OBSERVATIONAL STUDY PROTOCOL

Study Title:	Seroconversions, Resistance, Adverse Events and Drug Adherence among Subjects taking Truvada® for PrEP: A Nested Case Control study	
Sponsor:	Gilead Sciences, Inc. 333 Lakeside Drive Foster City, CA 94404	
Indication:	Pre-Exposure Prophylaxis of HIV-1 Infection	
Protocol ID:	GS-US-276-0104	
Gilead Sciences Study Director:	Name: Telephone: Mobile: Fax: E-mail:	PPD PPD PPD PPD PPD
Protocol Version/Date:	Original:	13 November 2012

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PROTOCOL SYNOPSIS
Gilead Sciences, Inc.
333 Lakeside Drive
Foster City, CA 94404, USA

Study Title:	Seroconversions, Resistance, Adverse Events and Drug Adherence among Subjects taking Truvada for PrEP: A Nested Case Control study
IND Number	This is a non-IND study.
Study Centers Planned:	Approximately 15 centers globally (North America, Latin America, Europe, Africa, and Asia) performing observational or clinical studies on Truvada for PrEP
Objectives:	• To assess levels of adherence, as measured by drug level, to the once-daily dosing regimen of Truvada for PrEP • To evaluate the association between levels of adherence, as measured by drug level, and the odds of seroconversion and resistance development including in pregnancy • To assess the association between levels of adherence as measured by drug level and the odds of renal and skeletal adverse events • To measure a gradient of adherence levels using available data on drug levels as the measure of adherence
Study Design:	This is a retrospective case control study nested among PrEP prospective observational or clinical studies. The study will assess level of adherence as measured by drug level and its association with renal and bone adverse events, risk of seroconversion, and resistance development in subjects taking Truvada for PrEP. In the protocols of the parent PrEP observational or clinical studies, subjects will have follow-up visits on average every 3 months for evaluation of adherence, HIV-1 status, renal and bone adverse events, and seroconversion. Adherence will be determined by the specific Truvada drug level measurement(s) outlined in the parent protocol. There are two case definitions: a) One-hundred-fifty subjects who seroconvert (become HIV-1 positive) and b) any subjects who

	either develop a protocol defined renal adverse event (stratified by DAIDS grading) or have a skeletal adverse event (any fracture) while taking Truvada for PrEP. For each case, 3 randomly chosen controls on Truvada will be selected from the same site, with a similar treatment duration (last dose date minus first dose date). Exposure will be defined as tenofovir drug levels, which will be measured for each case and control subject from dried blood spot (DBS) samples. DBS analysis will provide a gradient of intracellular tenofovir-diphosphate (TFV-DP) concentrations in red blood cells (RBC), along with plasma TFV concentration. This gradient will be compared to self-reported adherence, seroconversion and resistance data, renal and skeletal adverse events, and to the presence or absence of signs and symptoms of acute HIV-1 infection. At selected sites where peripheral blood mononuclear cells (PBMCs) are being collected as part of the parent PrEP observational or clinical study, plasma TFV and emtricitabine (FTC) concentrations, as well as intracellular TFV-DP and emtricitabine-triphosphate (FTC-TP) concentrations will be collected. Results of resistance analyses of plasma HIV-1 will be collected from the seroconversion visit. This includes population nucleotide sequence analysis, followed by ultrasensitive testing (such as deep sequencing or allele-specific PCR of either plasma or peripheral blood mononuclear cells) if no resistance is identified by population sequencing.
Number of Subjects Planned:	Among the estimated 7,000 subjects from Truvada for PrEP observational and clinical studies all *Cases,* defined as subjects who seroconvert and/or develop either renal or skeletal adverse events, will be identified. Randomly chosen controls on Truvada will be selected in a 3:1 ratio, from the same site, with a similar treatment duration.
Target Population:	HIV-1 negative adults (any sex/gender, including transgender, pregnancy) $\geq$ 18 years of age who are participating in observational or clinical studies on Truvada for PrEP
Duration of Study Participation:	Up to 3 years. There is no minimum or maximum duration of exposure to Truvada for PrEP.

Diagnosis and Main Eligibility Criteria:	• HIV-1 negative adults (any sex/gender, including transgender, pregnancy) ≥ 18 years of age • Participants in Truvada for PrEP in observational or clinical studies • HIV-1 negative and without signs or symptoms of acute HIV-1 infection
Study Procedures/ Frequency:	At the Screening/Baseline Visit: Evaluation of medical history (specifically related to previous fractures or renal impairment), signs and symptoms of acute HIV-1 infection, and sexual risk for HIV-1 acquisition will be collected. Additionally, HBsAg status and renal function status (creatinine clearance [CrCl] by Cockcroft-Gault formula) will be determined. Samples will be collected and stored for resistance analysis as part of the parent protocol. The method and frequency of HIV-1 screening will be collected from each site on all visits between baseline and seroconversion or development of adverse event. Follow-up visits are expected to occur on average every 3 months and correspond to the parent PrEP observational or clinical study visit schedule. During follow-up visits, any protocol defined renal adverse event (stratified by DAIDS grading) or skeletal adverse event (any fracture), along with signs and symptoms of acute HIV-1 infection, self reported adherence, and results of testing to confirm negative HIV-1 status, will be extracted. Per the parent protocol, DBS samples will be collected and stored for future Truvada drug level determination on *Cases* and matched *Control* subjects; plasma or PBMC samples will be collected and stored for future resistance analyses on all cases and controls.
Test Product, Dose, and Mode of Administration:	Truvada once daily will be provided as part of the subject's participation in a parent PrEP observational or clinical study.
Criteria for Evaluation:	This non-interventional study is observational in nature and does not evaluate the safety or efficacy of any treatment.

Statistical Methods:	Descriptive statistics will summarize the demographics, serology test results, measures of renal function, adherence and drug levels within each parent PrEP observational or clinical study and as much as possible across all studies. The association between exposure (drug levels) and disease (seroconversion, adverse events) will be explored using ordinal logistic regression and/or other generalized linear statistical modeling (for a continuous measure of adherence).

This study will be conducted in compliance with guidelines for Good Pharmacoepidemiology Practices (GPPs) and all essential documents will be archived {20900}.

GLOSSARY OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	adverse event
ART	antiretroviral therapy
BMD	bone mineral density
CRF	case report form
CrCl	creatinine clearance
DAIDS	Division of Acquired Immunodeficiency Syndrome
DBS	dried blood spot
DSPH	Drug Safety & Public Health
FDA	(United States) Food and Drug Administration
FTC	emtricitabine
FTC-TP	emtricitabine-triphosphate
FTC/TDF	emtricitabine/tenofovir disoproxil fumarate (Truvada®)
GPP	Good Pharmacoepidemiology Practices (guidelines for)
GSI	Gilead Sciences, Inc.
HIV, HIV-1	human immunodeficiency virus, human immunodeficiency virus type 1
ICH	International Conference on Harmonisation
IND	Investigational New Drug (Application)
iPrEx	Pre-exposure Prophylaxis Initiative (study)
IRB	institutional review board
MD	Medical Doctor
mITT	modified intent-to-treat
MSM	men who have sex with men
NR	not reported
NS	not significant
Parent protocol	original protocol of the PrEP observational or clinical study that is providing data to this analysis
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PrEP	pre-exposure prophylaxis
RBC	red blood cells
sNDA	Supplemental New Drug Application
Sponsor	Parent PrEP observational or clinical study sponsor
TDF	tenofovir disoproxil fumarate
TFV	tenofovir
TFV-DP	tenofovir-diphosphate
US, USA	United States, United States of America

1. INTRODUCTION

1.1. Background

In 2010, an estimated 47,129 people were diagnosed with HIV-1 infection in the 46 states with confidential name-based HIV-1 infection reporting. The CDC estimates 1.2 million people in the United States (U.S.) are living with HIV-1 infection. One in five (20%) of those people are unaware of their infection. Despite increases in the total number of people in the U.S. living with HIV-1 infection in recent years (due to better testing and treatment options), the annual number of new HIV-1 infections has remained relatively stable with approximately 50,000 Americans becoming infected with HIV-1 each year, despite widespread knowledge of the protective effects of abstinence, monogamy, and condoms {22170}. The principal interventions used to prevent HIV-1 transmission have been voluntary testing, counseling, and the promotion of condom use, and the effectiveness of these interventions has been variable {15464}, {15465}, {16996}. Given that HIV-1 prevalence and incidence remain high despite considerable prevention efforts and that no vaccine is available, the identification of novel approaches to decreasing the sexual transmission of HIV-1 using simplified antiretroviral therapy (ART) in high-risk populations, such as men who have sex with men (MSM) and heterosexual discordant couples, is timely and of critical importance.

Tenofovir disoproxil fumarate (TDF, Viread®) is a nucleotide reverse transcriptase inhibitor that was approved for the treatment of established human immunodeficiency virus type 1 (HIV-1) infection by the Food and Drug Administration (FDA) in 2001. Emtricitabine (FTC, Emtriva®) is a nucleoside reverse transcriptase inhibitor that was approved for treatment of HIV-1 infection by the FDA in July 2003. A fixed dose co-formulation of emtricitabine 200 mg and tenofovir disoproxil fumarate 300 mg (FTC/TDF or Truvada®) was approved for HIV-1 treatment by the FDA in August 2004 and for pre-exposure prophylaxis (PrEP) on July 16, 2012.

Demonstrated Efficacy

The safety and efficacy of once-daily oral Truvada in the prevention of HIV-1 acquisition among MSM have been demonstrated in a large (n = 2499), multinational, randomized, placebo-controlled, double-blind, Phase 3 study (also known as the Pre-exposure Prophylaxis Initiative [iPrEx] study) {16681}. The safety and efficacy of once-daily oral Truvada in the prevention of HIV-1 acquisition among East African heterosexual men and women in 4747 HIV-1 serodiscordant partnerships were demonstrated in a randomized, placebo-controlled, double-blind, Phase 3 study (also known as the Partners PrEP study) {21185}.

In the iPrEx study, daily Truvada reduced the risk of HIV-1 acquisition in MSM by 42* (95% CI: 18-60%)*-44% (95% CI: 15-63%). In addition, the prophylactic efficacy of Truvada was strongly correlated with adherence to treatment. The risk for HIV-1 acquisition was substantially reduced among those with high study drug adherence, and detectable concentrations of tenofovir were strongly predictive of a high degree of protection from HIV-1 acquisition, with a 92% reduction in HIV-1 acquisition risk (95% CI: 40% to 99%; $p < 0.001$) observed among subjects in the Truvada arm with detectable drug levels versus those without detectable drug levels. This corresponds to a 12.9 (95% CI: 1.7 to 99.3; $p < 0.001$) fold reduction in the odds of HIV-1 infection.

In the Partners PrEP Study, both Truvada and Viread reduced the risk for HIV-1 acquisition. Relative to the placebo group, Truvada-treated subjects had a 75% lower risk of HIV-1 acquisition (95% CI: 55% to 87%; $p < 0.0001$) {21185}. The study was not powered to show statistical significance, but the degree of HIV-1 protection relative to placebo was notable for both genders (ie, Truvada-treated females 66% lower risk; Truvada-treated males 84% lower risk; $p = 0.24$). As with the iPrEx Study, the risk for HIV-1 acquisition in the Partners PrEP Study was substantially reduced among those with high study drug adherence, and detectable concentrations of tenofovir were strongly predictive of a high degree of protection from HIV-1 acquisition, with a 90% reduction in HIV-1 acquisition risk (95% CI: 56% to 98%; $p = 0.002$) observed among subjects in the Truvada arm with detectable drug levels versus those without detectable drug levels.

* Per final datacut from Truvada PrEP sNDA

Table 1-1. Measures of Efficacy with Daily Oral FTC/TDF for PrEP, by Medication Adherence

Study	Population	mITTa % Reduction in HIV Incidence (95% CI)			Combined Self-Report and Pill-Count Medication Adherence (95% CI)	Pill-Count Medication Adherence (95% CI)	TFV Blood Detectionb (95% CI)
iPrEx	MSM	42% (18-60%)[e]-44% (15-63%)			≥50%:[c] 50% (18-70%) ≥90%:[c] 73% (41-88%)	NR	92% (40-99%)
		Overall	**Men**	**Women**			
Partners PrEP	Heterosexual discordant couples	75% (55-87%)	84% (54-95%)	66% (28-84%)	NR	100%[d] (87-100%)	90% (58-98%)

a Excluded only those enrolled subjects later found to be infected at randomization and those with no follow-up visit or HIV test

b The percentage of reduction in HIV incidence among those with TFV detected in blood, compared with those without detectable TFV

c The percentage of reduction in HIV incidence, compared with the placebo group, is presented for 2 groups: those with 50% medication adherence and those with 90% medication adherence

d In a substudy of participants who provided counts via home-based unannounced pill counts with supplementary adherence counseling if the counts were <80%

e Per final datacut from Truvada PrEP sNDA

In both the iPrEx and Partners PrEP studies, the rate of HIV-1 acquisition was substantially affected by study drug adherence as assessed by plasma tenofovir concentrations. These findings demonstrate the critical importance of good adherence to Truvada to the effectiveness of PrEP in preventing HIV-1 acquisition.

Low Development of Resistance

In the iPrEx study, resistance development did occur in individuals found to be infected at baseline; however, no resistance developed in individuals who seroconverted while on study drug. In an assessment using drug-resistance assays based on allele-specific polymerase chain reaction (lower limit of quantification 0.5%) sensitive for the detection of minor sequence variants (ie, RT K65R and K70E [which confer resistance to TDF] and M184V and M184I [which confer resistance to FTC]), no minor drug resistant variants were detected among the subjects in the Truvada group who had HIV-1 seroconversion during the iPrEx study {17134}. HIV-1 infections pre-existing at study enrollment were diagnosed during the study for 2 subjects in the Truvada group and 8 subjects in the placebo group (p-value for difference between treatment groups = 0.06) {16681}. Among the 10 subjects in whom plasma HIV-1 RNA was subsequently detected in specimens obtained at enrollment, 3 had

FTC-resistant infection (2 of 2 in the Truvada group and 1 of 8 in the placebo group). No TDF-resistant infections were observed in either treatment group. Among the subjects with treatment-emergent HIV-1 seroconversion in the primary analysis, no FTC or TDF resistance was detected.

Similarly in the Partners PrEP study, resistance development only occurred in individuals with undiagnosed HIV-1 infection at baseline. HIV-1 infections pre-existing at study enrollment were diagnosed during the study for 5 subjects in the Truvada group, 3 subjects in the Viread group, and 6 subjects in the placebo group. Among the 14 subjects in whom plasma HIV-1 RNA was subsequently detected in specimens obtained at enrollment, 1 had FTC-resistant infection (1 of 5 in the Truvada group) and 1 had TDF-resistant infection (1 of 3 in the Viread group). Among the subjects with treatment-emergent HIV-1 seroconversion, no FTC or TDF resistance was detected.

Truvada has certain characteristics that make it suitable for HIV-1 chemoprophylaxis, including ease of administration, once-daily dosing, a relatively long half-life, established tolerability and potent antiviral effects, penetration of FTC and tenofovir into genital and colorectal mucosal tissue, high barrier to resistance for TDF, and selection of drug-resistant variants that have mutations associated with diminished capacity for replication. Use of FTC and TDF together for chemoprophylaxis increased the activity of the regimen and the barrier to drug resistance, and the protective activity of FTC plus TDF has been shown in mice transplanted with human immune cells {12771} and in nonhuman primates {17}, {2983}, {13751}. The relative efficacy of Truvada compared with Viread (each versus placebo) as a potential alternative for HIV-1 prophylaxis was supported by comparative Phase 3 clinical study data from the Partners PrEP Study.

1.2. Rationale for the Current Study

Due to the fact that PrEP is an intervention based on antiretrovirals, there has been a concern that wide-scale usage could lead to an increase in the development of ARV-resistant strains {22369} or in higher levels of renal or skeletal adverse events then were seen in the PrEP clinical trials. The strong correlation between adherence to once daily dosing and the efficacy of PrEP warrants an assessment of the level of adherence across a broader population.

An increase in the level of resistance could exacerbate the difficulty in treating HIV-1 positive subjects and make the epidemic more difficult to control {22370}, particularly if risk compensation occurs. Nonetheless, data from the PrEP studies primarily showed that the resistance occurred only in individuals found to be infected at baseline; and no resistance developed in individuals who seroconverted while on study drug.

Table 1-2. Summary of Resistance Development in PrEP Clinical Studies

Study	Infected on Study		Baseline Infections	
	# Infected	# Resistant to FTC or TDF	# Infected	# Resistant to FTC or TDF
iPrEx	100 (36 on FTC/TDF, 64 on placebo)	None	10 (2 on FTC/TDF, 8 on placebo)	2 on FTC/TDF (M184V/I); 1 on placebo (M184V)[a]
Partners PrEP	82 (13 on FTC/TDF, 17 on TDF, and 52 on placebo)	None	14 (3 on FTC/TDF, 5 on TDF, 6 on placebo)	1 on FTC/TDF (M184V); 1 on TDF (K65R)
TDF2	33 (9 on FTC/TDF, 24 on placebo)	1 placebo (K65R <1%)[a]	3 (1 on FTC/TDF, 2 on placebo)	1 on FTC/TDF (K65R, M184V, A62V)
FEM-PrEP	68 (33 on FTC/TDF, 35 on placebo)	1 placebo (M184V)[a;] 4 FTC/TDF (M184V/I)[b]	5 (1 on FTC/TDF, 4 on placebo)	None

a Transmitted (primary) resistance can occur independent of PrEP, which likely explains resistance in the placebo arm

b One probable and 2 possible transmitted resistance; 1 uncertain timing of infection (HIV RNA detectable at first follow-up visit)

Development of resistance primarily in individuals infected at baseline underscores the importance of screening individuals for potential acute infection prior to initiating PrEP.

Mathematical modeling {18152} has shown that PrEP interventions may increase the proportion of new infections caused by resistant strains if risk compensation occurs. Nonetheless if risk behavior remains stable, the concern is likely to be unfounded.

In addition to characterizing individuals who seroconvert, the current study should provide a better understanding of the impact of HIV-1 screening methods and frequency on seroconversion and the development of resistance.

Table 1-3. Summary of Safety in PrEP Clinical Studies

	iPrEx	Partners PrEP	CDC TDF2	FEM-PrEP	CDC 4323
Overall Safety	Similar safety profile to placebo with more gastrointestinal events through Week 4	Similar safety profile to placebo	Similar safety profile to placebo with more dizziness, nausea and vomiting (N/V)	Similar to placebo; more N/V and AST/ALT elevations	Similar safety profile to placebo
D/C for Safety or Intolerance	Rare	Rare	Rare	NR	Rare
Renal Safety	Mild-moderate serum creatinine elevation: 2.4% FTC/TDF vs. 2.2% placebo	Infrequent serum creatinine abnormalities (<2%); similar across all arms	Elevated serum creatinine rare (<0.1%)	No difference compared to placebo	Infrequent serum creatinine abnormalities (<1%); similar to placebo
Bone Safety	No increase in fracture; small decrease in BMD at the spine and hip, which returned toward baseline post d/c	No increase in fracture	No increase in fracture	NR	No increase in fracture; small decrease in BMD at the spine and hip

The intent of this study is to collect and analyze data related to medication adherence from individuals who take Truvada for PrEP of sexually acquired HIV-1 infection and who seroconvert or develop renal or skeletal adverse events during follow-up.

2. OBJECTIVES

The objectives of this study are:

- To assess levels of adherence, as measured by drug level, to the once-daily dosing regimen of Truvada for PrEP
- To evaluate the association between levels of adherence, as measured by drug level, and the odds of seroconversion and resistance development including in pregnancy
- To assess the association between levels of adherence as measured by drug level and the odds of renal and skeletal adverse events
- To measure a gradient of adherence levels using available data on drug levels as the measure of adherence

3. STUDY DESIGN

This is an observational nested case control study among PrEP observational or clinical studies. The study will assess level of adherence as measured by drug level and its relationship to renal and bone adverse events, risk of seroconversion, and resistance development in subjects taking Truvada for PrEP.

In the protocols of the parent PrEP observational or clinical studies, subjects will have follow-up visits on average every 3 months for evaluation of adherence, HIV-1 status, renal and bone adverse events, and seroconversion. Adherence will be determined by the specific Truvada drug level measurement(s) outlined in the parent protocol.

There are two case definitions: a) One-hundred-fifty subjects who seroconvert (become HIV-1 positive) and b) any subjects who either develop a protocol defined renal adverse event (stratified by DAIDS grading) or have a skeletal adverse event (any fracture) while taking Truvada for PrEP.

For each case, 3 randomly chosen controls on Truvada will be selected from the same site, with a similar baseline date.

DBS analysis will provide a gradient of intracellular tenofovir-diphosphate (TFV-DP) concentrations in red blood cells (RBC), along with plasma TFV concentration.

This gradient will be compared to self-reported adherence, seroconversion and resistance, renal and skeletal adverse events, and to the presence or absence of signs and symptoms of acute HIV-1 infection.

At selected sites where peripheral blood mononuclear cells (PBMCs) are being collected as part of the parent PrEP observational or clinical study, plasma TFV and emtricitabine (FTC) concentrations, as well as intracellular TFV-DP and emtricitabine-triphosphate (FTC-TP) concentrations will be collected.

Results of resistance analyses of plasma HIV-1 will be collected from the seroconversion visit. This includes population nucleotide sequence analysis, followed by ultrasensitive testing (such as deep sequencing or allele-specific PCR of either plasma or peripheral blood mononuclear cells) if no resistance was identified by population sequencing.

4. SUBJECT POPULATION

4.1. Number of Subjects and Subject Selection

Seven thousand (7000) HIV-1 negative adults (any sex/gender, including transgender, pregnancy) ≥ 18 years of age who are participating in observational or clinical studies on Truvada for PrEP

4.2. Inclusion Criteria

To be eligible for study participation, an uninfected individual must satisfy all of the following criteria:

- Participant in a Truvada for PrEP observational or clinical study
- HIV-1 negative adults (any sex/gender, including transgender, pregnancy) ≥ 18 years of age at time of enrollment in the Truvada for PrEP observational or clinical study.
- HIV-1 negative and without signs or symptoms of acute HIV-1 infection

4.3. Exclusion Criteria

This is an observational study and will monitor all reported seroconversions, and renal or skeletal adverse events without intervention/exclusion.

5. STUDY DRUGS

5.1. Study Drugs

No study drug will be administered, as this study is an observational study. Truvada will be provided as part of participation in a PrEP clinical study as part of a comprehensive HIV-1 prevention strategy. The decision to continue or discontinue Truvada belongs jointly to the subject and their investigators in the underlying study.

6. STUDY PROCEDURES

6.1. Enrollment and collection of data

Subjects will be consented and enrolled by the parent PrEP observational or clinical study teams following enrollment procedures described in those protocols.

Subjects in parent PrEP observational or clinical studies that are on study drug will be followed according to the respective study protocol, on average every 3 months for evaluation of adherence, HIV-1 status, renal and bone adverse events, and seroconversion. Adherence will be determined by the specific Truvada drug level measurement(s) outlined in the parent protocol.

There will be two case definitions:

a one-hundred-fifty subjects who seroconvert (become HIV-1 positive), and

b any subjects who either develop a protocol defined renal adverse event (stratified by DAIDS grading) or have a skeletal adverse event (any fracture) while taking Truvada for PrEP.

For each case, 3 randomly chosen controls on Truvada will be selected from the same site, with a similar treatment duration (last dose date minus first dose date).

Exposure will be defined as drug levels, which will be measured for each case and control subject from dried blood spot (DBS) samples. DBS analysis will provide a gradient of intracellular tenofovir-diphosphate (TFV-DP) concentrations in red blood cells (RBC), along with plasma TFV concentration.

This gradient will be compared to self-reported adherence, seroconversion and resistance, renal and skeletal adverse events, and to the presence or absence of signs and symptoms of acute HIV-1 infection.

At selected sites where peripheral blood mononuclear cells (PBMCs) are being collected as part of the parent PrEP observational or clinical study, plasma TFV and emtricitabine (FTC) concentrations, as well as intracellular TFV-DP and emtricitabine-triphosphate (FTC-TP) concentrations will be collected.

Results of resistance analyses of plasma HIV-1 will be collected from the seroconversion visit. This includes population nucleotide sequence analysis, followed by ultrasensitive testing (such as deep sequencing or allele-specific PCR of either plasma or peripheral blood mononuclear cells) if no resistance was identified by population sequencing.

For the subjects who seroconvert during participation in the parent PrEP studies the following data will be collected from the assessments performed during enrollment visit: confirmation of the HIV-1 negative status, risk behavior assessments, medical history and physical exam, and baseline sample collection.

Documentation of signs and symptoms of acute infection, sexual risk for HIV-1 acquisition, and results of testing to confirm positive HIV-1 status at the seroconversion visit or since the last visit when the seroconversion occurred will be collected.

If a stored baseline sample is available and analyzed for viral resistance, the data will be collected.

To preserve subject's confidentiality, each consented participant will be assigned one subject ID.

6.2. Criteria for Discontinuation of Follow up

The decision to continue or discontinue Truvada belongs jointly to the subject and their investigators in the parent study. In cases where subjects discontinue Truvada, they will be followed according to the parent PrEP observational or clinical study protocol.

7. ADVERSE EVENTS AND SPECIAL SITUATION MANAGEMENT

7.1. Adverse Events, Special Situations, and Product Complaints

Adverse event (AE) or special situation reports (eg, reports of pregnancy with either maternal or paternal exposure, medication errors, abuse, misuse, overdose, lack of effect, adverse reactions in infants following exposure from breastfeeding, or adverse reactions associated with product complaints), collectively referred to as safety events, will not be solicited in this observational study. Any AE or special situation that arises should be reported as instructed in the Truvada for PrEP observational or clinical study in which the subject is participating.

The usual means for reporting post-marketing adverse events to Gilead's Drug Safety & Public Health (DSPH) department are available to prescribers or uninfected individuals who wish to report potential adverse effects to Gilead products. As a courtesy, prescribers are provided with the following contact information for Gilead's Drug Safety & Public Health department:

- Gilead DSPH: 1-800-GILEAD-5 (1-800-445-3235), Option 3

8. STATISTICAL CONSIDERATIONS

8.1. Analysis Objectives

One objective of the statistical analysis is to describe the adherence to Truvada and the association between the odds of seroconversion and the tenofovir drug levels after adjusting for potential confounders, and those that have bone or renal adverse events from all the parent PrEP observational and clinical studies.

The analysis will adjust for baseline resistance, renal impairment, and/or other prognostic factors or confounders. Moreover descriptive statistics will be used to show the timing and types of signs/symptoms of acute infection before and at seroconversion.

Additionally, person time exposed to Truvada among cases and controls will be used to describe the timing of seroconversion among the subjects in the study.

Another objective of the statistical analysis is to describe the association between the odds of having a renal and/or bone adverse event and the tenofovir drug levels after adjusting for potential confounders.

A separate analysis will be done for pregnant women who seroconvert during therapy.

8.1.1. Laboratory Evaluations

Resistance analyses of plasma HIV-1 will be collected from the seroconversion visit, including population nucleotide sequence analysis. If no resistance is identified by population sequencing, ultrasensitive testing results (such as deep sequencing or allele-specific PCR of either plasma or peripheral blood mononuclear cells) will be collected.

8.1.2. Sample Size

The seroconversion rate has varied from 2.3 per 100 person years in the iPrEx study to 0.5 cases per 100 person years in the Partners PrEP study. In order to have 150 seroconversion cases, one would have to accumulate approximately 10,000 person years of follow up. The number of expected subjects in the parent PrEP observational and clinical studies (7,000) will be sufficient when followed by an average of two years to accumulate the amount of person time needed.

The rate of mild to moderate serum creatinine elevation in the PrEP clinical trials was 2.4%. 7,000 subjects followed for an average of two years would contribute 168 Cases to the study.

9. RESPONSIBILITIES

9.1. Investigator Responsibilities

9.1.1. Good Clinical Practice

The sponsor of each parent PrEP observational or clinical study protocol will ensure that their study is conducted in accordance with the principles of the "Declaration of Helsinki" (as amended in Edinburgh, Tokyo, Venice, Hong Kong, and South Africa), International Conference on Harmonisation (ICH) guidelines, or with the laws and regulations of the countries in which the parent protocol is conducted, whichever affords the greater protection to the study subject.

9.1.2. Institutional Review Board/ Independent Ethics Committee (IRB/IEC) Approval

Each parent PrEP observational or clinical study sponsor is responsible for obtaining IRB/IEC approval for their respective protocol, protocol amendments and associated ICF (s).

9.1.3. Informed Consent (ICF)

Each parent PrEP observational or clinical study sponsor is responsible for obtaining subject informed consent.

9.1.4. Confidentiality

Each parent PrEP observational or clinical study sponsor must assure that subjects' anonymity will be strictly maintained and that their identities are protected from unauthorized parties. Only the subject ID identification code (ID) (i.e., not names) should be recorded on any form or dataset submitted to Gilead Sciences.

The sponsor of the parent PrEP observational or clinical study agrees that all information received from Gilead Sciences, including but not limited to this protocol, and any other study information, remain the sole and exclusive property of Gilead Sciences during the conduct of the study and thereafter. This information is not to be disclosed to any third party (except employees or agents directly involved in the conduct of the study or as required by law) without prior written consent from Gilead Sciences. Each parent PrEP observational or clinical study sponsor further agrees to take all reasonable precautions to prevent the disclosure by any employee or agent of any study site to any third party or otherwise into the public domain.

9.1.5. Study Files and Retention of Records

Each parent PrEP observational or clinical study sponsor must ensure that study sites maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into at least the following two categories: (1) Study file, and (2) subject source documents.

Their study file will contain the protocol/amendments, and query forms, governmental approval, if applicable, IRB/IEC approvals and correspondence, drug records, staff curriculum vitae, subjects' informed consents, and other appropriate documents and correspondence.

The required source data are the documentation of assessments and laboratory results from subject's visits.

9.1.6. Case Report Forms

Each parent PrEP observational or clinical study sponsor and site is responsible for maintaining study CRFs.

9.1.7. Inspections

The sponsor of each parent PrEP observational or clinical study should understand that source documents for this study should be made available to appropriately qualified personnel from Gilead Sciences or its representatives, to the IRB/IEC, or to regulatory authority or health authority inspectors.

9.1.8. Protocol Compliance

The sponsor of each parent PrEP observational or clinical study is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

9.2. Sponsor Responsibilities

9.2.1. Protocol Modifications

Modifications to this protocol may be made only by Gilead Sciences.

9.2.2. Study Report and Publications

A clinical study report will be prepared and provided to the regulatory agency(ies) on an annual basis. Gilead Sciences will ensure that the report meets the standards set out in the Guidelines for Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).

Each parent PrEP observational or clinical study sponsor and Gilead Sciences will review and agree on any proposed publication or presentation along with the respective scientific journal or presentation forum at least 30 days before submission of the publication or presentation. Each parent PrEP observational or clinical study sponsor will comply with Gilead Sciences' request to delete references to its confidential information (other than the study results) in any paper or presentation and agrees to withhold publication or presentation for an additional 60 days.

9.3. Joint Investigator/Sponsor Responsibilities

9.3.1. Access to Information for Monitoring

There will be no subject enrollment under this protocol. Therefore, each parent PrEP observational or clinical study sponsor will be responsible for study conduct and monitoring of data.

9.3.2. Access to Information for Auditing or Inspections

Representatives of regulatory authorities or of Gilead Sciences may conduct inspections or audits of the parent PrEP observational or clinical study. If the sponsor of the parent PrEP observational or clinical study is notified of an inspection by a regulatory authority they agree to notify the Gilead Sciences medical monitor immediately. The sponsor of the parent study agrees to provide to representatives of a regulatory agency or Gilead Sciences access to records, facilities, and personnel for the effective conduct of any inspection or audit.

9.3.3. Study Discontinuation

Both Gilead Sciences and parent PrEP observational or clinical study sponsor reserve the right to withdraw their participation from this protocol at any time. Should this be necessary, both parties will arrange discontinuation procedures and notify the appropriate regulatory authority(ies), IRBs, and IECs. In terminating the study, Gilead Sciences and the parent PrEP observational or clinical study sponsor will assure that adequate consideration is given to the protection of the subjects' interests.

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11. APPENDICES

Appendix 1. Investigator Signature Page

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GILEAD SCIENCES, INC.
333 LAKESIDE DRIVE
FOSTER CITY, CA 94404

STUDY ACKNOWLEDGEMENT
Seroconversions, Resistance, Adverse Events and Drug Adherence among Subjects taking Truvada® for PrEP: A Nested Case Control study
GS-US-276-0104 Original, 13 November 2012

This protocol has been approved by Gilead Sciences, Inc. The following signature documents this approval.

PPD

Medical Monitor

PPD

Si

NOVEMBER 15, 2012
Date

INVESTIGATOR STATEMENT

I have read the protocol, including all appendices, and I agree that it contains all necessary details for me and my staff to conduct this study as described. I will conduct this study as outlined herein and will make a reasonable effort to complete the study within the time designated.

I will provide all study personnel under my supervision copies of the protocol and access to all information provided by Gilead Sciences, Inc. I will discuss this material with them to ensure that they are fully informed about the drugs and the study.

Principal Investigator Name (Printed)

Signature

Date

Site Number