus type 1; IQR, interquartile range; ITT, intent-to-treat; LLOQ, lower limit of quantification; LOD, limit of detection; mITT, modified intent-to-treat; MSM, men having sex with men; NRTI, nucleoside reverse transcriptase inhibitor; NtRTI, nucleotide reverse transcriptase inhibitor; N(t)RTI, nucleoside/nucleotide reverse transcriptase inhibitor; PD, pharmacodynamics; PK, pharmacokinetic; PPY, per person year; SC, time to first (and subsequently confirmed) anti-HIV antibody rapid test; TDF, tenofovir disoproxil fumarate; TFV, tenofovir; TFV-DP, tenofovir diphosphate; TTIFN, time to infection;

Dispensed: Rx X OTC _____

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Virology Reviewer: Damon J. Deming, Ph.D.

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EXECUTIVE SUMMARY

The human immunodeficiency virus type 1 (HIV-1) epidemic in the United States has remained steady with approximately 48,000 to 58,000 new infections per year since the early 1990s ([Hall et al., 2008](#); [Holtgrave et al., 2009](#); [Prejean et al., 2011](#)) despite available protective measures including condoms and risk reduction behavior ([Johnson et al., 2008](#); [Underhill et al., 2007](#) and [2008](#)). Unfortunately, attempts to develop an effective vaccine have been unsuccessful to date. Approximately 75% of new U.S. HIV-1 infections occur in men, with African-American and Hispanic/Latino men experiencing the highest incidence rates ([Prejean et al., 2011](#)). The primary route of HIV-1 transmission in the U.S. is unprotected sex between men who have sex with men (MSM), accounting for 61% of new infections between 2006 and 2009. Despite the relatively stable rate of overall new infections in the U.S., the estimated number of new infections among young MSM has increased by 34% from 2006 to 2009, with a 48% increase among young African-American MSM. Among women, African-American women experience the highest HIV-1 incidence rates.

The suppression of HIV-1 replication with antiretroviral therapy in infected individuals has been shown to reduce transmission between serodiscordant couples ([Cohen et al., 2011](#)) and presents an attractive public health strategy for further reducing viral transmissions. However, more than 50% of sexually transmitted HIV-1 infections are from people who are unaware of their infection status ([Marks et al., 2006](#)). Even among people who are aware of their positive HIV-1 infection status, only 35% have suppressed viral loads ([CDC, 2011](#)). Further, the use of condoms, risk reduction behavior, and antiretroviral therapy for prevention requires negotiation and agreement between the uninfected and infected partners, which may not be possible in some circumstances.

This supplement to NDA 21-752 seeks to expand the indication of TRUVADA[®] to include pre-exposure prophylaxis of sexually transmitted HIV-1 infection in healthy individuals at high risk for infection. Currently, no drugs are approved for this indication. TRUVADA[®] is a fixed-dose combination of the approved nucleoside analogue reverse transcriptase inhibitor (NRTI) emtricitabine (FTC) and the approved acyclic nucleotide analogue reverse transcriptase inhibitor (NtRTI) tenofovir disoproxil fumarate (TDF). FTC (EMTRIVA[®]) and TDF (VIREAD[®]) were approved for the treatment of HIV-1 infected adults in combination with other antiretroviral drugs on July 02, 2003 and October 10, 2001, respectively. TRUVADA[®], indicated for the treatment of HIV-1 infected adults over 18 years of age in combination with other antiretroviral products, was first approved in the United States on August 02, 2004 (NDA 21-752).

The pivotal trials for this supplemental NDA application include iPrEx and Partner's PrEP. The iPrEx trial was a phase 3, randomized, double-blind, placebo-controlled study of TRUVADA[®] (FTC/TDF) chemoprophylaxis for HIV-1 prevention in uninfected MSM. The Partner's PrEP trial was a phase 3, randomized, double-blind, placebo-controlled study of Truvada[®] (FTC/TDF) and VIREAD[®] (TDF) chemoprophylaxis for HIV-1 infection between heterosexual serodiscordant couples. Both trials demonstrated statistically significant reductions in the rate of HIV-1 acquisition among subjects treated with FTC/TDF versus placebo, with risk reductions of 42% (95% CI, 22% to 63%) and 76% (95% CI, 55% to 87%) for the iPrEx and Partner's PrEP trials, respectively. Interestingly, pharmacokinetic/pharmacodynamic case-cohort analyses established a strong correlation between measurable drug levels and protection from infection. Subjects who received FTC/TDF and had quantifiable drug levels had risk reductions of 87.5% (95% CI, 66% to 95%) and 94% (95% CI, 56% to 98%) compared to subjects receiving placebo for the iPrEx and Partner's PrEP trials, respectively. These analyses provided an objective measure of adherence and indicated that good adherence was associated with high efficacy.

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The detection of resistant viruses in the iPrEx and Partner's PrEP trials was limited to isolates from subjects who were in the acute stage of HIV-1 infection at baseline and received active drug. Acute HIV-1 infection refers to the early stage of infection that occurs prior to the induction of an anti-HIV antibody response and is therefore undetectable by standard anti-HIV antibody detecting assays, such as those used to screen for infection in both of the pivotal trials. Collectively, 50% (5/10) of subjects who were acutely infected at baseline and received active drug had detectable levels of resistant virus by the time of seroconversion. The absence of detectable resistant variants among subjects who were not infected at baseline and failed prophylaxis may be attributable to lack of adherence and frequent testing for seroconversion, which would have limited drug exposures and minimized time for selection of resistant variants. If the supplemental NDA is approved, individuals who plan to use TRUVADA[®] for PrEP should confirm that they are uninfected using an assay that is sensitive for acute HIV-1 infection before initiating PrEP and continue to undergo frequent monitoring for infection for the duration of PrEP use.

1 RECOMMENDATIONS

1.1 Recommendation and Conclusion on Approvability

This supplemental NDA is approvable from a Clinical Virology perspective for the pre-exposure chemoprophylaxis (PrEP) of HIV-1–uninfected men and women who are at high risk of sexually transmitted HIV-1 infection.

1.2 Recommendation on Phase IV (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

This reviewer recommends the following post-marketing commitments:

1. Conduct analyses to assess the impact of TRUVADA[®] PrEP failures on the durability of subsequent antiretroviral treatment with regimens containing emtricitabine and/or tenofovir disoproxil fumarate. The study should include subjects who become infected while using TRUVADA[®] for PrEP and lack evidence of resistance by standard genotypic or phenotypic techniques at the time of HIV-1 diagnosis.
2. Conduct a study to assess the impact of TRUVADA[®] PrEP on the time to seroconversion. This commitment may be fulfilled by completing the HIV-1 RNA analysis of stored pre-seroconversion samples of Partner's PrEP participants. The time from HIV-1 infection to seroconversion should be determined for each subject and the median differences between subjects of each treatment group compared. The time of HIV-1 infection should be estimated from the times of the last viral negative and first viral RNA positive pre-seroconversion samples.
3. Periodically conduct and report surveillance analyses to determine if there are changes in the incidence of transmitted FTC and/or TDF resistant viruses among prophylaxis- and treatment-naïve individuals who become infected in communities where TRUVADA[®] for PrEP is used.
4. Conduct a study to compare the ability of different CDC-recommended acute HIV-1 screening algorithms to minimize initiation of TRUVADA[®] PrEP in infected individuals.

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2. SUMMARY OF OND VIROLOGY ASSESSMENTS

2.1 Nonclinical Virology

Nonclinical virology data describing the mechanism of action and antiviral activity for emtricitabine (Emtriva[®]), tenofovir disoproxil fumarate (Viread[®]), and the fixed combination of 200 mg emtricitabine with 300 mg tenofovir disoproxil fumarate (Truvada[®]) were previously reviewed under NDA 021500, NDA 021356, and NDA 021752, respectively. A brief overview of these is provided below.

Emtricitabine (FTC) is a synthetic nucleoside analog of cytidine that is phosphorylated by cellular kinases to its active triphosphate form, emtricitabine triphosphate (FTC-TP) ([EMTRIVA[®] label](#)). Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate (dCTP), and by being incorporated into nascent viral DNA, resulting in chain termination. Emtricitabine inhibited laboratory and clinical isolates of HIV-1 in lymphoblastoid cell lines, the MAGI-CCR5 cell line, and peripheral blood mononuclear cells with 50% effective concentration (EC₅₀) values in the range of 0.0013–0.64 μM (0.0003–0.158 μg/mL). FTC displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, and G (EC₅₀ values ranged from 0.007–0.075 μM) and showed strain specific activity against HIV-2 (EC₅₀ values ranged from 0.007–1.5 μM).

Tenofovir disoproxil fumarate (TDF) is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir disoproxil fumarate requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular kinases to form tenofovir diphosphate (TFV-DP), an obligate chain terminator ([VIREAD[®] label](#)). Tenofovir diphosphate inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate (dATP) and, after incorporation into DNA, by DNA chain termination. Tenofovir inhibited laboratory and clinical isolates of HIV-1 in lymphoblastoid cell lines, primary monocyte/macrophage cells, and peripheral blood lymphocytes with EC₅₀ values in the range of 0.04 μM to 8.5 μM. Tenofovir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC₅₀ values ranged from 0.5 μM to 2.2 μM) and strain specific activity against HIV-2 (EC₅₀ values ranged from 1.6 μM to 5.5 μM).

2.1.2 Prophylactic Activity in a Nonhuman Primate Model of HIV Transmission

Nonclinical studies using a macaque model of rectal chimeric simian immunodeficiency virus/human immunodeficiency virus (SHIV) infection have been conducted and support the proposed pre-exposure prophylaxis indication. The prophylactic activity of the combination of daily oral FTC and TDF at doses that yielded FTC-TP and TFV-DP concentrations in PBMCs equivalent to those observed in humans using TRUVADA[®] was evaluated in a controlled study of macaques inoculated weekly with SHIV for 14 weeks ([García-Lerma et al., 2008](#)). Of the 18 control animals, 17 became infected after a median of 2 rectal exposures. In contrast, 4 of the 6 animals treated daily with oral FTC and TDF remained uninfected, and the two infections that did occur were significantly delayed (9 and 12 exposures) with reduced viral titers and delayed viremia. An M184I-expressing FTC resistant variant emerged in one of the two macaques after 3 weeks of continued drug exposure. In order to evaluate the prophylactic effect of tenofovir dosed 3- to 4-fold higher than humans receiving TRUVADA[®] or VIREAD[®], additional macaques were treated subcutaneously with 20 mg/kg FTC and 22 mg/kg tenofovir 2 hours before and 24 hours after each weekly rectal challenge. All 6 animals treated subcutaneously with emtricitabine/high-dose tenofovir remained fully protected from infection.

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2.2 Clinical Virology

The clinical data supporting this new indication are derived from two pivotal phase 3 trials:

1. A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study of Chemoprophylaxis for HIV Prevention in Initially HIV-1-Uninfected Men Who Have Sex with Men (MSM) (CO-US-104-0288; iPrEx Study)
2. Parallel Comparison of Tenofovir and Emtricitabine/Tenofovir Pre-Exposure Prophylaxis to Prevent HIV-1 Acquisition within HIV-1 Discordant Couples (CO-US-104-0380; Partner's PrEP)

The iPrEx trial compared the prophylactic efficacy of daily TRUVADA[®] (FTC 200 mg/TDF 300 mg) versus placebo in uninfected men who have sex with men (MSM) who are at high risk of HIV-1 infection. The Partner's PrEP trial compared the prophylactic efficacies of daily FTC/TDF, TDF alone, and placebo in uninfected heterosexual men and women who have HIV-1 infected partners. TRUVADA[®] was shown to be effective in both trials for preventing HIV-1 infection, with a strong correlation between measurable drug concentrations and efficacy. Please see the reviews of Thomas Hammerstrom, Ph.D., the FDA's statistical reviewer, Jiang Liu, Ph.D., the FDA's pharmacometrics reviewer, and Ruben Ayala, Ph.D., the FDA's clinical pharmacology reviewer.

Genotypic resistance was not detected by population nucleotide sequence analysis, which has a limit of sensitivity for minority species comprising approximately 25% or more of the viral quasispecies, among the isolates of the 48 subjects who were enrolled into the FTC/TDF cohort of the iPrEx trial and became infected during the treatment phase. However, resistant virus was detected after 4 weeks of FTC/TDF prophylaxis in 2/2 subjects who were unknowingly infected (i.e., HIV-1 seronegative) at the time of enrollment. An FTC resistance-associated amino acid substitution in HIV-1 reverse transcriptase, M184V, was detected in the week 4 isolate of one subject but was absent in the baseline isolate, indicating that resistance emerged during the study. Another FTC resistance-associated substitution, M184I, was detected in the week 4 isolate of the second subject; however, the baseline sample did not yield genotypic data due to insufficient viral RNA in the sample, and therefore it is unclear if the M184I substitution was selected during the study or if it was borne by the transmitted virus. A second genotypic analysis using an allele-specific reverse-transcriptase polymerase chain reaction assay that is sensitive to the presence of low levels of variants (0.5% of the viral quasispecies) expressing specific resistance-associated substitutions (i.e., K65R, K70E, M184V, and M184I) was conducted. None of the assayed variants were detected among subjects in the FTC/TDF group who became infected during the trial. The results of the genotypic analyses are consistent with the pharmacokinetic finding of no quantifiable intracellular drug levels among most subjects who failed chemoprophylaxis.

Genotypic resistance was not detected by population nucleotide sequence analysis of viruses isolated from 15 subjects enrolled into the TDF cohort or 12 subjects enrolled into the FTC/TDF cohorts who became infected during the Partner's PrEP trial. However, resistant virus was detected among subjects who were unknowingly infected (i.e., HIV-1 seronegative) at the time of enrollment and received either TDF or FTC/TDF. Two of the five subjects infected at baseline and receiving TDF had detectable variants expressing resistance at the time of seroconversion, one with a K65R-expressing variant at week 16 and the other with a variant bearing the

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Microbiology Review

1. Introduction and Background

There is no cure for human immunodeficiency virus type 1 (HIV-1) infection, nor has an effective HIV-1 vaccine for prophylaxis of infection been developed. The HIV-1 epidemic in the United States has remained steady with approximately 48,000 to 58,000 new infections per year since the early 1990s ([Hall et al., 2008](#); [Holtgrave et al., 2009](#); [Prejean et al., 2011](#)) despite available protective measures including condoms and risk reduction behavior ([Johnson et al., 2008](#); [Underhill et al., 2007](#) and [2008](#)). Approximately 75% of new U.S. HIV-1 infections occur in men, with African-American and Hispanic/Latino men experiencing the highest incidence rates ([Prejean et al., 2011](#)). The primary route of HIV-1 transmission in the U.S. is unprotected sex between men who have sex with men (MSM), accounting for 61% of new infections between 2006 and 2009. Despite the relatively stable rate of overall new infections in the U.S., the estimated number of new infections among young MSM has increased by 34% from 2006 to 2009, with a 48% increase among young African-American MSM. Among women, African-American women experience the highest HIV-1 incidence rates.

The suppression of HIV-1 replication with antiretroviral therapy in infected individuals has been shown to reduce transmission between serodiscordant couples ([Cohen et al., 2011](#)) and presents an attractive public health strategy for further reducing viral transmissions. However, the estimated 20% of persons in the United States who are unaware that they are infected with HIV-1 ([Campsmith et al., 2010](#); [CDC, 2011](#)) disproportionately account for greater than 50% of HIV-1 sexual transmissions ([Marks et al., 2006](#)), and even among those who are aware of their positive infection status, only 35% have suppressed viral loads ([CDC, 2011](#)). Further, the use of condoms, risk reduction behavior, and antiretroviral therapy for prevention requires negotiation and agreement between the uninfected and infected partners, which may not be possible in some circumstances.

This supplement to NDA 21-752 seeks to expand the indication of TRUVADA[®] to include pre-exposure prophylaxis of sexually transmitted HIV-1 infection. TRUVADA[®] is a fixed-dose combination of the approved nucleoside analogue reverse transcriptase inhibitor (NRTI) emtricitabine (FTC) and the approved acyclic nucleotide analogue reverse transcriptase inhibitor (NtRTI) tenofovir disoproxil fumarate (TDF). FTC (EMTRIVA[®]) and TDF (VIREAD[®]) were approved for the treatment of HIV-1 infected adults in combination with other antiretroviral drugs on July 02, 2003 and October 10, 2001, respectively. TRUVADA[®], indicated for the treatment of HIV-1 infected adults over 18 years of age in combination with other antiretroviral products, was first approved in the United States on August 02, 2004 (NDA 21-752).

1.1 Important Milestones in Product Development

FTC was approved for treatment of HIV-1 infection on July 02, 2003 and marketed under the name EMTRIVA[®] (IND 053971 and NDA 021500). TDF was approved for treatment of HIV-1 infection on October 10, 2001 and is marketed under the name VIREAD[®] (IND 052849 and NDA 021356). TDF and FTC are also components of three approved fixed-dose, orally administered combination products for HIV-1 infection: TRUVADA[®] (FTC 200 mg/TDF 300 mg; NDA 021752, approved August 2, 2004); ATRIPLA[®] (efavirenz 600 mg/FTC 200 mg/TDF 300 mg; NDA 021937, approved July 12, 2006); and COMPLERA[®] (FTC 200mg/rilpivirine [RPV] 25 mg/TDF 300 mg; NDA 202123, approved August 10, 2011).

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1.2 Methodology

1.2.1 HIV-1 Diagnostics

iPrEx

Anti-HIV-1 antibody rapid testing was performed monthly and seroconversions were detected according to the algorithm presented in Figure 1 (Study CO-US-104-0288 Clinical Study Report, pg. 45). Rapid anti-HIV-1 antibody testing was performed with Oraquick® (OraSure Technologies) and Clearview® (Inverness Medical) in the U.S.A., and Bioline (Standard Diagnostics) and Determine® (Abbott/Inverness Medical) in other countries. Samples that were positive for anti-HIV-1 antibody by one or both of the rapid tests were also tested by Western blot analysis (Bio-Rad Laboratories). The enrollment samples of subjects who seroconverted within the first 12 weeks of the trial were tested for the presence of HIV-1 RNA using the RT-PCR-based RealTime HIV-1 (Abbott Molecular, Inc., Des Plaines, IL), which has a lower limit of quantification (LLOQ) of 40 copies/mL.



Figure 1. HIV-1 Testing Algorithm for Seroconversion in iPrEx

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Partner's PrEP

HIV-1 testing for partner subjects was performed monthly, according to the algorithm presented in Figure 2 (pg. 120, Partners PrEP Protocol, Version 3.0, 12 October 2007). Trained study personnel performed 2 rapid assays in parallel at the sites during the monthly subject visits. The brand of rapid tests used was determined by assay availability within each participating country; all assays must have passed proficiency testing and meet national HIV-1 testing approval. The rapid tests used in Kenya included the Determine[®] HIV 1/2 (Abbott/Inverness Medical), the Uni-Gold[™] (Trinity Biotech), and the Bioline (Standard Diagnostics). The rapid tests that were used at the Uganda study sites included the Determine[®] HIV 1/2, the Uni-Gold[™], and the HIV 1/2 STAT-PAK[®] (Chembio Diagnostic Systems). If one or both rapid tests were positive, blood was drawn for enzyme immunoassay (EIA) confirmation testing using assays meeting national policies, and the subject was taken off study drug. EIA tests used in Kenya included the Vironostika[®] HIV Ag/Ab 4th generation test (bioMérieux) and the Murex HIV Ag/AB Combo 4th gen (Abbott Murex), while those used in Uganda included the Vironostika[®] HIV Ag/Ab 4th gen (bioMérieux), Vironostika[®] HIV Uni-Form II plus O – 3rd generation test (bioMérieux), BioRad HIV 1/2 (Bio-Rad Laboratories), and the Murex HIV 1.2.0 AB 3rd generation test (Abbott Murex).



Figure 2. HIV-1 testing algorithm used in the Partners PrEP trial

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1.2.2 Detection of HIV-1 RNA in Baseline Samples

The baseline infection status of subjects in both the iPrEx and Partner's PrEP trials was confirmed using the RT-PCR-based RealTime HIV-1 (Abbott Molecular, Inc.) assay, which has a lower limit of quantification (LLOQ) of 40 copies/mL.

1.2.3 Plasma HIV-1 RNA Load Determinations

iPrEx

Plasma samples from seroconverters were retrospectively tested for HIV-1 RNA, starting with the sample immediately preceding that of the seroconversion visit. If a sample collected earlier than that of the seroconversion sample tested positive for HIV-1 RNA, then the date of that sample was considered the estimated time to infection (TTIFN). HIV-1 RNA was quantified using the RT-PCR-based Real-Time HIV-1 (Abbott Molecular, Inc.) assay, which has a lower limit of quantification (LLOQ) of 40 copies/mL, or the Amplicor HIV-1 Monitor (Roche Molecular Systems, Inc.) assay, which has a LLOQ of 50 copies/mL. The limit of detection (LOD) of both assays is equal to their respective LLOQ.

Partner's PrEP

HIV-1 RNA was quantified using the RT-PCR-based Real-Time HIV-1 (Abbott Molecular, Inc.) assay, which has a lower limit of quantification (LLOQ) of 40 copies/mL.

1.2.3 Genotypic Resistance Analyses

iPrEx

The initial genotypic evaluation was performed with the approved Trugene[®] HIV-1 Genotyping kit (Siemens Healthcare Diagnostics Inc., Deerfield, IL), which utilizes population nucleotide sequence analysis to identify HIV-1 variants expressing resistance-associated substitutions. Population nucleotide sequencing is limited in sensitivity, detecting variants that comprise >20-30% of the viral population within an individual. In order to detect lower levels of variants, the sponsor performed an allele-specific RT-PCR assay that has a LLOQ of 0.5% to measure K65R, K70E, M184V, and M184I-expressing variants within the viral quasispecies.

Multiple primer sets, each consisting of 1) a "cure" primer pair and 2) an allele-specific and universal primer pair, were validated for cross-clade amplification and minor variant quantification. Primer sets were selected by proximity to the drug resistance-associated codon site and closest match to the population sequence for that particular specimen (Table 1; ARI-UCSF Laboratory of Clinical Virology qMVA Description, pg. 6; submitted in SDN 710). Eight primer sets consisting of the cure, allele-specific (ARMS) primers (mutant and wild type), and the cognate universal primer were used. The primer sets are listed in Table 1 and include primer name, the HIV-1 reference strain HXB2 (Genbank #K03455) reverse transcriptase (RT) reference nucleotide location, and the 5' → 3' oligonucleotide sequence. The "cure" PCR primer tolerates polymorphic mismatches while still allowing for target PCR amplification, thereby minimizing the destabilizing effect from sequence heterogeneity in the primer binding regions ([Svarovskaia et al., 2006](#)).

(b) (4)

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(b) (4)



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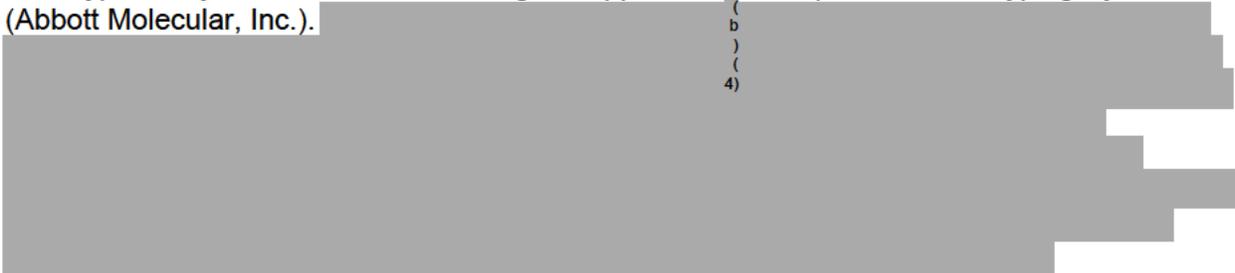
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(b) (4)



Partner's PrEP

Genotypic analysis was conducted using the approved ViroSeq[®] HIV-1 Genotyping System (Abbott Molecular, Inc.).



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Table 3. “In House” Primer Sets in Partner’s PrEP

(b) (4)

1.3 Prior FDA Virology Reviews

Supporting nonclinical and clinical virology studies for emtricitabine (EMTRIVA[®]), tenofovir disoproxil fumarate (VIREAD[®]), and the fixed combination of 200 mg emtricitabine with 300 mg tenofovir disoproxil fumarate (TRUVADA[®]) were previously reviewed under NDA 021500, NDA 021356, and NDA 021752, respectively.

1.4 Major Virology Issues that Arose during Product Development

1.4.1 Resistance

While the daily administration of FTC/TDF appears to be highly efficacious for preventing sexually transmitted HIV-1 infection, the drug combination is not a complete antiretroviral (ARV) treatment regimen capable of fully suppressing HIV-1 replication in people who are infected. As a result, the selection of resistant virus in HIV-1 infected people who use FTC/TDF is anticipated. Emtricitabine and tenofovir disoproxil fumarate are components of all U.S. Department of Health and Human Service recommended first-line antiretroviral regimens for HIV-1 infected adults and adolescents ([DHHS Guidelines, 2012](#)), and the selection of resistant HIV-1 due to misuse or failure of PrEP could result in the loss of these preferred options. Further, amino acid substitutions associated with FTC and/or TFV resistance may confer cross-resistance to other HIV-1 N(t)RTIs, further limiting treatment options.

The potential for an increase in the transmission rates of resistant viruses, which could not only limit the treatment options for treatment-naïve subjects but might also limit the future effectiveness of FTC/TDF or other HIV-1 N(t)RTI-containing prophylactics, is an additional concern. However, it is possible that a reduction in the replication efficiency of resistant variants may also limit the efficiency at which they are transmitted ([Wagner et al., 2012](#)), as has been observed in macaque models of mucosal infection using SHIV expressing K65R or M184V substitutions ([Cong et al., 2011](#)). Further, it has been argued that the contribution of PrEP to the transmission of resistant viruses is likely to be small within the context of resistance associated with ARV treatment failures ([Parikh and Mellors, 2012](#)).

Sophisticated stochastic mathematical models have been used to evaluate the principle factors that could contribute to the emergence and spread of resistant variants as a result of PrEP ([Abbas et al., 2010](#)) and to predict the impact of a PrEP roll-out on resistance within a resource-

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rich, ART-experienced community ([Supervie et al., 2010](#)). Modeling conducted by [Abbas et al., 2010](#) determined that the prevalence of PrEP-related resistance was likely to be primarily influenced by: 1) the extent and duration of inadvertent ARV use in people who are HIV-1 infected when initiating PrEP, 2) the persistence of the transmitted resistant variants, and 3) the duration of continued ARV use in people who become infected while using PrEP. [Supervie et al., 2010](#) predicted that rollout of an effective PrEP product could increase the proportion of sexually transmitted viruses but result in a reduction in the number of sexually transmitted variants due to an overall decrease in the absolute number of new infections. However, increased risk behavior and/or lower efficacy PrEP products could result in an increase in transmission of resistant strains.

Determining the impact of TRUVADA[®] for PrEP on the transmission of FTC and TDF resistance may require longer-term epidemiological studies of surveillance data collected from treatment- and PrEP-naïve individuals who become infected in communities where PrEP is used. Importantly, detection of transmitted resistant viruses in treatment- and PrEP-naïve individuals may be difficult if the resistance-associated substitution comes with a loss of fitness for the virus. In such cases, detection of the resistant variant may only be possible if genotyped early after infection before the resistant variant is overgrown by true revertant wild-type virus ([Johnson et al., 2008](#)). Unfortunately, even low levels of resistant virus that are undetectable by standard genotypic and phenotypic assays could impact the durability of future ARV regimens ([Johnson et al., 2008](#); [Little et al., 2002](#)). Determining the absolute concentrations of resistant variants using deep sequencing may prove useful in predicting response to subsequent therapies ([Goodman et al., 2011](#)).

1.4.2 Screening for HIV-1 infection prior to initiating PrEP

The selection of resistant variants in the iPrEx and Partner's PrEP trials was limited to those subjects who were acutely infected with HIV-1, i.e., seronegative but HIV-1 RNA positive, at baseline and received drug. Collectively, 50% (5/10) of subjects who were acutely infected at baseline and received active drug had detectable levels of resistant virus by the time of seroconversion. In an independent analysis of the genotypic data, FDA confirmed that uncommon N(t)RTI resistance pathways ([Stanford HIV-1 Drug Resistance Database](#)) were not found in isolates from the other 5 subjects. The absence of detectable resistant variants among subjects who were not infected at baseline and failed prophylaxis may be attributable to lack of adherence and frequent testing for seroconversion, conditions that may not be reflected in a "real world" setting where intermittent drug use and infrequent HIV-1 testing may occur. Alternatively, it is possible that the "real world" scenario will involve increased adherence given that individuals taking FTC/TDF for PrEP will know that the drug works, thereby preventing infection and abating concerns for the selection of ARV resistance. In either case, the use of HIV-1 chemoprophylaxis interventions should include frequent monitoring for infection, including sensitive HIV-1 RNA-specific assays at or near the time of prophylaxis initiation.

Minimizing the exposure times of subjects who are infected but seronegative at the time of PrEP initiation is an important consideration for preserving treatment options and limiting the transmission of N(t)RTI resistant viruses. Acute infections have been estimated to account for 5%-20% of all newly identified cases of HIV-1 infection ([Branson and Stekler, 2011](#); [CDC, 2009](#); [Patel et al., 2006](#); [Pilcher et al., 2005](#); [Stekler et al., 2009](#)). A complication for detecting the acute stages of HIV-1 infection is the "eclipse" or "window" phase, the period of time between infection and when the virus is first detectable ([Cohen et al., 2010](#); [Fiebig et al., 2003](#); [Tomaras et al., 2008](#)). Although all HIV-1 diagnostic assays suffer an eclipse phase, they do not suffer equally. Detection of acute HIV-1 infection, which includes the time before seroconversion, can

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only be identified by assays that detect viral RNA or viral p24 antigen. Based on the current understanding of the biology of early HIV-1 infections, RNA diagnostic assays are believed to be able to detect virus within 10 to 15 days of infection, while assays that can detect viral p24 antigen are sensitive to the presence of virus within 15-20 days of infection ([Cohen et al., 2010](#); [Fiebig et al., 2003](#)). Seroconversion typically occurs at least 20 days after infection, at which time the anti-HIV-1 antibody detecting assays become effective. Interestingly, the monthly rapid anti-HIV antibody testing and retrospective RT-PCR analyses of stored pre-seroconversion samples for viral RNA that were included in the iPrEx and Partner's PrEP studies demonstrated that anti-HIV-1 antibody rapid tests were initially unable to diagnose 21% (20/93) and 48% (28/58) of new infections, respectively. These results imply that the window period for some of the seroconversion assays may be closer to 4 weeks and support use of nucleic acid based tests for screening or retesting 4 weeks after the initial test for seroconversion.

1.4.3 Hypothetical delay in the time to seroconversion when infected and using ARVs

An additional concern is the possibility that the antiviral activity of FTC and TDF could indirectly diminish the sensitivity of rapid tests to detect seroconversion among people using PrEP. A reduction of viral replication could lower the production of immunogenic viral proteins and delay the timing, intensity, or quality of antibody response. Indeed, the suppression of HIV-1 replication through effective ARV therapies administered early during infection has been associated with reduced anti-HIV-1 antibody responses in some studies ([Binley et al., 2000](#); [Kassutto et al., 2005](#); [Morris et al., 1998](#); [Re et al., 2010](#); [Selleri et al., 2007](#)). It is unclear what effect, if any, inhibition of viral replication at the earliest stages of HIV-1 infection, such as is anticipated with use of the combination of FTC and TDF in acutely infected individuals, might have on the initial induction of anti-HIV antibody responses. A study in macaques infected with SHIV while receiving daily or intermittent FTC/TDF as prophylaxis did not experience a delay in the time to seroconversion, although their anti-SHIV antibodies exhibited lower affinities ([Curtis et al., 2011](#)).

1.5 State of Antiviral Used for the Indication(s) Sought

No antiviral products are currently approved for the prophylaxis of HIV-1 infection.

2 Nonclinical Virology

Sections 2.1-2.4 reproduce nonclinical virology information from the emtricitabine and tenofovir disoproxil fumarate labels. For more thorough analyses of the nonclinical data, see the original Clinical Virology NDA reviews of emtricitabine (NDA 021500), tenofovir disoproxil fumarate (NDA 021356), and the fixed combination of 200 mg emtricitabine with 300 mg tenofovir disoproxil fumarate (NDA 021752), by Narayana Battula, Ph.D.

2.1 Mechanism of Action

Emtricitabine (FTC) is a synthetic nucleoside analog of cytidine that is phosphorylated by cellular kinases to its active triphosphate form, emtricitabine triphosphate (FTC-TP). Emtricitabine 5'-triphosphate inhibits the activity of the HIV-1 reverse transcriptase by competing with the natural substrate deoxycytidine 5'-triphosphate and by being incorporated into nascent viral DNA which results in chain termination ([EMTRIVA® label](#)).

Tenofovir disoproxil fumarate (TDF) is an acyclic nucleoside phosphonate diester analog of adenosine monophosphate. Tenofovir disoproxil fumarate requires initial diester hydrolysis for conversion to tenofovir and subsequent phosphorylations by cellular kinases to form tenofovir diphosphate (TFV-DP), an obligate chain terminator ([VIREAD® label](#)). Tenofovir diphosphate

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inhibits the activity of HIV-1 reverse transcriptase by competing with the natural substrate deoxyadenosine 5'-triphosphate (dATP) and, after incorporation into DNA, by DNA chain termination.

2.2 Antiviral Activity

The antiviral activity in cell culture of emtricitabine against laboratory and clinical isolates of HIV was assessed in lymphoblastoid cell lines, the MAGI-CCR5 cell line, and peripheral blood mononuclear cells ([EMTRIVA® label](#)). The 50% effective concentration (EC₅₀) value for emtricitabine was in the range of 0.0013–0.64 µM (0.0003–0.158 µg/mL). FTC displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, and G (EC₅₀ values ranged from 0.007–0.075 µM) and showed strain specific activity against HIV-2 (EC₅₀ values ranged from 0.007–1.5 µM).

The antiviral activity of tenofovir against laboratory and clinical isolates of HIV-1 was assessed in lymphoblastoid cell lines, primary monocyte/macrophage cells and peripheral blood lymphocytes ([VIREAD® label](#)). The EC₅₀ (50% effective concentration) values for tenofovir were in the range of 0.04 µM to 8.5 µM. Tenofovir displayed antiviral activity in cell culture against HIV-1 clades A, B, C, D, E, F, G, and O (EC₅₀ values ranged from 0.5 µM to 2.2 µM) and strain specific activity against HIV-2 (EC₅₀ values ranged from 1.6 µM to 5.5 µM).

2.3 Combination Antiviral Activity

No antagonism was reported in combination studies of emtricitabine. Additive to synergistic effects were observed ([EMTRIVA® label](#)) with nucleoside reverse transcriptase inhibitors (abacavir, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, nevirapine), and protease inhibitors (amprenavir, nelfinavir, ritonavir, saquinavir).

No antagonism was reported in combination studies of tenofovir. Tenofovir demonstrated additive to synergistic effects ([VIREAD® label](#)) with nucleoside reverse transcriptase inhibitors (abacavir, didanosine, lamivudine, stavudine, zalcitabine, zidovudine), non-nucleoside reverse transcriptase inhibitors (delavirdine, efavirenz, nevirapine), and protease inhibitors (amprenavir, indinavir, nelfinavir, ritonavir, saquinavir).

2.4 Resistance Studies

Emtricitabine-resistant isolates of HIV-1 have been selected in cell culture and *in vivo* ([EMTRIVA® label](#)). Genotypic analysis of these isolates showed that the reduced susceptibility to emtricitabine was associated with a mutation in the HIV-1 reverse transcriptase gene at codon 184, which resulted in an amino acid substitution of methionine by valine or isoleucine (M184V/I).

Emtricitabine-resistant isolates of HIV-1 have been recovered from some patients treated with emtricitabine alone or in combination with other antiretroviral agents. In a clinical study of treatment-naïve patients treated with emtricitabine, didanosine, and efavirenz, viral isolates from 37.5% of patients with virologic failure showed reduced susceptibility to emtricitabine. Genotypic analysis of these isolates showed that the resistance was due to M184V/I substitutions in HIV-1 reverse transcriptase.

In a clinical study of treatment-naïve patients treated with either FTC, TDF, and efavirenz or zidovudine/lamivudine and efavirenz, resistance analysis was performed on HIV-1 isolates from

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all virologic failure patients with >400 copies/mL of HIV-1 RNA at Week 48 or early discontinuations. Development of efavirenz resistance-associated substitutions occurred most frequently and was similar between the treatment arms. The M184V amino acid substitution, associated with resistance to FTC and lamivudine, was observed in 2/12 (17%) analyzed patient isolates in the FTC + TDF group and in 7/22 (32%) analyzed patient isolates in the lamivudine/zidovudine group. Through 48 weeks of Study 934, no patients have developed a detectable K65R substitution in their HIV as analyzed through standard genotypic analysis. Insufficient data are available to assess the development of the K65R substitution upon prolonged exposure to this regimen.

HIV-1 isolates with reduced susceptibility to tenofovir have previously been selected in cell culture ([VIREAD[®] label](#)). These viruses expressed a K65R substitution in reverse transcriptase (RT) and showed a 2-4 fold reduction in susceptibility to TFV. Notably, K65R has also been identified among clinical isolates from subjects experiencing virologic failure of their TDF-containing regimens (Gilead Studies 902 and 907).

Tenofovir-resistant isolates of HIV-1 have been recovered from some patients treated with TDF in combination with certain antiretroviral (ARV) agents ([VIREAD[®] label](#)). In Study 903 of treatment-naïve subjects (Viread[®] + lamivudine + efavirenz versus stavudine + lamivudine + efavirenz), genotypic analyses of isolates from subjects with virologic failure through Week 144 showed development of efavirenz and lamivudine resistance-associated substitutions to occur most frequently and with no difference between the treatment arms. The K65R substitution occurred in 8/47 (17%) analyzed patient isolates on the Viread[®] arm and in 2/49 (4%) analyzed patient isolates on the stavudine arm. Of the 8 subjects whose virus developed K65R in the Viread[®] arm through 144 weeks, 7 of these occurred in the first 48 weeks of treatment and one at Week 96. Other substitutions resulting in resistance to Viread[®] were not identified in this trial.

In Study 934 of treatment-naïve subjects (TDF + FTC + efavirenz versus zidovudine (AZT)/lamivudine (3TC) + efavirenz), genotypic analysis performed on HIV-1 isolates from all confirmed virologic failure subjects with greater than 400 copies/mL of HIV-1 RNA at Week 144 or early discontinuation showed development of efavirenz resistance-associated substitutions occurred most frequently and was similar between the two treatment arms ([VIREAD[®] label](#)). The M184V substitution, associated with resistance to EMTRIVA and lamivudine, was observed in 2/19 analyzed subject isolates in the TDF + FTC group and in 10/29 analyzed subject isolates in the zidovudine/lamivudine group. Through 144 weeks of Study 934, no subjects have developed a detectable K65R substitution in their HIV-1 as analyzed through standard genotypic analysis.

In HPTN 050, which included an evaluation of the effect of tenofovir gel in HIV-1 infected women, no new resistance-associated RT substitutions were detected in isolates collected from plasma or cervicovaginal lavage (CVL) after 14 days of tenofovir gel use ([Mayer et al., 2006](#)). Samples were collected on Days 0, 7, and 14 and the genotypic analysis was conducted using population-based nucleotide sequencing.

Cross-resistance between emtricitabine and certain nucleoside analog reverse transcriptase inhibitors has been recognized ([EMTRIVA[®] label](#)). Emtricitabine-resistant isolates expressing RT M184V/I were cross-resistant to lamivudine and zalcitabine but retained sensitivity in cell culture to didanosine, stavudine, tenofovir, zidovudine, and NNRTIs (delavirdine, efavirenz, and nevirapine). HIV-1 isolates containing the K65R substitution, selected in vivo by abacavir, didanosine, tenofovir, and zalcitabine, demonstrated reduced susceptibility to inhibition by emtricitabine. Viruses harboring substitutions conferring reduced susceptibility to stavudine and

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zidovudine (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E) or didanosine (L74V) remained sensitive to emtricitabine. HIV-1 containing the K103N substitution associated with resistance to NNRTIs was susceptible to emtricitabine.

Similarly, cross-resistance between tenofovir and certain nucleoside/tide analog reverse transcriptase inhibitors has been observed ([VIREAD[®] label](#)). The K65R substitution selected by tenofovir is also selected in some HIV-1 infected subjects treated with abacavir, didanosine, or zalcitabine. HIV-1 isolates with this substitution also show reduced susceptibility to emtricitabine and lamivudine. Therefore, cross-resistance among these drugs may occur in patients whose virus harbors the K65R substitution. HIV-1 isolates from subjects (N=20) whose HIV-1 expressed a mean of 3 zidovudine-associated reverse transcriptase substitutions (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N), showed a 3.1-fold decrease in the susceptibility to tenofovir.

The virologic response to TDF therapy has been evaluated with respect to baseline viral genotype (N=222) in treatment-experienced subjects participating in Studies 902 and 907 ([VIREAD[®] label](#)). In these clinical trials, 94% of the participants evaluated had baseline HIV-1 isolates expressing at least one NRTI resistance-associated substitution. However, virologic responses for subjects in the genotype substudy were similar to the overall trial results.

Several exploratory analyses were conducted to evaluate the effect of specific substitutions and substitution patterns on virologic outcome ([VIREAD[®] label](#)). Because of the large number of potential comparisons, statistical testing was not conducted. Varying degrees of cross-resistance of VIREAD to pre-existing zidovudine resistance-associated substitutions (M41L, D67N, K70R, L210W, T215Y/F, or K219Q/E/N) were observed and appeared to depend on the type and number of specific substitutions. VIREAD-treated subjects whose HIV-1 expressed 3 or more zidovudine resistance-associated substitutions that included either the M41L or L210W reverse transcriptase substitution showed reduced responses to VIREAD therapy; however, these responses were still improved compared with placebo. The presence of the D67N, K70R, T215Y/F, or K219Q/E/N substitution did not appear to affect responses to TDF therapy. Subjects whose virus expressed an L74V substitution without zidovudine resistance associated substitutions (N=8) had reduced response to TDF. Limited data are available for subjects whose virus expressed a Y115F substitution (N=3), Q151M substitution (N=2), or T69 insertion (N=4), all of whom had a reduced response. In the protocol-defined analyses, virologic response to TDF was not reduced in subjects with HIV-1 that expressed the abacavir/emtricitabine/lamivudine resistance-associated M184V substitution. HIV-1 RNA responses among these subjects were durable through Week 48.

2.5 Oral Prophylaxis in Animal Models of HIV Transmission

A series of nonclinical studies using a repeated exposure macaque model of rectal transmission with chimeric simian immunodeficiency virus/human immunodeficiency virus (SHIV) infection have been conducted and support the proposed pre-exposure prophylaxis indication of TRUVADA[®]. The studies used SHIV_{SF162P3}, which bears the *tat*, *env*, and *rev* genes of HIV-1_{SF162}, a subtype B, CCR5-tropic strain of HIV-1. SHIV_{SF162P3} does not express the reverse transcriptase (RT) of HIV-1, although FTC and TDF do have activity against the RT of SIV and resistance to each drug is primarily mediated by M184V and K65R substitutions, respectively. Rectal inoculations were conducted by applying 10 median tissue culture infectious doses (TCID₅₀) to the rectal surface once weekly for up to 14 weeks.

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In the first study, groups of macaques were either untreated (n=4) or treated with an oral dose of TDF that yielded plasma concentrations similar to those observed in people using Viread® on a daily (n=4) or weekly (n=4) schedule ([Subbarao et al., 2006](#)). Interestingly, subsequent work with this model demonstrated that the same oral dose of TDF (22 mg/kg) also yielded intracellular diphosphate levels in peripheral blood mononuclear cells (PBMCs) comparable to those seen in humans using TRUVADA® or VIREAD® ([García-Lerma et al., 2008](#)). All animals were inoculated with 10 median tissue culture infectious doses (TCID₅₀) of SHIV_{SF162P3} once weekly for 14 weeks or until viral RNA was detected in plasma by RT-PCR. SHIV challenges took place two hours after receiving oral TDF (22 mg/kg). Of the 4 untreated control animals, 4 became infected after a median of 1.5 exposures. One of four animals treated with daily TDF became infected, although the median time to infection was increased to six weeks. None of the animals receiving weekly TDF were protected by infection, although the median time to infection was increased to 7 weeks. Interestingly, no difference in the viral loads of the treatment groups and no delay in the time to seroconversion from the initial time of SHIV RNA detection were observed, indicating low antiviral activity from either the daily or weekly TDF treatment regimen. Consistent with the observation of low antiviral activity, no resistance conferring substitutions were detected among SHIV isolated from infected animals who continued their TDF regimens until week 36 of the study.

In a second study, the prophylactic activity of the combination of daily oral FTC and TDF at doses that yielded FTC-TP and TFV-DP concentrations in PBMCs similar to those observed in humans using TRUVADA® was evaluated ([García-Lerma et al., 2008](#)). Macaques were inoculated rectally with SHIV once weekly for up to 14 weeks as described by [Subbarao et al., 2006](#). Of the 18 control animals, 17 became infected after a median of 2 rectal exposures. In contrast, 4 of the 6 animals treated daily with oral FTC and TDF (20 and 22 mg/kg/day, respectively) remained uninfected and the two infections that did occur were significantly delayed until weeks 9 and 12. Interestingly, the 2 animals that became infected in this study exhibited reduced viral titers and delayed viremia, indicating a higher level of antiviral activity than was observed for the TDF-only regimen described in the first study, and an M184I-expressing FTC resistant variant emerged in one of the two macaques after 3 weeks of continued drug exposure.

In order to evaluate the prophylactic effect of tenofovir (TFV) dosed 3- to 4-fold higher than humans receiving TRUVADA® or VIREAD®, additional macaques received daily subcutaneous administrations of 20 mg/kg FTC and 22 mg/kg TFV 2 hours before SHIV exposure ([García-Lerma et al., 2008](#)). All 6 animals treated with FTC/high-dose TFV remained fully protected from infection. In addition, 6 of 6 animals who received intermittent treatment with once weekly, two dose subcutaneously administered FTC/high-dose tenofovir 2 hours before and 22 hours post SHIV exposure were also fully protected from infection.

A third series of macaque studies began with an experiment to determine if subcutaneous FTC/high dose TFV (20 mg/kg FTC and 22 mg/kg TFV) could protect against 14 weeks of once weekly rectal SHIV exposures when administered as either pre- or post-exposure administrations ([García-Lerma et al., 2010](#)). Five of five untreated control animals became infected within 3 rectal exposures. Treatment with FTC/high-dose TFV 2 hours before SHIV exposure resulted in 2 of 6 infections, with infections occurring at exposures 8 and 13. A two treatment, post-exposure regimen of FTC/high-dose TFV at 24 and 48 hours post exposure was not protective, and this treatment group of the study was stopped for futility when 3 of 6 animals became infected after the first 2 exposures. These studies indicate that the full protection described by [García-Lerma et al., 2008](#) required FTC/high-dose TFV treatment both pre- and post-exposure.

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Having established that maximum protection from SHIV infection required the administration of drug before and after virus challenge, [García-Lerma et al., 2010](#) evaluated a series of pre- and post-exposure treatment schedules for their protective effect. Notably, this series of experiments used orally administered FTC/TDF (20/22 mg/kg) rather than the subcutaneously administered combination of FTC/high-dose TFV (20/22 mg/kg) that was able to achieve full protection when administered daily or once weekly at 2 hours pre- and 22 hours post-SHIV exposure ([García-Lerma et al., 2008](#)). Nine of nine untreated animals became infected at a median of 1 rectal exposure. One of six animals treated once weekly with oral FTC/TDF 22 hours pre- and 2 hours post-SHIV exposure became infected at the week 4 exposure. The same level of protection was observed in animals treated once weekly with two doses of FTC/TDF 3 days before and 2 hours after SHIV challenge, with 1 of 6 animals becoming infected at week 2. Two of six animals treated with FTC/TDF 7 days before and 2 hours after challenge became infected at weeks 3 and 14. These data indicate a statistically significant reduction in the risk of infection by all three pre- and post-challenge PrEP interventions. The mean peak SHIV RNA loads were lower in PrEP failures than untreated controls by 1.5 log₁₀ copies/mL. However, no M184I/V or K65R resistance-associated substitutions were detected among the viruses of PrEP failures, in contrast to the detection of two resistant variants among six PrEP failures in [García-Lerma et al., 2008](#). One potential explanation for the lack of resistance is that the once weekly, two-dose treatment regimen evaluated in the later study provided less selective pressure than that of the earlier study's daily treatment regimens.

Collectively, the macaque rectal challenge studies support the combination of FTC and TDF for PrEP over use of the individual drugs. Daily oral TDF (22 mg/kg) protected 25% of animals (1/4) ([Subbarao et al., 2006](#)) and daily oral FTC (20 mg/kg) protected 33% (2/6) animals ([García-Lerma et al., 2008](#)). In contrast, the combination of daily oral FTC/TDF (20/22 mg/kg) protected 67% (4/6) animals. While treatment with subcutaneously administered FTC/high dose TFV was required for full protection, a subsequent study demonstrated that TFV alone was unable to provide full protection, even when administered at very high concentrations. [García-Lerma et al., 2011](#) treated macaques with GS-7340 (IND 063737 for treatment of HIV-1 infection), a prodrug of tenofovir that results in intracellular phosphate concentrations higher than those achieved by TDF, three days before SHIV exposure. After 14 rectal exposures, 33% (2/6) animals remained uninfected despite TFV-DP levels that were from 50-100 fold-higher in PBMCs than those of animals treated with TDF. The researchers determined that the surprising lack of protection might be attributable to high intracellular dATP levels in rectal lymphocytes, the natural substrate that TFV-DP competes with for incorporation by viral reverse transcriptase into nascent DNA. These data indicate that it may not be possible to achieve TFV-DP concentrations within the SHIV-susceptible cells of the rectal mucosa high enough to confer complete protection. If so, then the addition of another antiretroviral drug, such as FTC, is further justified. Studies comparing the endogenous dATP levels of lymphocytes within the rectal, vaginal, and penile mucosa of macaques are ongoing. It should be noted that this study did have limitations: the PrEP regimen evaluated in this study was different from those of other studies (e.g., there were no post-exposure doses of GS-7340), the combination of FTC and GS-7340 was not evaluated to confirm the benefit of FTC to the prophylactic effect, and it is unclear if rectal lymphocytes of human tissue have comparably high endogenous dATP levels observed in those of macaques.

The combination of FTC and TDF may also provide prophylactic activity against transmission of HIV-1 variants that are resistant to either FTC or TDF. Recently, [Cong et al., 2011a](#) demonstrated that FTC/TDF PrEP maintained protection against challenge with a SHIV strain

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expressing the FTC-resistant M184V substitution, although protection against a K65R-expressing variant ([Cong et al., 2011b](#)) was not described.

Surprisingly, a macaque model of vaginal SHIV challenge does not appear to have been used to evaluate the effectiveness of orally administered ARVs for PrEP against this route of infection.

3 Relevant Findings from other Disciplines

3.1 Clinical Pharmacology, Pharmacometrics, and Statistics

Pharmacokinetic/pharmacodynamic (PK/PD) case-cohort analyses were conducted by the iPrEx and Partner's PrEP study teams that demonstrated a strong association between measurable drug levels and the absence of HIV-1 infection. In the iPrEx analysis, intracellular triphosphorylated drug components were measured in each of the samples from 34 subjects who became infected while receiving FTC/TDF and compared to FTC/TDF treated subjects who remained uninfected. The sponsor's estimated relative risk reduction between subjects with and without measurable TFV-DP levels who were enrolled into the active arm was 92% (95% CI, 72% to 99%) after adjusting for age, number of partners at baseline, self-reported receptive anal intercourse (at baseline and follow-up), education, and body mass index. In the Partner's PrEP analysis, plasma TFV levels were determined and the concentrations of those who became infected versus those who were uninfected compared. For subjects in the TDF treatment group, the sponsor's estimated relative risk reduction for subjects with measurable TFV levels relative to those without was 86% (95% CI, 67% to 95%). For subjects in the FTC/TDF treatment group, the sponsor's estimated relative risk reduction for subjects with measurable TFV levels relative to those without was 90% (95% CI, 56% to 98%).

An independent FDA analysis using a different methodology found that the relative risk reduction in iPrEx subjects with measurable TFV-DP was 87.5% (95% CI, 66% to 95%) versus placebo while those with unmeasurable concentrations had a relative risk reduction of 14.5% (95% CI, -22.3% to 40.3%), which was not significantly different from placebo. When subjects with measurable TFV-DP concentrations were further stratified into two groups based on whether or not their intracellular concentrations were measured above or below the median concentration of $15.6 \text{ fmol}/10^6 \text{ PMBCs}$, subjects with concentrations below the median had a relative risk reduction of 76% (95% CI, 34% to 91%) while those above the median concentration had a relative risk reduction of 100% (95% CI, 60% to 100%). When a similar analysis was conducted using data from the Partner's PrEP trial, the FDA found that subjects who had measurable plasma TFV levels at all tested time points had a relative risk reduction of 94% (95% CI, 75% to 98%) relative to subjects who received placebo. These results are consistent with those of the sponsor and support the strong correlation between adherence and efficacy.

Please see the reviews of Thomas Hammerstrom, Ph.D., the FDA's statistical reviewer, Jiang Liu, Ph.D., the FDA's pharmacometrics reviewer, and Ruben Ayala, Ph.D., the FDA's clinical pharmacology reviewer for descriptions of the full analysis and a discussion of their results.

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4. Clinical Virology

4.1 Overview of Phase 3 Trials

4.1.1 Pre-exposure Prophylaxis Initiative (iPrEx; CO-US-104-0288)

The iPrEx trial was a randomized, double-blind, placebo-controlled, phase 3 study of the safety and efficacy of oral TRUVADA® (FTC 200mg/TDF 300 mg) for chemoprophylaxis of sexually transmitted HIV-1 in seronegative men who have sex with men (MSM) and are at high risk for acquiring HIV-1 infection. The study duration was event driven, with study evaluations continuing until at least 85 seroconversion events were identified.

The trial enrolled 2499 HIV-1 seronegative men or transgender women who have sex with men to receive a combination of 200 mg emtricitabine and 300 mg tenofovir disoproxil fumarate (FTC/TDF; n=1251) or placebo (n=1248) once daily. This study was conducted at a total of 11 study sites in 6 countries: 3 in Peru (56.0% [1400/2499] of randomized subjects), 1 in Ecuador (12.0% [300/2499] of randomized subjects), 3 in Brazil (14.8% [370/2499] of subjects), 2 in the United States (9.1% [227/2499] of subjects), 1 in Thailand (4.6% [114/2499] of subjects), and 1 in South Africa (3.5% [88/2499] of subjects). This trial was designed with an event driven endpoint (i.e., HIV-1 seroconversions), and subjects participated for variable durations (median of 1.2 years and a maximum of 2.8 years). The initial efficacy assessment was conducted using a data cut-off date of March 01, 2010, when 100 seroconversion events had occurred ([Grant et al., 2010](#)), although the NDA submission included data from study visits through November 21, 2010.

HIV-1 seroconversions occurred in 147 subjects (54/1226 in the FTC/TDF group and 93/1226 placebo) by November 21, 2010, which corresponded to the minimum 8 week time point after the end of treatment (EoT) for all subjects. Ten subjects (2 in the FTC/TDF group and 8 in the placebo group) were subsequently determined to have been infected at baseline by retrospective detection of plasma HIV-1 RNA in stored specimens obtained during the enrollment visit. Among the remaining 137 subjects who became infected during the trial (52/1224 in the FTC/TDF and 85/1218 in the placebo groups), 6 seroconverted after the end of treatment (4 FTC/TDF and 2 placebo), which occurred no later than August 31, 2010, and were excluded from the modified intent-to-treat (mITT) population. The rate of infection among the mITT population was 2.4% (48/1224) per person year (PPY) for those enrolled into the FTC/TDF arm and 4.2% (83/1218) PPY in the placebo group, representing a risk reduction of 42% (95% CI, 22% to 63%; p=0.001).

Subject identification number (SubID), study country, the study day of infection and/or seroconversion, and HIV-1 subtype are summarized in Table 4 for the mITT population's seroconverters. The time to infection (TTIFN) was defined as the study day of the first reactive (and subsequently confirmed) rapid anti-HIV-1 antibody test or as the day of the first positive HIV-1 RNA test. The time to seroconversion (SC) was the day that anti-HIV antibodies were detected by at least one of the two rapid tests, and are only shown for subjects whose TTIFN and time to seroconversion dates were different.

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Table 4. Summary of iPrEx seroconverters (mITT)

	SubID	Country	TTIFN	SC	Subtype	SubID	Country	TTIFN	SC	Subtype
Placebo	8730088	Brazil	420		B	9116818	Peru	248		B
	8730446	Brazil	285		B	9117344	Peru	391		B
	8730721	Brazil	252		B/C	9117475	Peru	328		B
	8831018	Brazil	253		B	9117849	Peru	178	345	B
	8831678	Brazil	257	278	B	9117989	Peru	337		B
	8932007	Brazil	207		B	9123030	Peru	252		B/F
	8944080	Brazil	120		B	9123602	Peru	273		B
	8944264	Brazil	334	419	B	9150380	Peru	560	597	B
	8944433	Brazil	167	193	B	9212077	Peru	588		B
	8944918	Brazil	147		B	9212092	Peru	85		B
	9010398	Peru	252		B/F	9218169	Peru	792		B
	9010419	Peru	669		B	9218211	Peru	251		B
	9010488	Peru	1091		B	9218986	Peru	308		B
	9010609	Peru	336	408	B	9219175	Peru	562		B
	9010730	Peru	575		B	9219392	Peru	784		B
	9010838	Peru	339		B	9219700	Peru	509		B
	9010908	Peru	202		B	9219829	Peru	392		B
	9010963	Peru	475		F1	9313117	Ecuador	148		B
	9014206	Peru	392		B	9313352	Ecuador	812		B
	9014230	Peru	583		B	9313454	Ecuador	645		B
	9014717	Peru	560		B	9313612	Ecuador	445		B
	9015887	Peru	588		B	9313664	Ecuador	603		B
	9015957	Peru	418		B	9313956	Ecuador	591		B
	9022123	Peru	509		B	9320086	Ecuador	534		CRF02_AG
	9022371	Peru	305		B	9320666	Ecuador	364		B
	9022416	Peru	658		B	9320802	Ecuador	450		B
	9022677	Peru	332		B	9320965	Ecuador	333		B
	9022999	Peru	166		B	9320998	Ecuador	252		B
	9111000	Peru	983		B	9321117	Ecuador	507		CRF02_AG
	9111059	Peru	134		B	9321155	Ecuador	205		B
	9111064	Peru	840	896	B	9321393	Ecuador	253	280	B
	9111124	Peru	307		B	9321436	Ecuador	500	527	B
	9111280	Peru	280		B	9321553	Ecuador	161		B
9111417	Peru	364		B	9321891	Ecuador	418		B	
9111578	Peru	280		B	9433288	USA	331		B	
9111703	Peru	1009	1051	A1	9534415	USA	343		B/F	
9111848	Peru	472		B	9635258	South Africa	84		B	
9111966	Peru	995		B	9635621	South Africa	136		C	
9116160	Peru	168	196	B	9736101	Thailand	225		CRF01_AE	
9116351	Peru	673		B	9736381	Thailand	393		B	
9116413	Peru	843		B	9736778	Thailand	255		CRF01_AE	
9116736	Peru	336	395	B						
FTC/DF	8730001	Brazil	474	504	B	9150322	Peru	441	476	C
	8730104	Brazil	376		B	9150339	Peru	84	112	B/F
	8932010	Brazil	255		B	9150490	Peru	476		B
	8944631	Brazil	272		B	9212637	Peru	588	616	B
	9010202	Peru	837		B	9218116	Peru	475		B
	9010284	Peru	391		B	9218406	Peru	280		B
	9010691	Peru	189		B	9218719	Peru	217		B
	9014224	Peru	825		B	9218740	Peru	303		B
	9014560	Peru	907		B	9252420	Peru	326		B
	9015339	Peru	502		B/F	9252802	Peru	335		B
	9015536	Peru	752		B	9313697	Ecuador	419		B
	9022653	Peru	314		B	9313739	Ecuador	140		B
	9051738	Peru	203		B	9313886	Ecuador	316		B
	9111153	Peru	999		B	9313920	Ecuador	344	365	B
	9111213	Peru	815		B	9320851	Ecuador	32		B
	9111375	Peru	912		B/F	9320949	Ecuador	466		B
	9111831	Peru	518	672	B	9321108	Ecuador	588		B
	9111901	Peru	756		B	9321282	Ecuador	398		B
	9116369	Peru	301		B	9321623	Ecuador	162		CRF02_AG
	9116390	Peru	410		B	9433185	USA	586		B
	9116586	Peru	756	784	B	9635097	South Africa	293		C
9117355	Peru	868		B	9635826	South Africa	178		F2	
9117660	Peru	312		B	9736702	Thailand	279		CRF01_AE	
9150070	Peru	479		B	9736807	Thailand	142		CRF01_AE	

SC = time to first concordant or discordant (but subsequently confirmed) rapid test results if different from TTIFN

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The 10 subjects who were determined by RT-PCR assay for HIV-1 RNA to have been infected at baseline and were excluded from the mITT population are shown in Table 5. The 6 subjects who seroconverted after the drug stop date, which occurred no later than July 31, 2010, and were excluded from the mITT population are presented in Table 6.

Among the 131 infections that occurred in the mITT population, 79 (60.3%) took place in Peru, 26 (19.8%) in Ecuador, 14 (10.7%) in Brazil, 5 (3.8%) in Thailand, 4 (3.1%) in South Africa, and 3 (2.3%) in the U.S. Similar proportions were observed between the treatment arms, with Peru, Ecuador, Brazil, Thailand, South Africa, and the U.S. accounting for 59.0%, 20.5%, 12.0%, 3.6%, 2.4%, and 2.4% of infections in the placebo arm and 62.5%, 18.7%, 8.3%, 4.2%, 4.2%, and 2.1% of infections in the FTC/TDF arm, respectively. HIV-1 subtype B accounted for 84.7% (111/131) of infections, subtype C for 2.3% (3/131), subtype A1 and F1 for 0.8% (1/131) each, and the remainder with recombinant subtypes or co-infection with two subtypes. Definitive conclusions on the efficacy of TRUVADA[®] for PrEP in MSM are limited to subtype B given the preponderance of that subtype in the iPrEx trial's study sites. However, results from the Partners PrEP study indicate that TRUVADA[®] should be efficacious for subtypes A and D as well (see below).

Table 5. Summary of iPrEx baseline infections

	SubID	Country	TTIFN	SC	Subtype
Placebo	8730695	Brazil	0	26	ND
	8730824	Brazil	0	24	ND
	8831107	Brazil	0	26	ND
	9015791	Peru	0	58	ND
	9150288	Peru	0	28	ND
	9635569	South Africa	0	26	ND
	9635636	South Africa	0	10	ND
	9736657	Thailand	0	79	ND
FTC/TDF	9022624	Peru	0	34	ND
	9252180	Peru	0	28	ND

Table 6. Summary of iPrEx subjects who became infected after the drug stop date

	SubID	Country	Stop	TTIFN	Subtype
PBO	9022887	Peru	616	648	B
	9736067	Thailand	531	561	CRF01_AE
FTC/TDF	9111734	Peru	1008	1036	B
	9117924	Peru	861	861	B
	9150645	Peru	539	608	F1
	9635309	South Africa	442	457	C

No delay in the median time to infection from the start of the trial was observed between subjects who became infected within each treatment group, with median (IQR) times to infection of 56 (IQR, 11-143) weeks and 55 (IQR, 12-156) weeks for subjects treated with FTC/TDF and placebo, respectively (P=0.56, Mann-Whitney Test).

Drug components were quantifiable in 10% (5/48) of HIV-1 infected subjects of the FTC/TDF group near the TTIFN, which for this analysis included PK data measured at the TTIFN or within 4 weeks of TTIFN if the PK data for the TTIFN sample were missing. All available PK data for these five subjects are summarized in Table 7. Note that there were no PK data for intracellular triphosphate concentrations from Subject 9212637 at the TTIFN. The quantification ranges for FTC and TFV were 16 pM to 2.4 mM and 40 pM to 6 mM (10 to 1500 ng/mL), respectively. The quantifiable range for FTC-TP was 0.10 to 200 pmol/sample and that of TFV-DP was 2.5 to

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2000 fmol/sample. The lower limit of quantification for the HIV-1 RNA RT-PCR assay was 1.6 log₁₀ copies/mL (40 copies/mL).

Table 7. PK and HIV-1 RNA data from subjects with measurable drug levels near TTIFN

SubID	TTIFN	Day	RNA (log ₁₀ copies/mL)	FTC-TP (pmol/10 ⁶ PBMCs)	TFV-DP (fmol/10 ⁶ PBMCs)	FTC (nM)	FTC (ng/mL)	TFV (nM)	TFV (ng/mL)
8730001	474	342		BLQ	BLQ				
		474	2.4	3.25	10.5	16246	4016	1417	407
		504	5.7	BLQ	BLQ		BLQ		BLQ
9015536	752	167		BLQ	BLQ				
		335		BLQ	BLQ				
		504		BLQ	BLQ				
		671	BLQ	8.55	15.6	15777	3900	1442	414
		752	4.9	7.02	14.7	8940	2210	533	153
9212637	588	168		BLQ	BLQ				
		335		BLQ	BLQ				
		507	BLQ	BLQ	BLQ		BLQ		BLQ
		588	5.7				BLQ		BLQ
9218406	280	616	5.1	1.44	9.54	10057	2486	3095	889
		168		6.01	36.9	5672	1402	742	213
		280	5.9	0.29	BLQ	769	190	157	45
9321282	398	169		BLQ	BLQ		BLQ		BLQ
		337	BLQ	BLQ	BLQ		BLQ		BLQ
		405	4.4	0.15	4.19		BLQ		BLQ

The median (min-max) TFV-DP and FTC-TP PBMC concentrations near the estimated time to infection (TTIFN) of the five subjects with measurable drug levels were 9.54 (range, BLQ-14.7) fmol/10⁶ cells and 1.44 (range, 0.15-7.02) pmol/10⁶ cells, respectively. In contrast, drug levels were BLQ at the time to infection in 83% (40/48) of the subjects who became HIV-1 infected during the trial, while 6.2% (3/48) of the subjects did not have PK measurements of samples collected within 4 weeks of the estimated TTIFN. Interestingly, the 5 subjects who failed prophylaxis but had measurable triphosphate concentrations of FTC and/or TDF near the TTIFN were below the lower limit of quantification for at least one other time point, indicating intermediate levels of adherence. It should be noted that the TTIFN is an estimate of the time of infection and the actual concentrations of drug components in PBMCs at the time of HIV-1 exposure are unknown.

The results of a pharmacokinetic study measuring TFV-DP concentrations among HIV-uninfected subjects after 6 weeks of receiving once-daily, directly-observed TDF at either 2 (n=21), 4 (n=21), or 7 (n=22) times per week were recently reported in an oral presentation at CROI 2012 ([Anderson et al., 2012](#)). TDF-DP concentrations in viable PMBCs were measured at median (IQR) of 11 (IQR, 6-13), 32 (IQR, 25-39), and 42 (IQR, 31-47) fmol/10⁶ cells for subjects receiving the 2, 4, and 7 doses per week regimens, respectively. Assuming that the presence of FTC does not alter the pharmacokinetics of TFV-DP, the results of the PK trial indicate that subjects who failed prophylaxis in iPrEx but still had measurable TDF-DP levels were using FTC/TDF less than twice weekly.

Although the combination of FTC and TDF is not expected to completely suppress HIV-1 replication within individuals with established infections, the ARVs are expected to exhibit antiviral activity and reduce viral loads. As such, confirmation of lower viral loads in subjects who were using FTC/TDF after becoming infected could serve as a surrogate marker for drug adherence. RNA load data of samples collected within 7 days of seroconversion were available for 110 mITT subjects, including 47 who received FTC/TDF and 63 who received placebo. The

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median (IQR) RNA load for subjects treated with FTC/TDF were 5.3 (IQR, 4.9-5.8) log₁₀ copies/mL while that of subjects treated with placebo were comparable, at 5.2 (IQR, 4.7-5.6) log₁₀ copies/mL (P=0.25; Wilcoxon Test). These data indicate that there was no evidence of antiviral activity from FTC/TDF by virologic response, which is consistent with poor adherence.

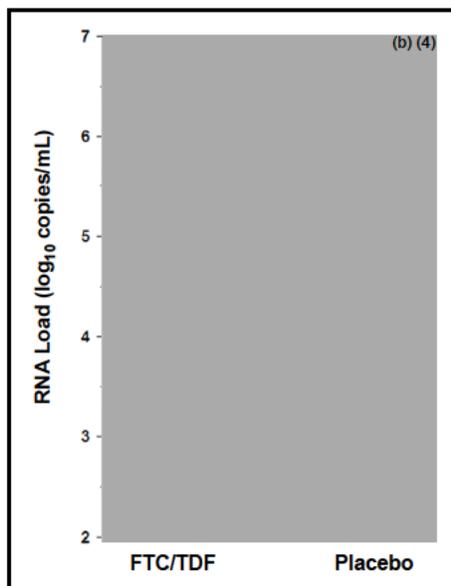


Figure 3. HIV-1 RNA loads at the TTIFN in iPrEx

4.1.2 Partner's PrEP (CO-US-104-380)

The Partner's PrEP trial was a Phase 3, multicenter, randomized, double-blind, placebo-controlled, 3 group study designed to evaluate the safety and efficacy of PrEP with either TDF (VIREAD®) or FTC/TDF (TRUVADA®) administered orally, once daily for the prevention of HIV-1 transmission between serodiscordant heterosexual couples. Among the 4747 serodiscordant couples who comprised the ITT population, 96 seroconversion events were observed. It was later determined that 14 site-reported seroconversions occurred in partner subjects who were HIV-1 RNA-positive by RT-PCR assay at enrollment, with 3 in the FTC/TDF group, 5 in the TDF group, and 6 in the placebo group. Of the 82 subjects who seroconverted during the trial and comprised the mITT population, 13/1576, 17/1579, and 52/1578 occurred in partner subjects randomized to FTC/TDF, TDF, and placebo treatment groups, respectively. The rate of infection among the mITT population was 0.50% (13/2616), 0.65% (17/2604), and 1.99% (52/2607) PPY for the FTC/TDF, TDF, and placebo treated subjects, respectively. Both FTC/TDF and TDF demonstrated protection against infection, with relative risk reductions of 75% (95% CI, 60% to 90%) and 67% (95% CI, 49% to 85%) for the FTC/TDF and TDF treated subjects relative to those receiving placebo, respectively. Importantly, FTC/TDF and TDF provided statistically significant protection from HIV-1 acquisition in both men and women, with relative risk reductions of 66% (95% CI, 40% to 92%) and 84% (95% CI, 67% to 101%) for women and men treated with FTC/TDF relative to those treated with placebo, respectively, and 71% (95% CI, 49% to 94%) and 62% (95% CI, 34% to 91%) for women and men treated with TDF relative to those treated with placebo, respectively.

Subject identification number (SubID), study country, gender, time to seroconversion, and HIV-1 subtype are summarized in Table 8 for the mITT seroconverters and Table 9 for subjects who were determined to have been infected at baseline. The time to seroconversion was defined as the study day of the first reactive (and subsequently confirmed) anti-HIV-1 antibody rapid test.

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Table 8. Summary of Partner's PrEP seroconverters (mITT)

	SubID	Country	Sex	SC	Subtype		SubID	Country	Sex	SC	Subtype	
Placebo	5001419	Uganda	F	196	A	Placebo	5629410	Kenya	M	294	A	
	5004719	Uganda	M	476	CRF01_AE		5637016	Kenya	M	224	CRF01_AE	
	5007013	Uganda	F	616	D		5640215	Kenya	M	52	CRF01_AE	
	5035412	Uganda	F	224	A		5643817	Kenya	F	56	D	
	5036314	Uganda	M	588	A		5645812	Kenya	M	331	D	
	5038416	Uganda	M	169	A		5647914	Kenya	F	199	A	
	5049818	Uganda	F	84	G		5711912	Kenya	M	645	D	
	5055719	Uganda	F	173	CRF01_AE		5747916	Kenya	M	140	D	
	5062917	Uganda	F	80	D		5802718	Uganda	F	574	A	
	5111318	Kenya	F	165	C		5811315	Uganda	F	252	B	
	5131610	Kenya	F	472	D		5824014	Uganda	F	280	CRF01_AE	
	5135219	Kenya	M	332	A		FTC/TDF	5017613	Uganda	F	646	D
	5200612	Uganda	M	672	A			5204215	Uganda	F	113	CRF01_AE
	5206914	Uganda	F	280	A	5237817		Uganda	M	419		
	5209816	Uganda	F	616	A	5312715		Kenya	F	112	A	
	5230114	Uganda	F	252	D	5408214		Uganda	F	610	A	
	5242013	Uganda	F	308	A	5507219		Uganda	F	448	A	
	5250115	Uganda	F	252	A	5522417		Uganda	F	112	A	
	5254919	Uganda	M	168	CRF01_AE	5612214		Kenya	M	419	A	
	5311712	Kenya	F	224	A	5623212		Kenya	F	726	D	
	5313317	Kenya	M	280	CRF01_AE	5631410		Kenya	F	448	CRF01_AE	
	5314015	Kenya	M	672	A	5700916		Kenya	M	168	A	
	5327312	Kenya	M	447	A	5707914		Kenya	F	839	A	
	5332513	Kenya	F	23	A	5727117		Kenya	M	576	A	
	5336716	Kenya	M	391	A	TDF	5015117	Uganda	F	422	A	
	5341219	Kenya	M	84	D		5025815	Uganda	M	420	A	
	5348013	Kenya	M	228	A		5037515	Uganda	F	308	A	
	5416915	Uganda	M	560	D		5053119	Uganda	F	294	A	
	5429317	Uganda	F	588	A		5100415	Kenya	M	537	A	
	5431113	Uganda	M	560	D		5124214	Kenya	M	448	A	
	5433510	Uganda	M	504	CRF01_AE		5241418	Uganda	F	392	A	
	5438713	Uganda	F	280			5250917	Uganda	F	140	A	
	5441815	Uganda	F	504	A		5303216	Kenya	M	284	CRF01_AE	
5451314	Uganda	F	175	A	5303417		Kenya	M	368	A		
5537319	Uganda	F	364	A	5426914		Uganda	M	308	A		
5543712	Uganda	F	336	D	5438419		Uganda	M	196	D		
5544013	Uganda	M	308	D	5504813		Uganda	M	560	A		
5546018	Uganda	M	280	A	5510313	Uganda	F	336	D			
5605910	Kenya	M	193	D	5611111	Kenya	F	195				
5615310	Kenya	F	57	A	5651813	Kenya	M	364	A			
5628112	Kenya	F	724	A	5657618	Kenya	F	337				

The times to seroconversion were not significantly different between groups of the mITT analysis group, with median (IQR) of 448 (IQR, 140.5-628), 337 (IQR, 289-421), and 280 (IQR, 179.5-497) days for FTC/TDF, TDF, and placebo groups, respectively (P=0.26; Mann-Whitney Test).

The majority of HIV-1 cases in the mITT population were associated with subtype A (58.9% [46/78]), subtype D (24.3% [18/78]), and CRF01_AE (12.8% [11/78]) viruses, with single cases of infections by subtypes B, C, and G. The proportions of subjects infected with subtypes A and D were consistent between the treatment arms and placebo, with subtype A comprising 61.5% (8/13), 80% (12/15), and 52% (26/50) of the FTC/TDF, TDF, and placebo-receiving subjects, respectively (P=0.16; Fisher Exact Test), as well as for subtype D comprising 15.4% (2/13), 13.3% (2/15), and 28% (14/50) of the FTC/TDF, TDF, and placebo-receiving arms, respectively (P=0.46; Fisher Exact Test). These data indicate that there were no apparent discrepancies between the efficacies of FTC/TDF or TDF against the primary HIV-1 subtypes encountered during the Partner's PrEP trial. The results of this analysis are consistent with those of a similar analysis conducted by the sponsor in response to a query from the DAVP (SDN 739, [Question](#)

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2). Results from the iPrEx trial indicate that oral PrEP for prevention of heterosexual transmission should be effective for subtype B viruses.

Table 9. Summary of Partner's PrEP baseline infections

	SubID	Country	Sex	SC	Subtype
Placebo	5046518	Uganda	F	28	A
	5254015	Uganda	M	84	A
	5414913	Uganda	M	24	C
	5649015	Kenya	F	28	K
	5657314	Kenya	M	56	A
	5742211	Kenya	M	28	A
FTC/ TDF	5230312	Uganda	M	84	A
	5530314	Uganda	M	28	D
	5652413	Kenya	M	22	D
TDF	5044313	Uganda	M	420	D
	5235716	Uganda	F	28	A
	5248810	Uganda	M	84	A
	5329316	Kenya	F	28	A
	5425710	Uganda	F	112	D

Interestingly, different subtypes were identified in 9 subjects when evaluating virus subtype in samples collected at a different time point (Table 10). These changing subtype assignments are likely attributable to infection with a recombinant strain and to changes in the population nucleotide sequence of HIV-1 *pol* gene that occurred between sample dates.

Table 10. Disparate HIV-1 subtype determinations

	SubID	GT	Subtype
Placebo	5200612	673	A
		701	CRF01_AE
	5250115	253	A
		273	CRF01_AE
	5615310	58	A
		62	CRF01_AE
5629410	295	A	
	421	CRF01_AE	
FTC/TDF	5507219	449	A
		463	CRF01_AE
	5522417	94	A
		113	C
	5530314	29	D
		43	A
5631410	449	CRF01_AE	
	457	D	
TDF	5250917	141	A
		155	CRF01_AE

In contrast to the pharmacokinetic evaluation of subjects in iPrEx, Partner's PrEP only evaluated tenofovir in plasma, which may provide a less accurate assessment of subject compliance due to TFV's shorter half-life (approximately 19 hours) in plasma versus that of TFV-DP in PBMCs (approximately 87-150 hours). For example, a poorly-compliant subject who used FTC/TDF hours before sample collection might have plasma TFV levels comparable to those of a subject who was highly compliant. However, the TFV-DP levels of the poorly- and highly-compliant subjects would be markedly different.

All available PK data for the 3 subjects treated with FTC/TDF and 7 subjects treated with TDF who had quantifiable levels of TFV near their time to seroconversion, which for this analysis included PK data measured within 4 weeks of the seroconversion visit, are summarized in Tables 11 and 12, respectively. The lower limit of quantification for TFV was 1 nM (0.3 ng/mL) and that of HIV-1 RNA was 1.6 log₁₀ copies/mL (40 copies/mL).

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Table 11. PK and HIV-1 RNA data from FTC/TDF-treated subjects with measurable drug levels near SC

SubID	SC	Day	RNA	TFV	
			(log ₁₀ copies/mL)	(nM)	(ng/mL)
5204215	113	30		572	1991.6
		84		169	588.4
		113	BLQ	158	550.1
		127	2.7		
		197	3.2		
		281	3.3		BLQ
		365	3.3		
		449	3.2		BLQ
		533	3.1		
		701	2.8		
		785	3.5		BLQ
5237817	419	32		14.7	51.2
		84		70.8	246.5
		168		66.3	230.8
		419		65.5	228.1
		447	3.0		
		504	3.4		
5522417	112	93	5.2		BLQ
		112	4.3	74.2	258.4

The median (min-max) TFV concentrations near the time to seroconversion for the 3 subjects who received FTC/TDF was 74.2 (range, 65.5-158) nM while that of the 7 subjects who received TDF was 48.8 (range, BLQ-433) nM. In contrast, drug levels were below the lower limit of quantification at the time of seroconversion in the remainder of the subjects who became HIV-1 infected during the trial. Interestingly, 8/10 of these subjects consistently had measurable plasma levels of TFV until they became infected. However, TFV's short half-life in plasma only reflects very recent compliance but does not provide insight into the compliance of these subjects over longer periods of time.

RNA load data of samples collected at the time of seroconversion were available for 76 subjects in the mITT population, including 10 who received FTC/TDF, 15 who received TDF, and 51 who received placebo (Figure 4). The median (IQR) RNA loads for subjects treated with FTC/TDF were 4.4 (IQR, 3.5-4.7) log₁₀ copies/mL, 4.0 (IQR, 3.4-4.8) log₁₀ copies/mL for those treated with TDF, and 4.7 (IQR, 4.2-5.4) log₁₀ copies/mL for those treated with placebo. While the HIV-1 RNA loads were comparable between the FTC/TDF and TDF treatment groups (P=0.7), those of FTC/TDF and TDF treatment groups were significantly different from placebo-treated loads, with median differences of -0.3 (P=0.04) and -0.7 (P=0.01) log₁₀ copies/mL, respectively. In contrast to the results of HIV-1 RNA loads in the iPrEx trial, these data indicate that there was virologic evidence of antiviral activity at the seroconversion visit. However, these data should be interpreted carefully as the sample numbers from the treatment groups are small and it is unknown if comparable levels of drug were present at the actual time of infection.

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Table 12. PK and HIV-1 RNA data from TDF-treated subjects with measurable drug levels near SC

SubID	SC	Day	RNA	TFV	
			(log ₁₀ copies/mL)	(nM)	(ng/mL)
5037515	308	28		89.9	313
		81		79.5	276.8
		168	2.4	103	358.6
		252	4.1	60.1	209.3
		308	3.9	83.8	291.8
		326	5		
		421	4.2		
5241418	392	28		140	487.5
		88		18.5	64.4
		168		192	668.5
		339	2.8	23.3	81.1
		392	3.4	80.5	280.3
		409	4.5		
		462	4.1		
5250917	140	28		112	390
		84	4.2	82.6	287.6
		140	3.5		BLQ
		230	4.7		
		314	5.5		BLQ
5426914	308	28		203	706.8
		84		189	658.1
		168		253	880.9
		252	4.9	42.3	147.3
		308	4.3	433	1507.7
		316	5.1		
		392	5.9		
476	5.3				
5510313	336	24		78.6	273.7
		84		63.7	221.8
		168		52.6	183.1
		252		41.8	145.5
		336	3.5	48.8	169.9
		355	4		
		404	3.5		
		475	3.6		
		563	3.4		
		670	2.9		
756	3.7				
5611111	195	31		21.6	75.2
		79		50.9	177.2
		163		72.5	252.4
		195	2.5	25.7	89.5
		202	2.6		
		279	2.4		
		358	1.6		
		504			BLQ
696			BLQ		
5657618	337	28		51.7	180
		83	2.5	20.4	71
		170		56.5	196.7
		337		8.43	29.4

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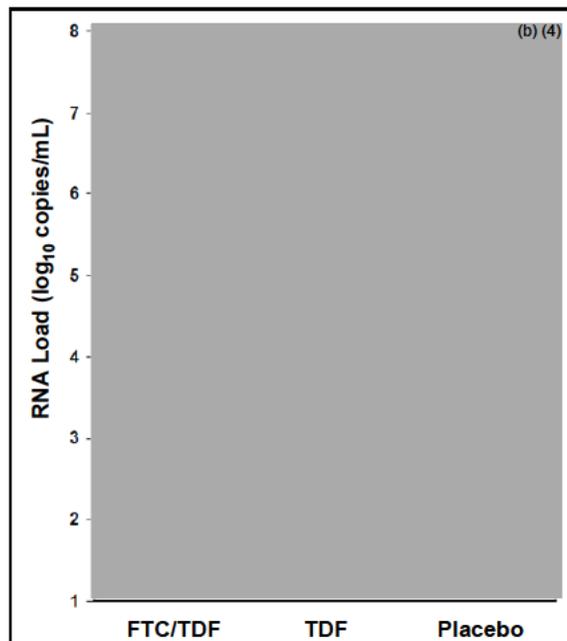


Figure 4. HIV-1 RNA loads at the seroconversion visit of Partner's PrEP

4.2 Resistance Analyses of iPrEx and Partner's PrEP

Partial HIV-1 RT amino acid sequences of isolates collected from subjects of the mITT populations of iPrEx and Partner's PrEP were determined by translation of the consensus nucleotide sequence and submitted to the FDA for review. The FDA analysis included a review of substitutions at amino acids of the HIV-1 RT associated with reduced susceptibility to N(t)RTIs (i.e., M41, A62, K65, D67, T69, K70, L74, V75, F77, Y115, F116, Q151, M184, L210, T215, and K219). No N(t)RTI-resistance-associated substitutions were identified among the isolates. The results of the genotypic analysis, which used the reverse transcriptase of HIV-1 LAV-1 (GenBank #K02013) as reference, are summarized in Table 13. Tables identifying all substitution differences from the reference sequence are provided in [Appendix B](#).

An exploratory analysis was conducted to identify amino acid positions within the HIV-1 RT that exhibited a higher frequency of substitutions among isolates of FTC/TDF (iPrEx and Partner's PrEP trials) and TDF (Partner's PrEP) treated subjects relative to those isolated from subjects receiving placebo ([Appendix C](#)). Amino acid positions showing 2-fold or greater frequency differences were tested for significantly different ratios between isolates of the study cohorts by the Fisher Exact test. There were no statistically significant substitution frequency differences observed between FTC/TDF and placebo treated isolates from the iPrEx trial. However, isolates from Partner's PrEP subjects receiving TDF had a 2.9-fold higher frequency of RT K20R and V21I compared to those from placebo treated subjects, a difference that was statistically significant ($P=0.03$ for both positions, Fisher Exact Test). The isolates from the TDF group bearing K20R and/or V21I included 3 subtype A viruses expressing K20R and V21I, 2 subtype A viruses expressing K20R, 1 subtype A expressing V21I, and one subtype CRF01_AE expressing K20R and V21I, while six subtype A viruses expressed the wild type amino acid at both positions. According to the [Stanford University HIV Resistance Database](#), K20R and V21I are common polymorphisms in subtype A viruses, occurring at frequencies of 18% and 17% in N(t)RTI-naïve subjects, respectively. Restricting the analysis to only include subtype A and CRF01_AE variants, the ratio of variants expressing K20R and V21I between the TDF and placebo treatment groups loses statistical significance ($P= 0.065$ and $P= 0.25$, respectively).

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Although interesting, it is possible that the initial observation was related to the small TDF-treatment sample size.

Table 13. Summary of genotypic resistance observed in the PrEP Trials

Trial	On Treatment Infections			Baseline Infections		
	Arm (Cases)	Week	Genotype	Arm (Cases)	Week	Genotype
iPrEx	Placebo (0/82)			Placebo (1/8)	0	M184V+T215Y
					4	M184V+T215Y
	FTC/TDF (0/48)			FTC/TDF (2/2)	0	Wild-type
					4	M184V
					0	Indeterminate
Partners PrEP	Placebo (0/51)			Placebo (0/6)		
	FTC/TDF (0/12)			FTC/TDF (1/3)	0	Wild-type
					12	M184V
	TDF (0/15)			TDF (2/5)	0	Wild-type
					16	K65R
					0	No Data
TDF2	Placebo (0/24)			Placebo (0/2)		
	FTC/TDF (0/9)			FTC/TDF (1/1)	0	Wild-type
					4	M184V
					28	A62V+K65R+M184V

Genotypic resistance was not detected by population nucleotide sequence analysis, which has a limit of sensitivity for minority species comprising approximately 25% or more of the viral quasispecies, among the isolates of the 48 subjects who were enrolled into the FTC/TDF cohort of the iPrEx trial and became infected during the treatment phase. The results of the genotypic resistance analysis of the Partner's PrEP trial were similar to those of the iPrEx trial. No genotypic resistance was detected by population nucleotide sequence analysis of viruses isolated from 12 subjects enrolled into the FTC/TDF cohort or from the 15 subjects enrolled into the TDF cohort who became infected during the Partner's PrEP trial and were included in the analysis.

A concern is that clinically significant populations of resistant virus could be present among the viral quasispecies of subjects who failed PrEP at levels below the limit of detection of population sequencing. Therefore, a second genotypic analysis using an allele-specific reverse-transcriptase polymerase chain reaction assay that is sensitive to the presence of low levels of variants (0.5% of the viral quasi-species) expressing specific resistance-associated substitutions (i.e., K65R, K70E, M184V, and M184I) was conducted for isolates from the iPrEx trial. None of the assayed variants were detected among subjects in the FTC/TDF group who became infected during the trial (Table 14). The results of the genotypic analyses are consistent with those of the population nucleotide sequence data and pharmacokinetic finding of no measurable drug concentrations among most subjects who failed chemoprophylaxis. The individual data for each subject are presented in [Appendix D](#).

Table 14. Summary of genotypic analysis using allele-specific RT-PCR

	K65R		K70E		M184V		M184I	
	n	median (min-max) %	n	median (min-max) %	n	median (min-max) %	n	median (min-max) %
FTC/TDF	34	0.12 (0.06-0.30)	33	0.13 (0.03-0.29)	33	0.27 (0.1-0.42)	33	0.08 (0.04-0.25)
Placebo	60	0.11 (0.06-0.69)	56	0.14 (0.02-0.32)	58	0.26 (0.12-1.26)	58	0.09 (0.04-0.21)

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Despite the lack of resistance observed in isolates of subjects who became infected during the PrEP trials, resistance was frequently detected among viruses isolated from subjects who were acutely infected at time of enrollment and received FTC/TDF or TDF. In the iPrEx trial, 2/2 subjects who were acutely infected and received FTC/TDF had detectable levels of resistant virus by week 4 of the study. An FTC resistance-associated amino acid substitution, M184V, was detected in the week 4 isolate of one subject but was absent in the baseline isolate, indicating that resistance emerged during the study. Another FTC resistance-associated substitution, M184I, was detected in the week 4 isolate of the second subject; however, the baseline sample did not yield genotypic data due to insufficient viral RNA in the sample, and therefore it is unclear if the M184I resistance-associated substitution was selected during the study or if it was borne by the transmitted virus. In the Partner's PrEP trial, 1 of the 3 subjects acutely infected at baseline who received FTC/TDF had an M184V-expressing variant detected at week 12. Two of the five subjects infected at baseline and receiving TDF had detectable variants expressing resistance at the time of seroconversion, one with a K65R-expressing variant at week 16 and the other with a variant bearing the combination of D67N and K70R at week 60. Genotypic analyses of the baseline isolates of the subjects with the M184V and K65R-expressing viruses indicated that resistance emerged by weeks 12 and 16 of the study, respectively. No genotypic analysis of the baseline isolate of the subject with the D67N plus K70R-expressing virus—or that of an isolate from the infected index partner—was conducted, and it is unclear if the resistant virus was transmitted or emergent.

The TDF2 study was conducted by the U.S. CDC to evaluate the safety and efficacy of once daily oral TRUVADA[®] for HIV-1 PrEP in heterosexual men and women in Botswana. (b) (4)

One subject enrolled into the FTC/TDF arm of the trial was acutely infected at enrollment and was not diagnosed as infected until 7 months later. Retrospective RT-PCR analysis verified that the subject had been infected at baseline, and genotypic analysis of the baseline, month 1, and month 7 isolates indicated that the subject's virus developed a M184V resistance-associated substitution by month 1 and that K65R and A62V resistance-associated substitutions were also present on the same genome by month 7.

The results of the genotypic analyses are consistent with the pharmacokinetic findings of no measurable drug among most subjects who failed chemoprophylaxis. However, the daily administration of FTC/TDF is not expected to fully suppress HIV-1 replication in an established infection. As a result, the selection of resistant virus in HIV-1 infected subjects who use FTC/TDF is anticipated. Interestingly, the selection of resistant variants in the iPrEx and Partner's PrEP trials was limited to those subjects who were seronegative and presumed HIV-1-negative at baseline and received drug. Collectively, 55% (6/11) of subjects who were acutely infected at baseline and received active drug had detectable levels of resistant virus at the time of seroconversion. The absence of detectable resistant variants among subjects who were not infected at baseline and failed prophylaxis may be attributable to lack of adherence and frequent testing for seroconversion. Surprisingly, there are no data that indicate the presence of subjects who were acutely infected at baseline who were within the window period of the RT-PCR assay. These subjects would be expected to have been HIV-1 RNA negative in their baseline sample, seroconverted early during the trial, and harbor resistant virus. Possible reasons for the absence of these subjects include inadequate trial sizes for detecting these rare events, clearance of virus due to post-exposure prophylactic (PEP) activity, or suppression of viral replication and delay of seroconversion in subjects who were adherent to the PrEP regimen. In the latter case, it is possible that FTC/TDF could significantly delay the time to viremia, which represents the earliest time that an HIV-1 RNA-detecting assay is expected to be

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able to diagnose infection. Further, it is possible that the emergence of resistant viruses from the hypothetical low-level, persistent infection could be rare given the combination of the low frequency of the resistance-conferring substitutions and a very small viral population from which it could be selected.

4.3 Analyses of the Time to Seroconversion in the iPrEx and Partner's PrEP Trials

The iPrEx and Partner's PrEP studies included retrospective analyses of HIV-1 RNA in stored samples that had been collected at pre-seroconversion timepoints. Ideally, these data would permit the determination of the number of months that passed between the time of infection, identified as an estimated time between the last HIV-1 RNA negative and first RNA positive samples, and seroconversion, identified by the first detection of anti-HIV antibodies. The results of such analyses could be useful for determining the impact of FTC/TDF PrEP on the amount of time required for seroconversion following infection and/or to inform a recommendation for HIV testing frequency for individuals using TRUVADA[®] for PrEP. However, the frequency of sampling and/or HIV-1 RNA testing on samples collected during the pre-seroconversion visits was inadequate to allow for sensitive analyses.

Among the 48 iPrEx subjects who were randomized into the FTC/TDF cohort and seroconverted during the iPrEx trial, 46 subjects had at least 1 sample collected prior to seroconversion tested for the presence of HIV-1 RNA. Of the 46 seroconverters whose earlier samples were tested for viral RNA, only 28 included samples that were confirmed HIV-1 RNA negative, thereby allowing for an estimation of the earliest time of infection. The median (range) amount of time between the collection times of the last HIV-1 RNA negative sample and seroconversion was 83.5 (range, 21-203) days. The median (range) amount of time between the latest confirmed HIV-1 RNA negative sample and seroconversion for the 37/83 placebo-treated subjects who became infected during the trial and had available data was 85 (range, 29-273) days, which was not significantly different from that of subjects who received FTC/TDF (P=0.7, Mann-Whitney Test). While these results are encouraging, it should be noted that very few subject samples were sequentially tested for viral RNA in short enough intervals (i.e., monthly) to allow an accurate determination of the time of acute infection. This infrequent testing creates a large window of uncertainty for estimating the actual time of infection and makes the analysis less sensitive to smaller shifts in time to seroconversion. However, the poor adherence observed among the subjects who became infected while receiving FTC/TDF makes the observation of any delay in time to seroconversion unlikely. Notably, the 2 subjects who were acutely infected at enrollment and administered FTC/TDF seroconverted within 34 days.

Pre-seroconversion HIV-1 RNA data for subjects who became infected during the Partner's PrEP study are limited and a comparison of seroconversion times between the treatment groups is difficult. Only 0/4, 1/11, and 6/25 the subjects from the FTC/TDF, TDF, and placebo-arms, respectively, had pre-seroconversion samples that were confirmed RNA negative. The duration of infection prior to seroconversion for the majority of the subjects therefore remains unknown. The median (range) of seroconversion times for subjects who were acutely infected and administered FTC/TDF (n=3) or placebo (n=6) were similar, at 28 (range, 22-84) days and 28 (range, 24-84) days, respectively. However, 2/5 subjects who were acutely infected at enrollment and received TDF showed longer times to seroconversion (112 and 420 days) than the remaining three subjects of that group (28, 28, and 84 days). It is unclear if the delayed times to seroconversion are attributable to TDF or to other viral or host factors.

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5 Conclusions

This supplemental NDA is approvable from a Clinical Virology perspective for the pre-exposure chemoprophylaxis (PrEP) of HIV-1–uninfected men and women who are at high risk of sexually transmitted HIV-1 infection.

6 Package Insert

This section includes the virology relevant changes that have been proposed to the TRUVADA® label by the sponsor as well and the proposed edits and additions of the FDA Clinical Virology reviewer. The label's current text is presented in black text, the sponsor's proposed changes in red text, and this reviewer's edits in blue text.

(b) (4)

4 Page(s) of Draft Labeling have been Withheld in Full as b4 (CCI/TS) immediately following this page

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7 Recommendations

1. Conduct analyses to assess the impact of TRUVADA[®] PrEP failures on the durability of subsequent antiretroviral treatment with regimens containing emtricitabine and/or tenofovir disoproxil fumarate. The study should include subjects who become infected while using TRUVADA[®] for PrEP and lack evidence of resistance by standard genotypic or phenotypic techniques at the time of HIV-1 diagnosis.
2. Conduct a study to assess the impact of TRUVADA[®] PrEP on the time to seroconversion. This commitment may be fulfilled by completing the HIV-1 RNA analysis of stored pre-seroconversion samples of Partner's PrEP participants. The time from HIV-1 infection to seroconversion should be determined for each subject and the median differences between subjects of each treatment group compared. The time of HIV-1 infection should be estimated from the times of the last viral negative and first viral RNA positive pre-seroconversion samples.
3. Periodically conduct and report surveillance analyses to determine if there are changes in the incidence of transmitted FTC and/or TDF resistant viruses among prophylaxis- and treatment-naïve individuals who become infected in communities where TRUVADA[®] for PrEP is used.

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4. Conduct a study to compare the ability of different CDC-recommended acute HIV-1 screening algorithms to minimize initiation of TRUVADA[®] PrEP in infected individuals.

Appears this way on the original

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8 Appendices

Appendix A. Clinical Virology-Relevant Communications with Sponsor during NDA Review

The DAVP comments are presented in bold and the sponsor's responses are italicized.

Response to FDA Information Request of January 20, 2012 (SDN 710; eCTD 0396)

11. Please submit a line-item virology dataset that consolidates pharmacokinetic and virologic data. Specifically, the file should allow for the comparison of drug concentrations, virus titers, and genotypic data of samples collected at, or near, the same time point(s). We request that the file include the following data:

- **Subject ID**
- **Trial (i.e., iPrEx or Partners PrEP)**
- **Cohort**
- **Time of seroconversion (Days from Baseline)**
- **HIV-1 subtype**
- **Time of PK Sample Collection (Days from Baseline)**
- **TFV concentration**
- **TFV-DP concentration**
- **FTC concentration**
- **FTC-TP concentration**
- **Time of virology sample collection (Days from Baseline)**
- **HIV-1 RNA load**
- **Genotypic data (complete HIV-1 RT amino acid sequence: if deep sequencing was used, provide a consensus amino acid sequence, identify variants that occurred in more than 1 individual and their percent within an individual's population); blank cells should be used for positions matching reference RT sequence (see guidance on submission of HIV-1 resistance data). Provide a separate dataset for allele-specific RT-PCR.**
- **Genotypic data of the Index Subject if resistance-associated substitutions are identified in the Partner Subject (if available from Partners PrEP). Include an identifier for matching with the appropriate partner and a column with "Y" or "N" as to whether a phylogenetic comparison of the index and partner viruses indicates that the partner was the probable source of the subject's infection.**

Multiple rows may be included for the same subject if samples collected at multiple time points were evaluated. Also, please identify the assays used for RNA load determination and genotypic data analysis (e.g., allele-specific, ultra-deep, or population-based nucleotide sequencing assay).

Virology datasets consolidating pharmacokinetic and virology data are being provided for CO-US-104-0288 (DERRESI) and CO-US-104-0380 (ADVIRO).

For Study CO-104-0288, the dataset provided is relevant to the iPrEx study design in that variable site name rather than cohort is included. The DERPCR is the dataset providing allele specific RT-PCR. Please note that DRUGRESU raw dataset (updated version of the DRUGRESI dataset) is used as the source for the DERPCR dataset. Prior analysis (those

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submitted in the original application) of the RT-PCR data was based on the DRUGRESI dataset which has been included as a raw dataset for reference. In the original application an Exceldata.pdf file was provided to document the source of DRUGRESI dataset because at that time, this dataset was transferred to Gilead via an Excel file. Since that time, the study team has incorporated this information into the database, making the reference to this dataset in the Exceldata.pdf file no longer relevant.

For Study CO-US-104-0380, the dataset (ADVIRO) provided is relevant to the study design (ie seroconverters, as virology data are only available on seroconverters). Of note, only tenofovir plasma concentrations were analyzed for this study and therefore, the dataset does not include the TFV-DP, FTC or FTC-DP concentration data. In addition, neither resistance testing in the index subject nor phylogenetic comparison of index partner virus has been conducted. RNA load determination was done using the Abbott Real-Time HIV-1 RNA assay, performed at the [REDACTED] (b) (4). All genotypic data are by population-based nucleotide sequencing only, as detailed in response to comment 12, Section 1.12. A separate dataset for allele specific RT-PCR is not being provided, as these analyses were not conducted for Study CO-US-104-0380. Additionally, neither resistance testing in the index subject nor phylogenetic comparison of index and partner virus has been conducted for Study CO-US-104-0380. A Resistance Interpretation Guidance document included in Module 5.3.5.1 indicates how mutations were interpreted for reporting.

COMMENT: **Not to be communicated.** Response noted.

12. Please provide study reports for the phenotypic and genotypic studies that were conducted for each trial, including detailed methodologies and a description of the performance parameters of assays that have not been approved. The assay descriptions should include primer and probe sequences (when applicable), a description of the sensitivity limits for minority populations, and—in the case of allele-specific RT-PCR—the detection limits for each of the degenerate bases within a codon for each resistance-associated substitution that was evaluated).

Virology study reports are not available for Studies CO-US-104-0288 and CO-US-104-0380; however, detailed information regarding methodologies used and performance parameters of the assays are included in Module 5.3.5.4.

For Study CO-US-104-0288, the FDA-approved TruGene assay was utilized for the standard sequencing and an in-house assay for allele-specific PCR of specific mutations relevant to TDF and FTC was utilized for added sensitivity as described in the Virology Method Description included in Module 5.3.5.4. The sensitivity limits for the allele-specific PCR varied from 0.1 to 0.6% depending on the specific mutations analyzed.

For Study CO-US-104-0380, a standard algorithm for genotypic testing was applied as described below (specific details are described in the Virology Methods Summary included in Module 5.3.5.4). All of the genotyping procedures for Study CO-US-104-0380 provided sequencing results from population-based analyses and hence have similar sensitivity cut-offs as described for the ViroSeq HIV-1 assay.

- a. *Each specimen was first assayed using the FDA-approved ViroSeq HIV-1 Genotyping System (Abbott), using methods provided by the package insert and*

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operating manual. All steps of the ViroSeq kit were followed to generate amplicons. If successful, the amplicons were sequenced using the primers provided in the ViroSeq kit.

- b. In instances where the ViroSeq PCR product was insufficient for sequencing based on criteria in ViroSeq kit, an additional nested PCR using “non-subtype B” primers from the Frenkel Lab CLIA-certified “in house” assay was performed, and the amplicon was sequenced with the “in house” primers.*
- c. Specimens that did not amplify with ViroSeq primers were processed using the Frenkel Lab CLIA-certified “in house” assay, using “non-subtype B” primers; see attached protocol.*
- d. For three specimens that did not amplify by Frenkel Lab “in house” assay, additional testing was attempted in the [REDACTED] (b) (4) using the CAP-certified protocol with “master-mix 1;” see attached protocol.*

COMMENT: **Not to be communicated.** Response noted.

13. Please conduct an expanded HIV-1 resistance analysis for subjects who failed prophylaxis and had detectable drug levels or who were missing those pharmacokinetic data. The analysis should include a genotypic characterization of reverse transcriptase using an assay that is sensitive to minority species (e.g., 454 sequencing) and a phenotypic characterization for emtricitabine and tenofovir susceptibility if no known resistance-associated substitutions are identified.

The extent of the information that the CO-US-104-0288 and CO-US-104-0380 study teams have available with regard to the HIV-1 resistance analysis has been submitted to this application to date and is presented in the enclosed virology datasets. Neither team anticipates being able to conduct phenotypic analyses.

COMMENT: **Not to be communicated.** Response noted.

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Information Amendment submitted on February 22, 2012 (SDN 711; eCTD 0397)

During the process of responding to the Agency's request for biostatistics and clinical virology information of 20 January 2012, the Partners PrEP Study Team have identified a few errors in the safety, laboratory and resistance tables of the CSR which we wish to bring to the FDA review team's attention. Gilead and the Partners PrEP team have discussed the impact of the errors and agree that impact on the CSR is minimal and that there are no changes in the interpretation of the data.

CSR Table 11-14. Primary Study Resistance Mutations

In the original table, two errors were included: one seroconverter in the placebo arm was reported as having the non-primary mutation T215C and one seroconverter in the TDF arm was reported as having the non-primary mutation K65N. The cause of the errors in the original table was that the sequences were reported in error. In the course of organizing the resistance data per FDA guidelines, the testing laboratory reviewed all reports of resistance and noted the discrepancy between the sequence file and the results reported, for these two cases. After reviewing the sequence data, the testing laboratory reported that the mutation pattern was misread and reported in error. Corrected source documents have been issued by the testing laboratory. Additional mutations, some causing low-level resistance and most unrelated to the study products, were present in the dataset.

COMMENT: **Not to be communicated.** Submission noted.

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Response to FDA Information Request of March 29, 2012 (SDN 725; eCTD [0407](#))

- 1. Please verify that the pharmacokinetic (i.e., PKDT) and RNA sampling dates of the iPrEx datasets (e.g., DERRESI) are equivalent. If not, please provide the date/DAY of sample collection for both the PK and RNA assays.**

The DERRESI dataset previously submitted in Sequence No. 0396 dated 12 February 2012 included data from subjects in the mITT analysis set (excluded baseline seropositive subjects). The pharmacokinetic (ie PKDT) and RNA sampling dates of the iPrEx datasets (eg DERRESI) are not equivalent for all subjects due to the manner by which the DERRESI dataset was generated. The DERRESI dataset was generated by merging multiple analysis datasets and specifying visit windows with a 90-day span. Subjects could have had multiple plasma PK and/or PBMC samples, which would potentially result in multiple records in the DERRESI dataset. As such, Table 1 includes those subjects with a difference between the PK sampling date and RNA sampling date (both non-missing).

The DERRESI dataset includes subjects in the mITT dataset. Therefore, the 10 subjects considered seropositive at baseline are not included in the DERRESI dataset, but are listed by subject ID (ptid) in Table 2.

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Table 1. Study CO-US-104-0288: Date/Day of Sample Collection for PK and RNA Assay for Subjects where Sample Dates are Not Equivalent

Participant ID	PK Specimen Date	PK Specimen Day	Subtype Specimen Date	Subtype Specimen Study Day	Genotype Resistance Date	Genotype Resistance Day
(b) (4)						

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Participant ID	PK Specimen Date	PK Specimen Day	Subtype Specimen Date	Subtype Specimen Study Day	Genotype Resistance Date	Genotype Resistance Day
(b) (4)						

Table 2. Study CO-US-104-0288: Subjects Seropositive at Baseline

Subject ID
(b) (4)

COMMENT: **Not to be communicated.** Response noted. Data included in the Clinical Virology review.

- Please verify that a value of “40” copies/mL in the “OTHERCOP” column of the DERRESI dataset indicates a negative result (i.e., below the lower limit of quantification and with no HIV-1 RNA detected by RT-PCR).**

Gilead has verified with the iPrEx study team that a value of 40 copies/mL in the “OTHERCOP” column of the DERRESI dataset indicates a negative result.

COMMENT: **Not to be communicated.** Response noted.

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- 3. Please include data from the 10 subjects (2 FTC/TDF and 8 Placebo) who were seronegative at enrollment but subsequently determined to be infected at baseline by RT-PCR in the DERRESI file.**

Gilead confirms that the DERRESI dataset previously submitted in Sequence No. 0396 dated 12 February 2012 included data from subjects in the mITT analysis set (excluded baseline seropositive subjects). Drug level testing was conducted for the 2 subjects on the active arm (subject IDs: 9022624, 9255180) and 2 subjects on the placebo arm (subject IDs: 8730695, 8730824) out of the 10 subjects (2 FTC/TDF and 8 placebo) who were seronegative at enrollment, but subsequently determined to be infected at baseline by RT-PCR.

Per the protocol for Study CO-US-104-0288, genotyping was not conducted for any of the 10 subjects (2 FTC/TDF and 8 placebo) who were seronegative at enrollment, but subsequently determined to be infected at baseline by RT-PCR.

As such, a replacement DERRESI dataset containing all available data (including the newly added drug level testing data for subjects 9022624, 9255180, 8730695, 8730824) is included in this submission.

COMMENT: **Not to be communicated.** Response noted. A working copy of this dataset was labeled "DERRESI 2." Another updated dataset was submitted on April 20, 2012 (eCTD [0412](#)) and a working copy was labeled "DERRESI 3."

- 4. It is unclear if the time to infection (TTINF in the DERRESI dataset) of the iPrEx trial was defined by the earliest detected HIV-1 RNA or by the time of seroconversion. Please provide the dates of seroconversion (e.g., concordant rapid tests or discordant rapid tests with confirmatory EIA, WB, or RT-PCR) if different from those associated with earliest detectable HIV-1 RNA.**

The time to infection (ITINF in the DERRESI dataset) in Study CO-US-104-0288 was defined by the earliest available HIV-1 RNA (Attachment 3 of the iPrEx Protocol). Included in Attachment 2 is a complete a list of all 147 infections (10 at baseline, 131 modified ITT and 6 post-stop) that occurred in Study CO-US-104-0288 through 21 November 2010. The seroconversions described in Attachment 2 are listed by subject ID (ptid) and include the date of first positive RNA, date of first positive rapid (concordant or discordant) and an indicator (0=No, 1=Yes) of when the dates differ.

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Attachment 2. Study CO-US-104-0288: Seroconversions through 21 November 2010

ptid	date positive rna	date positive rapids	dates differ (0=No, 1=Yes)
(b) (4)			

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ptid	date positive rna	date positive rapids	dates differ (0=No, 1=Yes)
(b) (4)			

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ptid	date positive rna	date positive rapids	dates differ (0=No, 1=Yes)
(b) (4)			

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ptid	date positive rna	date positive rapids	dates differ (0=No, 1=Yes)
(b) (4)			

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ptid	date positive rna	date positive rapids	dates differ (0=No, 1=Yes)
(b) (4)			

COMMENT: **Not to be communicated.** Response noted. These data were used to differentiate between time to infection (TTIFN) and seroconversion (SC) when summarizing results for the iPrEx trial.

5. In Supplementary Figure 4 of Grant et al., 2010 (N Engl J Med. Dec 30; 363(27) 2587-99), the authors indicate that plasma RNA levels at the seroconversion visit were comparable between subjects treated with FTC/TDF or placebo. Please provide those data and a description of the analysis.

The following response is confined to the data on which the NEJM manuscript was based.

The analysis included all 110 HIV-infected participants determined by positive antibody test results through 01 May 2010 (100 mITT seroconverters + 10 infected at enrollment).

The iPrEx study team reviewed all HIV-RNA values available at the time of positive antibody test results + 14 days. The logarithm base 10 of these values was recorded. If there was more than 1 value within 14 days, these values were averaged within participants. These (possibly averaged values) were then compared between the treatment arms by a t-test as shown in the raw Stata output below. The two-sided p-value is 0.7184.

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```
. ttest hiv_rna if coded_week==0, by(rx)

Two-sample t test with equal variances
```

Group	Obs	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
FTC/TDF	38	5.15463	.135092	.8327628	4.880908	5.428352
Placebo	72	5.097625	.0896945	.7610831	4.91878	5.276471
combined	110	5.117318	.0746784	.7832341	4.969308	5.265328
diff		.0570048	.1576771		-.2555386	.3695481

```

diff = mean(FTC/TDF) - mean(Placebo)          t = 0.3615
Ho: diff = 0                                degrees of freedom = 108

Ha: diff < 0                                Ha: diff != 0                                Ha: diff > 0
Pr(T < t) = 0.6408                          Pr(|T| > |t|) = 0.7184                          Pr(T > t) = 0.3592

```

Attachment 3 provides the data listings of the plasma RNA levels for HIV infected subjects (100 MITT seroconverters + 10 infected at enrollment) with positive antibody tests through 1 May 2010.

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Attachment 3. Study CO-US-104-0288: Plasma RNA Levels for HIV Infected Subjects with Positive Antibody Tests Through 1 May 2010 (100 MITT seroconverters + 10 infected at enrollment)

ptid ¹	Hivrnaavg ²	rnadate1 ³	hivrna1 ⁴	rnadate2 ⁵	hivrna2 ⁶	dateposab ⁷	posbase ⁸	rx ⁹
(b) (4)								

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ptid ¹	Hivrnaavg ²	rnadatel ³	hivrnal ⁴	rnadate2 ⁵	hivrna2 ⁶	dateposab ⁷	posbase ⁸	rx ⁹
(b) (4)								

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ptid ¹	Hivrnaavg ²	rnadate1 ³	hivrnal ⁴	rnadate2 ⁵	hivrna2 ⁶	dateposab ⁷	posbase ⁸	rx ⁹
(b) (4)								

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ptid ¹	Hivrnaavg ²	rnadate1 ³	hivrna1 ⁴	rnadate2 ⁵	hivrna2 ⁶	dateposab ⁷	posbase ⁸	rx ⁹
(b) (4)								

- 1 ptid = participant ID
- 2 rx = treatment group
- 3 dateposab = date of positive antibody test
- 4 posbase = 1=Participant was infected at enrollment 2=Participant not infected at enrollment
- 5 rnadate1 = date of HIV-RNA closest to infection
- 6 hivrna1 = HIV-RNA (logbase10) on rnadate1
- 7 rnadate2 = date of HIV-RNA after infection but less than or equal to 14 days after (if available)
- 8 hivrna2 = HIV-RNA (logbase10) on rnadate2
- 9 hivrnaavg = average of hivrna1 and hivrna2 if both available, otherwise hivrna1 -- this was the variable compared between groups using the t-test.

COMMENT: **Not to be communicated.** Response noted. The DAVP conducted a similar analysis of HIV-1 RNA loads at or near the time of seroconversion. However, the FDA analysis only included the viral load datum that was closest to the seroconversion visit (i.e., only one viral titer was included from each subject).

6. According to the SAS dataset BASICS, using a stop date of July 31, 2010, there are 78 infections in the placebo group out of 1218 eligible subjects and 45 infections in the FTC/TDF group out of 1224 eligible subjects. Please concur that these are the numbers you will be using in your Advisory Committee presentation and in the label. Otherwise, provide an explicit algorithm in English, not in SAS code, for obtaining different results using the dataset BASICS.

Of the 147 subjects with HIV-infection and the first positive antibody tests prior to 21 November 2010 (iPrEx results were announced on 23 November 2010): 10 were infected at enrollment, 6 post-stop infections and the remaining 131 are considered to be the modified intention to treat (MITT) seroconverters.

The stop date is not 31 July 2010; instead, 31 July 2010 is the date when the last bottle of study drug was dispensed to any subject. Therefore, last dose of study drug is considered to be the first visit after 31 July 2012 within each individual subject (visits were scheduled every 4 weeks). End of study treatment was the next visit after the last dose of study drug (as defined earlier).

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Therefore, 83 subjects on placebo and 48 subjects on FTC/TDF in the mITT have been reported as seroconverted subjects as described in Table 4-1 of the CO-US-104-0288 Addendum Report.

The 6 subjects who seroconverted between end of treatment and 21 Nov 2010 are listed in Table 3.

Table 3. Study CO-US-104-0288: Infections after the End of Study Treatment

Date of Positive Test Result	Subject ID
03-Sep-2010	9022887
10-Sep-2010	9111734
29-Oct-2010	9117924
19-Oct-2010	9150645
03-Sep-2010	9635309
16-Sep-2010	9736067

Gilead plans to use the 01 May 2010 data cut as the primary analysis in the US labeling for Truvada as this was the first formal unblinded analysis of the data, after achieving the pre-specified number of events, and it is the data that was published in Grant et al., 2010 (N Engl J Med. Dec 30; 363(27) 2587-99).

The iPrEx study team also plans to present data up to the 21 Nov 2010 in the Advisory Committee presentation and Gilead has included this information in the Advisory Committee Briefing Document submitted to NDA 21-752 in Sequence No. 0409 dated 10 April 2012.

COMMENT: **Not to be communicated.** This response to a Biostatistics comment has been noted.

- 8. Please provide a subject narrative for the subject in Study CDC TDF2 who was found to have high levels of K65R, M184V, and A62V reverse transcriptase resistance mutations in the FTC/TDF group. Indicate treatment course, including dates of study drug initiation, study drug discontinuation, HIV seroconversion, and confirmation testing of HIV infection.**

The following description contains details regarding the subject in Study CDC TDF2 who was found to have high levels of K65R, M184V, and A62V reverse transcriptase resistance mutations in the FTC/TDF group.

This 25-year old male study participant with HIV infection was enrolled in Study CO-US-104-0294, "Prevention of HIV infection in heterosexually active young adults in Botswana" and commenced blinded Truvada (emtricitabine/tenofovir DF) or placebo on 15 October 2008. Study medication was discontinued on 18 May 2009 when he was found to be HIV positive. Subsequent testing, as per the protocol's retrospective sampling algorithm, revealed that the subject had entered the study with unrecognized, acute, wild-type HIV-1 infection at enrollment. Within one month, the subject developed majority-level M184V. By the time the

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infection was recognized at month 7, K65R and A62V were also present on the same genome. The subject responded to subsequently initiated protease inhibitor-based therapy.

COMMENT: Not to be communicated. This response to a Clinical comment has been noted.

Draft of Sponsor Presentation for the May 10, 2012 Advisory Committee Meeting (submitted on April 20, 2012; SDN 732; eCTD [0413](#))

Response to FDA Information Request of April 13, 2012 (SDN 733; eCTD [0414](#))

- 1. Please verify that the RT-PCR assays that were used to detect and quantify plasma HIV-1 RNA (i.e., the Abbott RealTime HIV-1 or the Roche Amplicor HIV-1 Monitor tests for the iPrEx trial and the Abbott RealTime HIV-1 for Partner's PrEP) were also used to detect HIV-1 RNA in baseline samples of early seroconverters. If these assays were not used, please identify the assay(s) and provide a detailed description of its methodology and performance parameters if that information has not been submitted.**

In the Partners PrEP Study (CO-US-104-0380) plasma HIV-1 RNA concentrations from samples were collected at enrolment and quantified in batch testing at the [REDACTED] (b) (4) [REDACTED] using the Abbott Real-Time HIV-1 RNA assay (Abbott); the limit of quantification was 80 copies/mL.

In the iPrEx Study (CO-US-104-0288) the Abbott Real Time HIV-1 assay was used for the enrollment viral load reports for the early seroconverters ("infected at baseline").

COMMENT: Not to be communicated. Response noted. The higher LLOQ reported for the assay by the Partner's PrEP study team could be related to the use of lower volumes of plasma from banked enrollment samples relative to those used during the treatment phase of the trial.

- 2. Please identify the HIV-1 strain used as the reverse transcriptase amino acid reference for the genotypic data from the iPrEx trial as presented in the "DERRESI" dataset.**

The HIV-1 LAV-1 genome served as the reference strain (GenBank # K02013) using TRUGENE (Siemens) for the analysis for the genotypic data presented in the DERRESI dataset.

COMMENT: Not to be communicated. Response noted. The RT amino acid sequence of HIV-1 LAV-1 appears to be identical to that of HXB2 across the RT region included in the genotypic analyses.

- 3. We note that the resistance changes reflected in Amendment 1 of the Clinical Study Report of CO-US-104-0380, dated 20 February 2012, were not included in the preliminary manuscript submitted on 21 March 2012. Please verify that the authors intend to remove the identification of [REDACTED] (b) (4) [REDACTED] as a TDF resistance-associated substitution from the manuscript prior to submission for publication.**

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The Partners PrEP Study team confirms the removal of the identification of (b) (4) as a TDF resistance-associated substitution from the manuscript prior to publication.

COMMENT: Not to be communicated. Response noted.

4. There were long delays between the detection of seroconversion by rapid anti-HIV-1 antibody tests and the detection of viral RNA in some subjects of Study CO-US-104-0380 (i.e., Subjects 5044313 and 5657618 had 420 and 254 days, respectively, between the time of seroconversion and the earliest time of RNA detection). Notably, these two subjects had quantifiable levels of drug at multiple time points prior to seroconversion, indicating at least intermittent compliance. This observation raises concerns that partial suppression of viral replication could delay the induction and/or maturation of antiviral antibodies to levels necessary for detection by rapid antibody tests. Please provide alternative explanations for these results (e.g., reduced sensitivity of the diagnostic test for specific subtypes of virus, test defect, misuse, or misinterpretation, etc.) along with supporting data, if available.

The question of whether the use of mono, or dual-antiretroviral medications as HIV-1 prophylaxis results in partial viral suppression and delayed development of antiviral antibodies in individuals who have breakthrough infection is of importance. Data from two subpopulations of seroconverters in the Study CO-US-104-0380 can be interrogated to explore this question. Of the two subjects in this query, one belongs to the group who were found to have been infected at time of enrollment and the other subject was infected during study.

First, for the 14 individuals who were retrospectively found to be already HIV-1 infected at the time of randomization (i.e., who had seronegative acute infection on the day of enrollment), the time to seroconversion is provided in Table 1.

Table 1. Month of Seroconversion of Subjects HIV-1 Infected at Baseline

Randomization Arm	Study Month of Seroconversion
TDF	1, 1, 3, 4, 15
FTC/TDF	1,1,3
Placebo	1, 1, 1, 1, 2, 3

The seroconversion of 13 out of the 14 subjects occurred within a few months of enrollment, regardless of randomization arm. One subject (5044313, randomized to the TDF arm) had prolonged time to seroconversion (15 months). However, the other seven subjects randomized to the active study arms (TDF and FTC/TDF) had comparable times to seroconversion to subjects randomized to placebo. Innate reasons in this individual leading to delayed development of antibody response or reduced sensitivity of the diagnostic test for the specific virus infecting this subject are possible reasons for delayed development of a positive antibody test, but data are not available to support such hypotheses at this time.

There is no evidence of test defect, misuse, or misinterpretation; this subject would have been tested by different individuals between enrollment and study month 15 and the study site participated in ongoing quality assurance procedures for HIV-1 rapid testing.

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Eighty-two subjects seroconverted after the time of study enrollment. HIV-1 RNA was detected in samples collected prior to seroconversion (development of antiviral antibodies) in 37/82 (45%) subjects and was not associated with the treatment assignment Table 2.

Table 2. Proportion of Subjects with HIV-1 RNA Detection Prior to Seroconversion

Randomization Arm	Total Number of Seroconversions	HIV-1 RNA Detected prior to Seroconversion
TDF	17	11 (65%)
FTC/TDF	13	4 (31%)
Placebo	52	22 (42%)

For those subjects with HIV-1 RNA detected prior to seroconversion, the time from the first visit at which RNA was detected to the visit at which seroconversion occurred ranged from 18 days to 254 days, and subjects with more than 60 days occurred in all three study arms as summarized in Table 3.

Table 3. Days from First Detection of HIV-1 RNA to Seroconversion

Randomization Arm	Days to Seroconversion
TDF	28, 28, 32, 42, 53, 56, 56, 56, 115, 140, 254
FTC/TDF	19, 28, 33, 99
Placebo	18, 21, 25, 28, 28, 28, 28, 29, 31, 50, 53, 56, 56, 56, 56, 57, 80, 84, 84, 84, 84, 112

Subject 5657618 had the longest time span, 254 days, from first detection of HIV-1 RNA to seroconversion and was in the TDF treatment group. Excluding this case, the range of durations from first detection of HIV-1 RNA to seroconversion is broadly comparable across the three study arms. Even when restricting to cases in which quantifiable levels of study drug were detected, the range of times is still comparable to that seen for subjects on placebo (when excluding subject 5657618) as shown in Table 4.

Table 4. Days from First Detection of HIV-1 RNA to Seroconversion for Subjects with Detectable Tenofovir Drug Levels

Randomization Arm	Days to Seroconversion
TDF	53, 56, 56, 140, 254
FTC/TDF	19

Specifically, subject 5657618 appears to have notable viral control with a single viral load measurement of 346 copies/mL several visits prior to seroconversion, multiple measurements below the limit of quantification (including at the visit at which seroconversion was detected), and a viral load of just 176 copies/mL at a visit 3 months after seroconversion, when study when study medication had already been withdrawn (this last measurement occurred after 10 July 2011, the cut-off for data reported in this Clinical Study

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Report and is only recently available). The reason for this degree of viral control is at this time unknown, but low viremia would be a reasonable explanation for prolonged time to development of an antibody response – and innate or viral factors appear to be at least somewhat contributory, given continued viral control after study medication was discontinued.

In addition, it is important to consider the time to seroconversion for subjects who did not have HIV-1 RNA detected at a visit prior to the seroconversion visit. For these subjects, there is again a wide spread of time to seroconversion, regardless of study arm – some of the longer times reflect repeatedly missed visits (Table 5).

Table 5. Days to Seroconversion from the Last Seronegative Visit for Subjects with no HIV-1 RNA Detected Prior to Seroconversion

Randomization Arm	Days to Seroconversion from the last Seronegative visit
TDF	23, 32, 84, 86, 86, 193
FTC/TDF	29, 83, 84, 87, 167, 223, 526, 548, 646
Placebo	23, 27, 28, 28, 28, 42, 51, 55, 56, 56, 56, 56, 56, 56, 57, 57, 58, 70, 79, 83, 84, 84, 84, 84, 84, 85, 87, 89, 168

Restricting the above list to those subjects from the active arms for whom quantifiable levels of study drug were detected, the range of times is still comparable to that seen for subjects on placebo, as shown in Table 6 below:

Table 6. Days to Seroconversion from the Last Seronegative Visit of Subjects with no HIV-1 RNA Detected Prior to Seroconversion and Quantifiable Tenofovir Drug Levels

Randomization Arm	Days to Seroconversion from the last Seronegative visit
TDF	32, 84
FTC/TDF	29, 167

Thus, overall, while two subjects with prolonged time to seroconversion are notable, it does not appear that prolonged seroconversion is common among individuals with breakthrough infection, including when restricted to those with documented use of study medication around the time of HIV-1 acquisition. The two cases are from the TDF arm. Similar prolonged seroconversion was not seen in the FTC/TDF arm, suggesting potentially that the two cases in question may have additional features that make them unusual, since viral suppression and prolonged time to antibody response might be theoretically more likely to occur when two medications are used for prophylaxis compared to one.

In summary, prolonged seroconversion was not commonly seen in this study among individuals with breakthrough infection in spite of ongoing exposure to the study medication.

COMMENT: **Not to be communicated.** Response noted. The data used in the sponsor’s analyses were included in the supplemental NDA submission. Although the sponsor identified the earliest tested HIV-1 RNA positive samples relative to the time to seroconversion, the retrospective testing of samples was not continued

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until the last HIV-1 RNA negative sample was identified and the seronegative/RNA positive period of the infection remains undetermined.

- 5. Please identify the rapid anti-HIV-1 rapid antibody and EIA tests that were used in each country that participated in Study CO-US-104-0380.**

The different rapid antibody and EIA tests used by country are listed in Table 7 and Table 8.

Table 7. Study Site Laboratory Assays in Kenya

	Eldoret	Kisumu	Nairobi	Thika
HIV-1 rapids	Determine HIV 1/2 (Abbott/Inverness Medical)			
	Unigold (Trinity Biotech)		Bioline (Standard Diagnostics)	
HIV-1 enzyme immunoassays	Vironostika HIV Ag/Ab 4 th gen (bioMe'rieux)			
	Murex HIV Ag/AB Combo 4th gen (Abbott Murex)			

Table 8. Study Site Laboratory Assays in Uganda

	Jinja	Kampala	Kabwohe	Mbale	Tororo
HIV-1 rapids	Determine HIV 1/2 (Abbott/Inverness Medical)				
	Unigold (Trinity Biotech)		HIV 1/2 STAT-PAK (Chembio Diagnostic Systems)		
HIV-1 enzyme immunoassays ¹	Vironostika HIV Ag/Ab 4th gen (bioMe'rieux)		Vironostika HIV Uni-Form II plus O – 3rd gen (bioMe'rieux)		
	BioRad HIV 1/2 (Bio-Rad Laboratories)			Murex HIV 1.2.0 AB 3rd gen (Abbott Murex)	

¹ Jinja & Kampala used Vironostika 3rd gen until March 2010.

COMMENT: Not to be communicated. Response noted.

- 6. There appears to have been a high degree of discordance in the HIV-1 subtyping assay used in CO-US-104-0380, including different subtype determinations within a single sample when analyzed by HIV-1 protease or reverse transcriptase sequence and, less frequently, when the analysis was conducted between samples collected from the same subject at different time points. Please describe the subtyping assay that was used and comment on the reliability of the technique. If the results are considered reliable, please speculate on possible mechanisms (e.g., recombinant strains, superinfection) and summarize other available data that may help explain the results.**

HIV-1 subtypes were determined from consensus sequences generated to assess antiretroviral resistance. The sequences derived were submitted to the Stanford University HIV DRUG RESISTANCE DATABASE, Genotypic resistance Interpretation Algorithm (<http://hivdb.stanford.edu>). For reference the Laboratory Methods to Genotype HIV-1 pol Partners PrEP Study in Module 5.3.5.4 was submitted on 10 February 2012 (Seq. No. 0396) (please see page 18 of Human Immunodeficiency Virus (HIV) Genotypic Resistance Assay and page 12 of Human Immunodeficiency Virus (HIV) Genotypic Resistance Assay from Plasma).

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Two considerations are important in interpreting the subtype information.

First, a number of subtypes circulate in East Africa, including A and D (the predominant subtypes), and C, as well as a few less frequent subtypes. These have established circulating recombinant forms (CRFs). As a result, recombinant strains are common in this study, in which all participants were from East Africa.

Due to a population mobility and circulation of multiple subtypes in the community, recombinant forms are becoming more common. From a different cohort of HIV-1 seroconverters from East Africa, the frequency of subtype disagreement between the env and gag genes was recently described (Campbell, M., et al. HIV-1 Subtype C Is Not Associated with Higher Viremia during Early Infection Compared to Other Subtypes in an African Cohort [Paper #549]. Presented at: 19th Conference on Retroviruses and Opportunistic Infections (CROI). 2012. Seattle, WA).

		Gag subtype			
		A	C	D	Other
Env subtype	A	58	0	2	8
	C	2	1	1	3
	D	3	0	11	7
	Other	0	0	0	3

These data demonstrate that a substantial fraction (26/99) of individuals with recent HIV-1 infection had different subtypes for different parts of the viral genome. Among the Partners PrEP Study subjects, infection with a recombinant strain likely caused the subtype discrepancies noted in this question. Dual or super-infection (and the potential for de novo recombination within the study subjects) is a possibility as well, although likely more rare.

Second, because the genetic variability of pol, especially encoding protease, is relatively low, subtyping of this gene is less precise compared to other HIV-1 genes (e.g., env). Thus, subtyping results may also be less definitive for pol.

COMMENT: Not to be communicated. Response noted.

Response to FDA Clinical Information Request of April 25, 2012 (SDN 735; eCTD [0418](#))

1. For each trial, iPrEx and Partners PrEP, please conduct an analysis of sexually transmitted infection (STI) rates over time that includes:
 - RPR positive and confirmed syphilis rates at baseline and over time. The analysis of post-baseline syphilis infection rates should be limited to new RPR cases.
 - Gonorrhea infection rates at baseline and over time.
 - Chlamydia infection rates at baseline and over time.
 - Rates of genital ulcerative disease at baseline and over time.
 - HSV-2 infection rates at baseline and rates of new HSV-2 diagnoses over time.
 - Overall STI rates at baseline and over time. Overall STI includes any of the aforementioned individual diagnoses.

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Study CO-US-104-0288 (iPrEx)

An analysis of sexually transmitted infection (STI) rates over time based on the 01 May 2010 data cut has been previously presented by the iPrEx study team in Table S4 of the New England Journal of Medicine Supplement publication of the study results presented in the original sNDA in Table 33 of Module 2.7.4. The iPrEx study team has prepared an updated analysis of the STI rates over time based on the 21 November 2010 data cut as described in Table 1.

Table 1 Study CO-US-104-0288: Analysis of Sexually Transmitted Infection Rates over Time (21 November 2010 data cut)

	Baseline n (%)		Post-Baseline Incident n (rate per 100 PY)		Comparing Post- Baseline Incident
	Placebo	FTC/TDF	Placebo	FTC/TDF	P-value ^b
Syphilis RPR ^c	180 (14%)	180 (14%)	106 (6.4) ^a	99 (6.2) ^a	0.77
Syphilis (Confirmed)	163 (13%)	167 (13%)	79 (4.8) ^a	81 (5.0) ^a	0.80
Gonorrhea PCR ^d	5 (0.4%)	7 (0.6%)	30 (1.4)	18 (0.8)	0.09
Chlamydia PCR ^d	8 (0.6%)	8 (0.6%)	26 (1.2)	17 (0.8)	0.18
Genital Ulcer	8 (0.6%)	9 (0.7%)	48 (2.2)	55 (2.6)	0.44
HSV-2 ^e					
Negative	691 (55%)	692 (55%)			
Positive	431 (35%)	461 (37%)	117 (10.0) ^f	115 (10.3) ^f	0.84
Indeterminate	126 (10%)	97 (8%)			
Any of the above STI	198 (16%)	197 (16%)	245 (12.6)	236 (12.2)	0.70

a. Incidence among those who were negative at baseline

b. Logrank test

c. RPR = rapid plasma reagin

d. PCR = polymerase chain reaction

e. Baseline prevalence of HSV-2 was age dependent as described in Table 2

f. Incidence among those who were either negative or indeterminate at baseline

Table 2 Study CO-US-104-0288: Age Dependence of Baseline Prevalence of HSV-2

Age Category	HSV-2	Placebo	FTC/TDF
< 25 years	Negative	448 (68%)	411 (69%)
	Positive	147 (22%)	135 (23%)
	Indeterminate	67 (10%)	45 (8%)
≥ 25 years	Negative	243 (41%)	281 (43%)
	Positive	284 (48%)	326 (49%)
	Indeterminate	59 (10%)	52 (8%)

Study CO-US-104-0380 (Partners PrEP)

Laboratory screening for *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and syphilis (screening [RPR] and confirmatory testing) was done at baseline and annually, or more frequently if clinically indicated. Syndromic assessment of genital ulcer

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disease was done quarterly. Subjectss [sic] were treated for symptomatic sexually transmitted infections and asymptomatic sexually transmitted infections found as a result of scheduled screening. Post-seroconversion visits were excluded from these analyses.

In Table 3, the Partners PrEP Study team have provided prevalences of the infections described above at baseline and rates over time, except for HSV-2 infection, for which testing was done at baseline only. For “any sexually transmitted infection” at baseline and over time, the study team has provided a version both with and without including trichomonas (TV), since this infection was not specifically requested. The study team has also provided a combined any infection prevalence at baseline only which includes HSV-2 (with and without TV); for any infection analyses post-baseline HSV-2 is not included since we do not have post-baseline HSV-2 data at this time.

Table 3 Study CO-US-104-0380: Analysis of Sexually Transmitted Infection Rates over Time

STI	During months	TDF				FTC/TDF				Placebo		
		n positive	n tests	%	p-value vs placebo	n positive	n tests	%	p-value vs placebo	n positive	n tests	%
RPR reactive and confirmed	Enrollment	59	1,570	3.8	0.782	60	1,572	3.8	0.854	62	1,569	4.0
	1-12 Months	14	1,086	1.3		15	1,107	1.4		10	1,099	0.9
	13-24 Months	6	539	1.1	0.759	2	546	0.4	0.767	5	542	0.9
	25-36 Months	2	28	7.1		0	21	0.0		0	14	0.0
<i>Chlamydia trachomatis</i> (CT)	Enrollment	16	1,520	1.1	0.161	20	1,520	1.3	0.458	25	1,517	1.6
	1-12 Months	19	1,110	1.7		12	1,143	1.0		5	1,129	0.4
	13-24 Months	6	602	1.0	0.324	3	582	0.5	0.072	3	581	0.5
	25-36 Months	1	31	3.2		2	29	6.9		0	35	0.0

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STI	During months	TDF				FTC/TDF				Placebo		
		n positive	n tests	%	p-value vs placebo	n positive	n tests	%	p-value vs placebo	n positive	n tests	%
<i>Neisseria gonorrhoeae</i> (NG)	Enrollment	17	1,521	1.1	0.519	10	1,520	0.7	0.049	21	1,518	1.4
	1-12 Months	14	1,111	1.3		11	1,143	1.0		17	1,129	1.5
	13-24 Months	8	602	1.3	0.656	6	581	1.0	0.514	5	581	0.9
	25-36 Months	0	31	0.0		1	29	3.4		0	35	0.0
<i>Trichomonas vaginalis</i> (TV)	Enrollment	59	1,552	3.8	0.018	67	1,557	4.3	0.098	87	1,547	5.6
	1-12 Months	33	1,108	3.0		31	1,134	2.7		33	1,126	2.9
	13-24 Months	24	601	4.0	0.165	12	582	2.1	0.253	22	579	3.8
	25-36 Months	1	32	3.1		1	27	3.7		1	36	2.8
Genital ulcerative disease (GUD)	Enrollment	15	1,579	0.9	1.000	19	1,575	1.2	0.389	14	1,577	0.9
	1-12 Months	64	5,123	1.2		64	5,183	1.2		56	5,171	1.1
	13-24 Months	27	3,346	0.8	0.770	27	3,369	0.8	0.765	23	3,357	0.7
	25-36 Months	0	829	0.0		2	851	0.2		9	867	1.0
Any of syphilis, CT, NG, TV, GUD	Enrollment	156	1,590	9.8	0.024	168	1,583	10.6	0.133	196	1,589	12.3
	1-12 Months	148	5,158	2.9		143	5,211	2.7		130	5,205	2.5
	13-24 Months	77	3,362	2.3	0.788	59	3,387	1.7	0.762	61	3,376	1.8
	25-36 Months	4	833	0.5		6	854	0.7		10	870	1.1

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STI	During months	TDF				FTC/TDF				Placebo		
		n positive	n tests	%	p-value vs placebo	n positive	n tests	%	p-value vs placebo	n positive	n tests	%
Any of syphilis, CT, NG, GUD	Enrollment	102	1,590	6.4	0.327	108	1,583	6.8	0.628	116	1,589	7.3
	1-12 Months	117	5,157	2.3		114	5,211	2.2		98	5,205	1.9
	13-24 Months	54	3,361	1.6	0.562	49	3,387	1.4	0.313	40	3,376	1.2
	25-36 Months	3	833	0.4		5	854	0.6		9	870	1.0

At enrollment only

HSV2	Enrollment	835	1,506	55.4	0.186	814	1,507	54.0	0.034	875	1,512	57.9
Any of syphilis, CT, NG, TV, GUD, HSV2	Enrollment	897	1,590	56.4	0.091	869	1,583	54.9	0.011	944	1,589	59.4
Any of syphilis, CT, NG, GUD, HSV2	Enrollment	877	1,590	55.2	0.163	850	1,583	53.7	0.027	916	1,589	57.6

NOTE: Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis and syphilis (RPR positive and confirmed) were assessed at baseline, 12, 24, and 36 months, and between annual visits as clinically indicated.

NOTE: Syndromic diagnosis (yes/no) of GUD was asked quarterly.

NOTE: Results of confirmed syphilis after baseline is limited to tests of persons not confirmed positive at enrollment.

NOTE: P-values comparing enrollment STI prevalences by arm were generated using Fisher's exact test.

NOTE: P-values comparing follow-up STI rates by arm used all available tests after enrollment and were generated using GEE with logistic link and robust standard errors to adjust errors for correlation within responses from the same participant over time.

NOTE: HSV-2 testing was done using the HerpeSelect-2 EIA (Focus Technologies) at enrollment only; an index value of ≥ 3.5 was used to define a positive result.

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Response to FDA Information Request of April 26, 2012 (SDN 739; eCTD 0419)

- 1. Please provide a detailed discussion about the original misidentification of K65N in Subject 5241418 of the Partner's PrEP trial, including additional information on the collection dates of the samples (if different), the relative sensitivities of the assays to minority variants (if different), or other methodological changes that might have affected assay sensitivity.**

In the original clinical study report (CSR) for CO-US-104-0380 (Partners PrEP Study) submission (dated 14 November 2011), one seroconverter subject (5241418) was identified as having the non-primary mutation K65N – reported as K65KN, due to a minor secondary peak, in an incomplete and preliminary analysis that did not meet the threshold for finalization in both directions of the sequence. However, by mistake, this preliminary report was released from the testing laboratory to the Partners PrEP study team.

In the course of organizing the resistance data in February 2012 for formal submission per FDA request, two experienced technicians from the testing laboratory reviewed all chromatograms of specimens with resistance and noted a discrepancy between the sequence file and the result reported. Specifically, the re-review of the sequence data showed only K65K without evidence for the sequence encoding "N" – i.e., the final FASTA file did not include the K65N mutation. It was then determined that a preliminary report, based on incomplete data, had inadvertently not been corrected before it was issued to the Partners PrEP study team. Upon discovery that the preliminary report with incorrect information had been issued, a corrected report was generated and the original report was retracted. No additional testing of the specimen or of additional specimens was done related to this case. Of note, this subject had two samples from near the time of seroconversion tested for antiretroviral resistance: from 14 February 2011, the visit at which seroconversion was detected and study medication was withdrawn, and from 21 February 2011, the protocol-defined first post-seroconversion visit. The incorrectly-identified K65N mutation was from the latter visit only – the discrepancy across these two closely-timed visits would also be consistent with the error in reporting of the K65N mutation.

COMMENT: Not to be communicated. Response noted.

- 2. Please indicate if the frequencies of the individual HIV-1 subtypes that were observed among subjects of the Partner's PrEP study (for both partner and index subjects, if available) were representative of those prevalent in the areas of the study sites and comment on the relative efficacies of chemoprophylaxis against the different clades.**

HIV-1 subtypes have been determined only for HIV-1 seroconverters in the Partners PrEP Study, using methods as described in previous requests; HIV-1 sequencing to assess subtype among HIV-1 infected index participants has not been done. HIV-1 subtype for seroconverters is presented in Table 1.

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VIROLOGY REVIEW**

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Virology Reviewer: Damon J. Deming, Ph.D.

Table 1. Study CO-US-104-0380: Overall HIV-1 Subtype for Seroconverters

RT subtype	Randomization arm		
	TDF	FTC/TDF	Placebo
A	15 (75.0%)	8 (57.1%)	29 (51.8%)
C	0 (0.0%)	1 (7.1%)	2 (3.6%)
D	4 (20.0%)	3 (21.4%)	14 (25.0%)
Other	1 (5.0%)	2 (14.3%)	11 (19.6%)

Note: Subtype presented here is as determined by assessment of the RT region and, for individuals with >1 consensus genotype available, from the first post-seroconversion sample

In Kenya and Uganda, the predominant circulating HIV-1 subtypes are A and D, with A generally being more common than D in most surveillance studies, and then subtype C, circulating recombinant forms (CRFs), and other subtypes also seen. For example, from a different cohort of 99 HIV-1 seroconverters within HIV-1 serodiscordant couples from East Africa that we conducted (in which subtyping was done by analysis of env sequences), HIV-1 subtype A accounted for 68% of infections and D for 21% of infections (Campbell M, Kahle E, Celum C, Lingappa J, Kapiga S, Mujugira A, et al. HIV-1 Subtype C Is Not Associated with Higher Viremia during Early Infection Compared to Other Subtypes in an African Cohort [Paper #549]. Presented at: 19th Conference on Retroviruses and Opportunistic Infections (CROI); 2012 March 5-8; Seattle, WA.).

Thus, the frequencies of HIV-1 subtypes observed among subjects in the Partners PrEP Study – with subtype A seen in the majority of those acquiring infection, followed by D and then other subtypes in a minority – were representative of those prevalent in the areas of the study sites.

The Partners PrEP study team was unable to assess relative efficacy of chemoprophylaxis against different HIV-1 subtypes – to do so would require performing HIV-1 subtyping on all 4700+ HIV-1 infected index subjects in the study and then assessing efficacy of TDF and FTC/TDF versus placebo for each subgroup defined by subtype, much in the same way efficacy has been presented in the CSR for subgroups defined by other baseline covariates (e.g., gender). In addition, to maximize specificity, viral linkage of transmission pairs would also need to be done. As noted above, HIV-1 sequencing has not been done for index participants in the Partners PrEP Study.

However, the relative distribution of HIV-1 subtypes in HIV-1 seroconverters, if markedly different across randomization arms, could give some indication of differential efficacy. Restricting to the Partners PrEP Study group of seroconverters analyzed in the primary modified intention-to-treat analysis (mITT), Table 2 demonstrates that the distribution of subtypes is not markedly different (and is not statistically significantly different) for TDF versus placebo or FTC/TDF versus placebo, recognizing that the number of infections to consider is small. Thus, the data presented in Table 2 do not provide suggestion of differential efficacy by subtype.

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Virology Reviewer: Damon J. Deming, Ph.D.**

Table 2. Study CO-US-104-0380: Distribution of Subtypes in HIV-1 Seroconverters (mITT Population)

RT subtype	Randomization arm				
	TDF	P-value vs. placebo	FTC/TDF	P-value vs. placebo	Placebo
A	12 (80.0%)	0.27 Fisher's exact test	7 (63.6%)	0.31 Fisher's exact test	25 (50.0%)
C	0 (0.0%)		1 (9.1%)		1 (2.0%)
D	2 (13.3%)		1 (9.1%)		14 (28.0%)
Other	1 (6.7%)		2 (18.2%)		10 (20.0%)

COMMENT: Not to be communicated. Response noted.

3. The figures presented in slide #22 of the iPrEx presentation (slide 47/147 of the submission) are confusing and potentially misleading. For example, at Week 8 of FTC/TDF #1, the RNA load is approximately 2×10^3 copies/mL with approximately 100% of the virus expressing M184V. At Week 12 the viral load has increased to approximately 2×10^5 copies/mL with M184V-expressing virus comprising approximately 1% of the total population. While the relative proportion of M184V-expressing virus has diminished relative to wild-type virus, the titer of the variant may not have significantly decreased ($\sim 1\%$ of $2 \times 10^5 = \sim 2 \times 10^3$ copies/mL). In addition, given the long duration the variants may persist as provirus and the lack of any data on the efficacy of retreatment for these subjects, the clinical relevance of decreased relative proportions of drug-resistant HIV-1 is unclear. Please remove or modify the slide to avoid giving the impression that FTC/TDF exposure in patients who are unaware of being infected with HIV-1 will have no impact on subsequent therapy.

Based on the Agency's comments, the iPrEx study team confirms that slide 47/147 of those submitted to NDA 21-752 in Sequence No. 0413 dated 23 April 2012 has been removed from the final slide deck the study team intends to present at the Advisory Committee Meeting scheduled for 10 May 2012. In lieu of including this slide in the final presentation, the iPrEx study team will be placing a comment on the prior slide that the frequency of the drug resistant population wanes to lower than 0.5% within 6 months of stopping PrEP. The clinical significance of this drug resistance is not clear at any time.

COMMENT: Not to be communicated. Response noted.

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Virology Reviewer: Damon J. Deming, Ph.D.

Appendix B. HIV-1 RT substitutions identified among iPrEx Isolates

Table B1. HIV-1 RT substitutions of FTC/TDF treated iPrEx mITT subjects. The study day of infection as determined by earliest detection of viral RNA (TTIFN) or seroconversion (SC; only shown if different from TTIFN), day of isolate collection for genotypic analysis (GT), and viral subtype are indicated.

SubID	TTIFN	SC	GT Day	Subtype	T39	E40	K43	S48	I50	V60	K64	S68	R83	V90	A98	K101	K102	K103	K104	S105	V106
(b) (4)																					

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Table B1. HIV-1 RT substitutions of FTC/TDF treated iPrEx mITT subjects (2)

SubID	V108	V118	D121	E122	D123	I135	T139	I142	S162	T165	K166	E169	F171	R172	K173	Q174	D177	I178	V179	I180	V189	I195	G196	Q197	T200	
(b) (4)																										

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Table B1. HIV-1 RT substitutions of FTC/TDF treated iPrEx mITT subjects (3)

SubID	I202	E204	Q207	H208	R211	L214	K238	I244	V245	L246
(b) (4)										

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Table B2. HIV-1 RT substitutions of placebo treated iPrEx mITT subjects. The study day of infection as determined by earliest detection of viral RNA (TTIFN) or seroconversion (SC; only shown if different from TTIFN), day of isolate collection for genotypic analysis (GT), and viral subtype are indicated

SubID	TTIFN	SC	GT Day	Subtype	T39	E40	M41	K43	E44	S48	K49	I50	E53	V60	K64	S68	T69	R83	F87	V90	A98	K101
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(b) (4)



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Table B2. HIV-1 RT substitutions of placebo treated iPrEx mITT subjects (2)

SubID	TTIFN	SC	GT Day	Subtype	T39	E40	M41	K43	E44	S48	K49	50	E53	V60	K64	S68	T69	R83	F87	V90	A98	K101
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(b) (4)



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Table B2. HIV-1 RT substitutions of placebo treated iPrEx mITT subjects (3)

SubID	K102	K103	K104	V106	V108	V118	D121	E122	D123	135	E138	T139	142	S162	T165	K166	E169	K173	Q174	V175	P176	D177	178	V179	180
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(b) (4)



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Table B2. HIV-1 RT substitutions of placebo treated iPrEx mITT subjects (4)

SubID	102	103	104	106	108	118	121	122	123	135	138	139	142	162	165	166	169	173	174	175	176	177	178	179	180
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(b) (4)



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Table B2. HIV-1 RT substitutions of placebo treated iPrEx mITT subjects (5)

SubID	V189	I195	G196	Q197	T200	K201	I202	E203	E204	Q207	R211	L214	E224	L228	K238	P243	I244	V245	P247
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(b) (4)



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Table B2. HIV-1 RT substitutions of placebo treated iPrEx mITT subjects (6)

SubID	V189	196	G196	Q197	T200	K201	202	E203	E204	Q207	R211	L214	E224	L228	K238	P243	244	V245	P247
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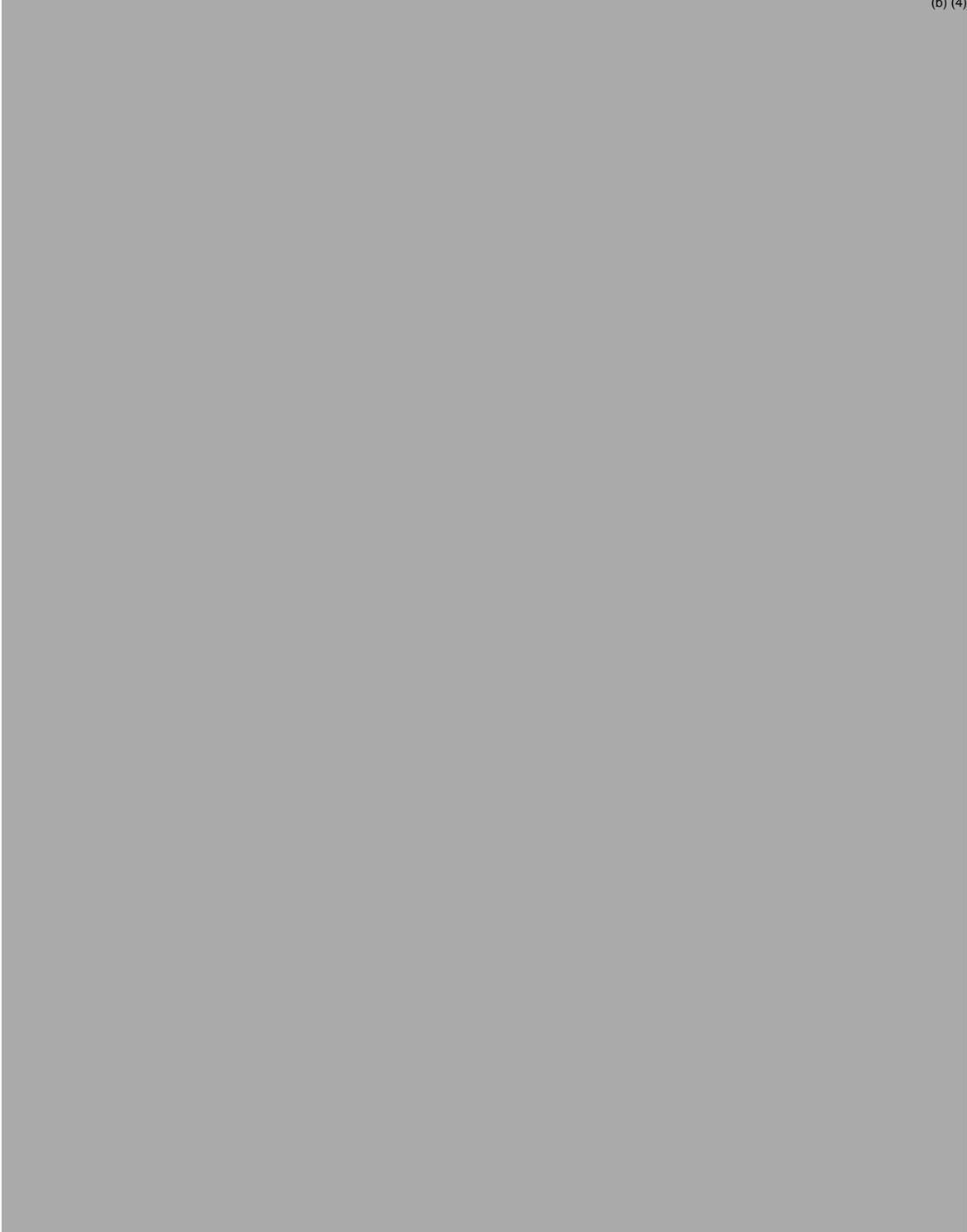
(b) (4)

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Table B3. HIV-1 RT substitutions of FTC/TDF and TDF treated Partner's PrEP mITT subjects. The subject ID (Sub ID), subject gender (Sex), study day of infection as determined by earliest detection of seroconversion (SC), day of isolate collection for genotypic analysis (GT Day), and viral subtype are indicated

Arm	SubID	Sex	SC	GT Day	Subtype	P4	I5	E6	K11	K20	V21	K32	V35	E36	T39	E40	K43	G45	S48	K49	I50
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(4)

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Table B3. HIV-1 RT substitutions of FTC/TDF and TDF Partner's PrEP mITT subjects (2)

Arm	SubID	V60	K64	A98	K103	V106	D121	D123	I35	E138	I42	Y144	A158	S162	T165	F171	K173	Q174	N175	D177	I78	V179	E194	G196	T200	I202	E203	Q207
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(b) (4)



Table B3. HIV-1 RT substitutions of FTC/TDF and TDF Partner's PrEP mITT subjects (3)

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Arm	SubID	R211	P225	P226	F227	L228	M229	M230	G231
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(b) (4)

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Table B4. HIV-1 RT substitutions of placebo-treated Partner's PrEP mITT subjects. The study day of infection as determined by earliest detection of seroconversion (SC), day of isolate collection for genotypic analysis (GT Day), and viral subtype are indicated

Arm	SubID	Sex	SC	GT Day	Subtype	P4	S	E6	V8	V10	K11	P14	M16	K20	V21	E28	K32	V35	E36	T39	E40
(b) (4)																					

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Table B4. HIV-1 RT substitutions of placebo-treated Partner's PrEP mITT subjects (2)

Arm	SubID	Sex	SC	GT Day	Subtype	P4	I5	E6	V8	V10	K11	P14	M16	K20	V21	E28	K32	V35	E36	T39	E40
(b) (4)																					

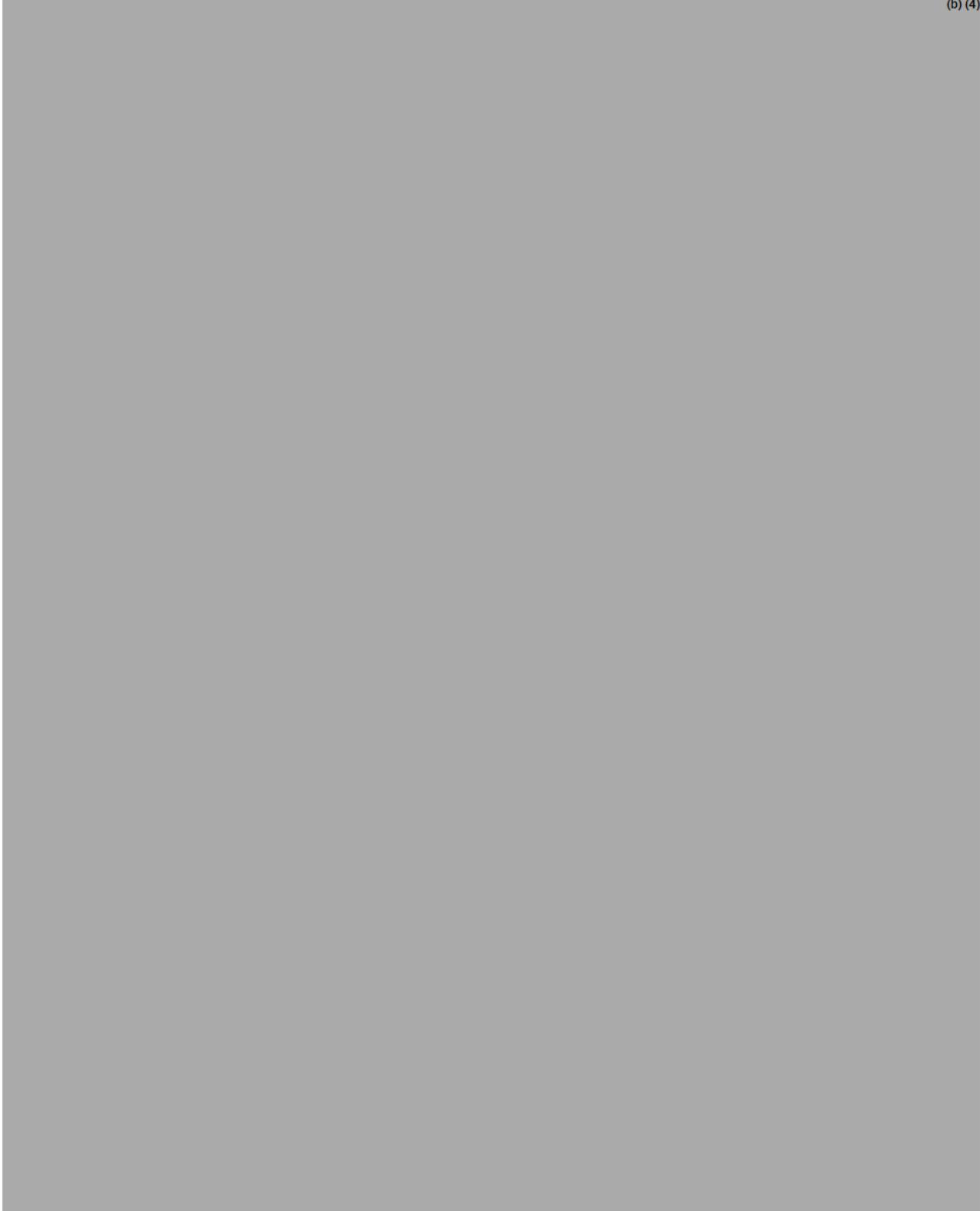
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Virology Reviewer: Damon J. Deming, Ph.D.

Table B4. HIV-1 RT substitutions of placebo-treated Partner's PrEP mITT subjects (3)

SubID	43	44	48	49	55	60	68	82	90	102	103	104	118	121	122	123	126	135	138	139	142	143	158	159	160	162	165	166	169	171
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(b) (4)



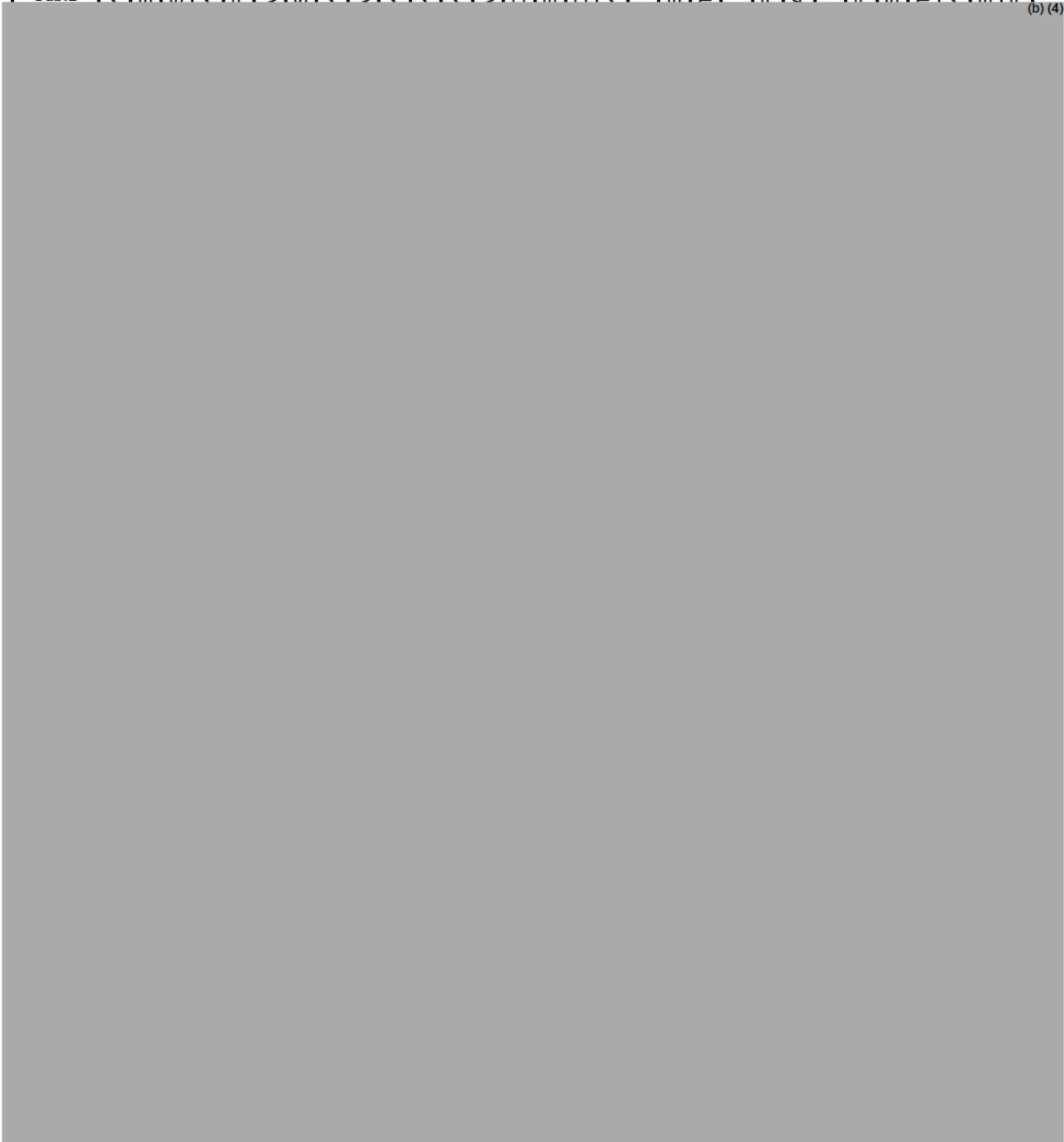
**DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
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Virology Reviewer: Damon J. Deming, Ph.D.

Table B4. HIV-1 RT substitutions of placebo-treated Partner's PrEP mITT subjects (4)

SubID	K43	E44	S48	K49	P55	V60	S68	K82	V90	K102	K103	K104	V118	D121	E122	D123	K126	I35	E138	T139	I42	R143	A158	I59	E160	S162	T165	K166	E169	E171
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(b) (4)



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Table B4. HIV-1 RT substitutions of placebo-treated Partner's PrEP mITT subjects (5)

SubID	173	174	177	178	179	195	196	197	200	202	203	207	211	224
(b) (4)														

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Table B4. HIV-1 RT substitutions of placebo-treated Partner's PrEP mITT subjects (5)

SubID	K173	Q174	D177	178	V179	195	S196	Q197	T200	202	E203	Q207	R211	E224
(b) (4)														

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Appendix C. HIV-1 RT substitution frequency figures

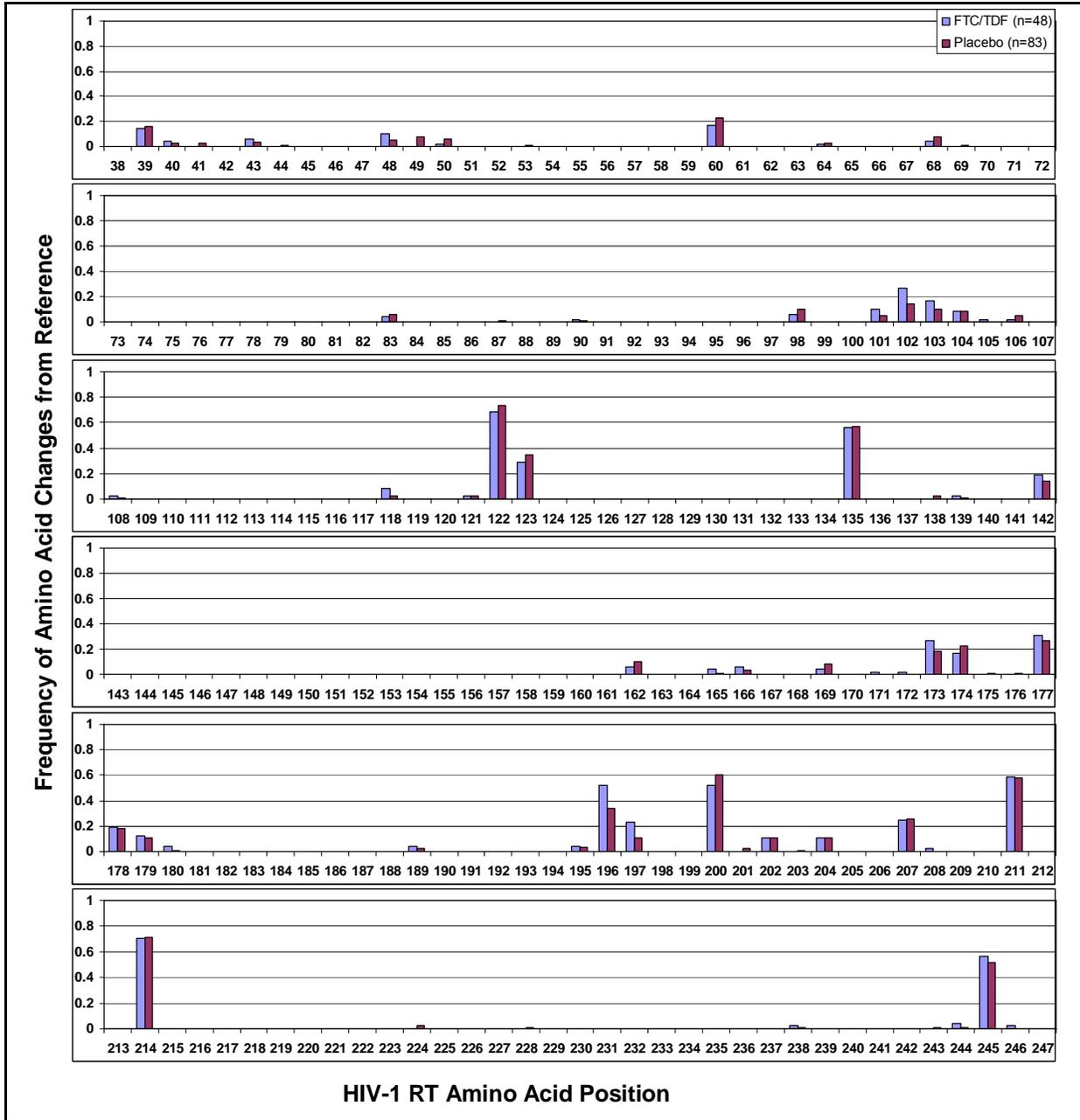


Table C1. Frequency of amino acid changes from the HIV-1 reference sequence for HIV-1 isolates from the iPrEx trial.

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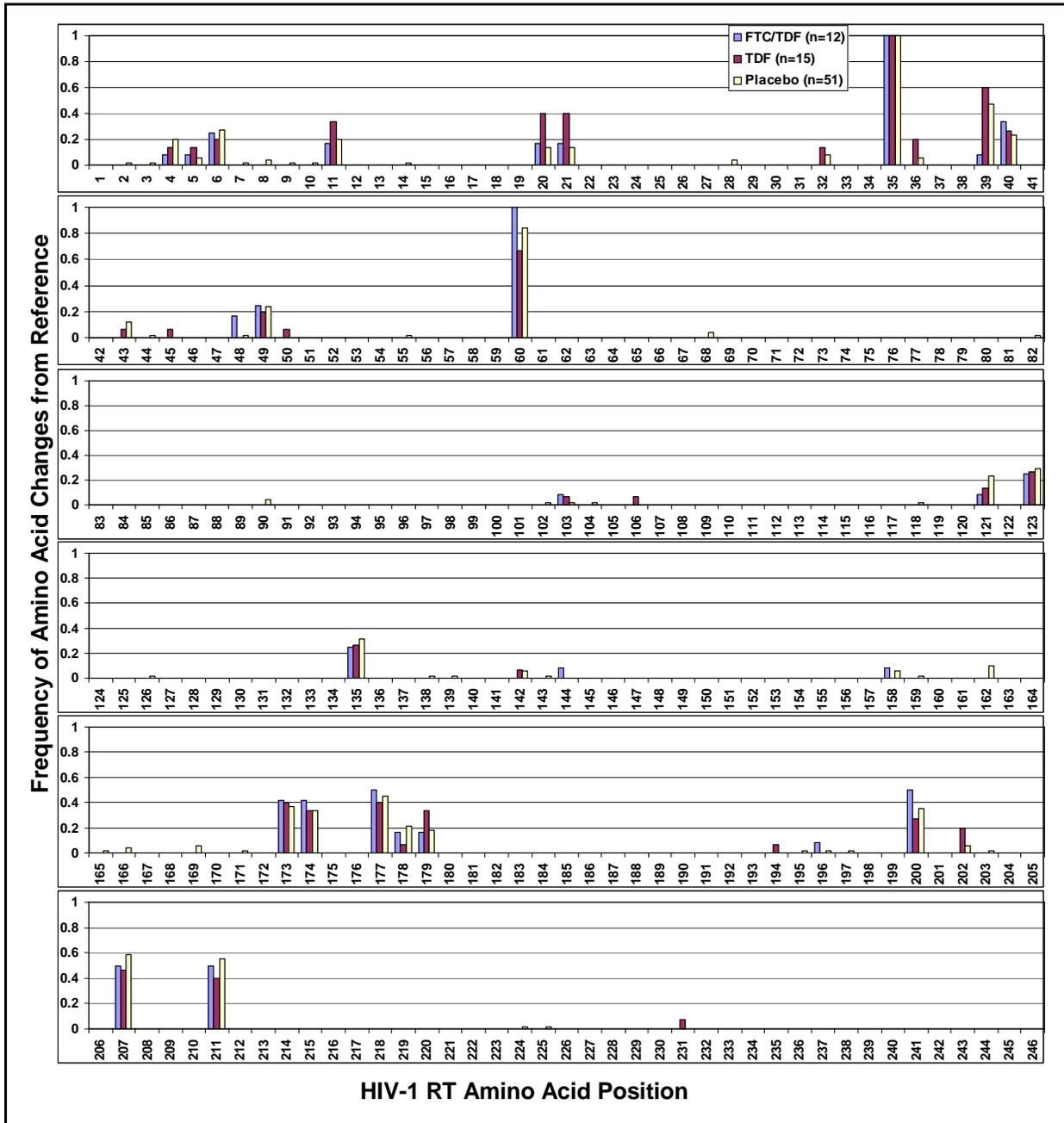


Table C1. Frequency of amino acid changes from the HIV-1 reference sequence for HIV-1 isolates from the Partner's PrEP trial.

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Appendix D. Allele-specific RT-PCR Data from the iPrEx Trial

Table D1. Percentage of minority variants expressing K65R, K70E, M184V, or M184I substitutions

	SubID	TTIFN	SC	GT	K65R	K70E	M184V	M184I		SubID	TTIFN	SC	GT	K65R	K70E	M184V	M184I
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(b) (4)



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**DIVISION OF ANTIVIRAL PRODUCTS (HFD-530)
VIROLOGY REVIEW**

NDA: 021752 **SN:** SLR 30 **DATE REVIEWED:** 4/30/2012

Virology Reviewer: Damon J. Deming, Ph.D.

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VIROLOGY REVIEW

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/s/

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06/01/2012

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