

Risk of Drug Resistance Among Persons Acquiring HIV Within a Randomized Clinical Trial of Single- or Dual-Agent Preexposure Prophylaxis

Dara A. Lehman,^{1,4} Jared M. Baeten,^{4,5,6} Connor O. McCoy,² Julie F. Weis,¹ Dylan Peterson,¹ Gerald Mbara,^{1,4} Deborah Donnell,^{3,4} Katherine K. Thomas,⁴ Craig W. Hendrix,^{8,9,10} Mark A. Marzinke,^{8,9,10} Lisa Frenkel,⁷ Patrick Ndase,⁴ Nelly R. Mugo,^{4,11} Connie Celum,^{4,5,6} Julie Overbaugh,^{1,2} and Frederick A. Matsen²; the Partners PrEP Study Team^a

¹Division of Human Biology, ²Division of Public Health Sciences, ³Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, ⁴Department of Global Health, ⁵Department of Medicine, ⁶Department of Epidemiology, University of Washington, and ⁷Seattle Children's Research Institute, Seattle, Washington; ⁸Department of Medicine, ⁹Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, and ¹⁰Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; and ¹¹Kenyatta National Hospital, University of Nairobi, Kenya

Background. Preexposure prophylaxis (PrEP) with emtricitabine plus tenofovir disoproxil fumarate (FTC/TDF) or TDF alone reduces the risk of human immunodeficiency virus (HIV) acquisition. Understanding the risk of antiretroviral resistance selected by PrEP during breakthrough infections is important because of the risk of treatment failure during subsequent antiretroviral use.

Methods. Within the largest randomized trial of FTC/TDF versus TDF as PrEP, plasma samples were tested for HIV with resistance mutations associated with FTC (K65R and M184IV) and TDF (K65R and K70E), using 454 sequencing.

Results. Of 121 HIV seroconverters, 25 received FTC/TDF, 38 received TDF, and 58 received placebo. Plasma drug levels in 26 individuals indicated PrEP use during or after HIV acquisition, of which 5 had virus with resistance mutations associated with their PrEP regimen. Among those with PrEP drug detected during infection, resistance was more frequent in the FTC/TDF arm (4 of 7 [57%]), compared with the TDF arm (1 of 19 [5.3%]; $P = .01$), owing to the FTC-associated mutation M184IV. Of these cases, 3 had unrecognized acute infection at PrEP randomization, and 2 were HIV negative at enrollment.

Conclusions. These results suggest that resistance selected by PrEP is rare but can occur both with PrEP initiation during acute seronegative HIV infection and in PrEP breakthrough infections and that FTC is associated with a greater frequency of resistance mutations than TDF.

Keywords. HIV; pre-exposure prophylaxis; antiretroviral resistance; HIV prevention.

The use of daily oral emtricitabine plus tenofovir disoproxil fumarate (FTC/TDF) or TDF alone as preexposure prophylaxis (PrEP) has been demonstrated to

protect against human immunodeficiency virus (HIV) acquisition [1–4]. These results led the US Food and Drug Administration to approve FTC/TDF for PrEP use in the United States, and both FTC/TDF and TDF are being implemented as PrEP worldwide among high-risk populations [5, 6]. However, PrEP is not 100% protective, and antiretroviral resistance could develop in individuals who acquire HIV while taking PrEP. Specifically, PrEP use between HIV acquisition and detection of seroconversion (when PrEP would be discontinued) has the potential to select for drug-resistant virus. In analogous use of antiretrovirals for HIV prevention, resistance commonly occurs in infants

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^aStudy group members are listed at the end of the text.

Correspondence: Dara Lehman, PhD, MHS, 1100 Fairview Ave N, C3-168, Seattle, WA 98109 (dlehman@fhcrc.org).

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who acquire HIV despite prophylaxis used to prevent mother-to-child transmission [7–9].

Data from PrEP efficacy trials indicate that cases of antiretroviral resistance have been rare and predominately limited to individuals who started PrEP during unrecognized, seronegative acute HIV infection [1–4, 10–13]. However, these studies had important limitations. First, most used standard consensus sequencing [1, 2, 4, 11] that only detects resistant variants present at high frequencies (>20%) within the viral population, and HIV treatment studies have shown that resistance present at frequencies as low as 1% can be associated with subsequent treatment failure [14–16]. Intermittent PrEP use could result in low and fluctuating drug levels, an environment in which selective pressure for resistance is not continuous, and thus resistant variants could be present only at low frequencies. Second, in several PrEP studies, many cases of HIV acquisition in the active PrEP arms appeared to occur in the absence of PrEP exposure. For example, one recent study showed resistance was rare, but only 8 seroconverters had evidence of PrEP use within 90 days of seroconversion, and only 4 had drug detected in a sample with documented HIV infection [13].

We performed highly sensitive resistance testing among HIV seroconverters within the largest oral PrEP trial, the Partners PrEP Study, a randomized, placebo-controlled trial of FTC/TDF and TDF PrEP among HIV-uninfected partners in African HIV-serodiscordant couples. In that trial, FTC/TDF and TDF reduced the risk of HIV acquisition by 75% ($P < .001$) and 67% ($P < .001$), respectively [2]. Adherence, measured by detection of PrEP drug in plasma, was the highest of all PrEP trials, and there were cases of HIV acquisition that occurred at times when PrEP medication was detected [17]. Standard consensus sequencing revealed that only 2 individuals in Partners PrEP had evidence of PrEP-related resistance; both had unrecognized acute HIV infection at the time of PrEP initiation [2]. Here we use a more sensitive assay, 454 sequencing, to detect mutations that could be selected by FTC (K65R and M184IV) or TDF (K65R and K70E) in seroconverters from the Partners PrEP Study that have been missed by standard sequencing.

METHODS

Study Population

The Partners PrEP Study was a phase 3, randomized, double-blinded, placebo-controlled trial of oral FTC/TDF and TDF PrEP, the details of which were described previously [2]. Briefly, HIV-uninfected partners of 4747 HIV-serodiscordant couples in Kenya and Uganda were randomly assigned to receive FTC/TDF, TDF, or placebo. Monthly clinic visits included HIV testing. Plasma specimens were stored quarterly, when seroconversion was first detected, and 1 month after seroconversion. In July 2011, the data and safety monitoring board recommended public report of results and discontinuation of

placebo, owing to demonstrated efficacies for HIV protection of 75% for FTC/TDF and 67% for TDF. All placebo-arm participants were subsequently randomly assigned to receive FTC/TDF or TDF [18], and follow-up continued until December 2012. The present analysis includes individuals who seroconverted to HIV through December 2012. Identification numbers are delinked from patient identifiers.

HIV Testing and Tenofovir Concentrations

Rapid HIV tests were conducted at each clinic visit. HIV seroconversions were confirmed by Western blot. To more precisely determine the timing of HIV infection, the Abbot RealTime HIV-1 assay (lower limit of detection, 40 copies/mL) was used to detect HIV RNA in archived plasma specimens obtained before seroconversion. Dates of infection were estimated as the midpoint between the visit during which seroconversion was detected and the prior visit during which the participant tested negative for HIV RNA and HIV antibody, or as 17 days before the first visit during which the participant tested positive for HIV RNA and negative for HIV antibody.

Tenofovir concentrations in stored plasma samples collected every 3 months plus during the first HIV antibody-positive visit from seroconverters assigned to receive FTC/TDF or TDF were determined with liquid chromatography tandem mass spectrometry (lower limit of detection, 0.31 ng/mL) as described previously [17].

Antiretroviral Resistance Testing

All resistance testing was conducted and finalized while blinded to randomization arm. Resistance testing by standard Sanger consensus sequencing was performed during the parent study, and findings are reported elsewhere [2]. 454 sequencing was performed as described previously [15, 19], with modifications described in the [Supplementary Materials](#). 454 sequences are available online (<http://www.ncbi.nlm.nih.gov/sra>; accession no. SRP049715).

Statistical Analysis

The Fisher exact test was used to determine associations with >1% resistance. Analyses were performed with Stata 9.2 and R 3.0.2.

RESULTS

In total, 122 HIV seroconversions occurred during the Partners PrEP Study: 25 had been randomly assigned to receive FTC/TDF; 39, to TDF; and 58, to placebo. Eighteen of the 122 were retrospectively determined to have acute seronegative HIV infection (HIV RNA positive and HIV antibody negative) at the time of PrEP randomization. In the active PrEP arms, including those infected at randomization, the time between the estimated HIV infection date and detection of seroconversion (when PrEP

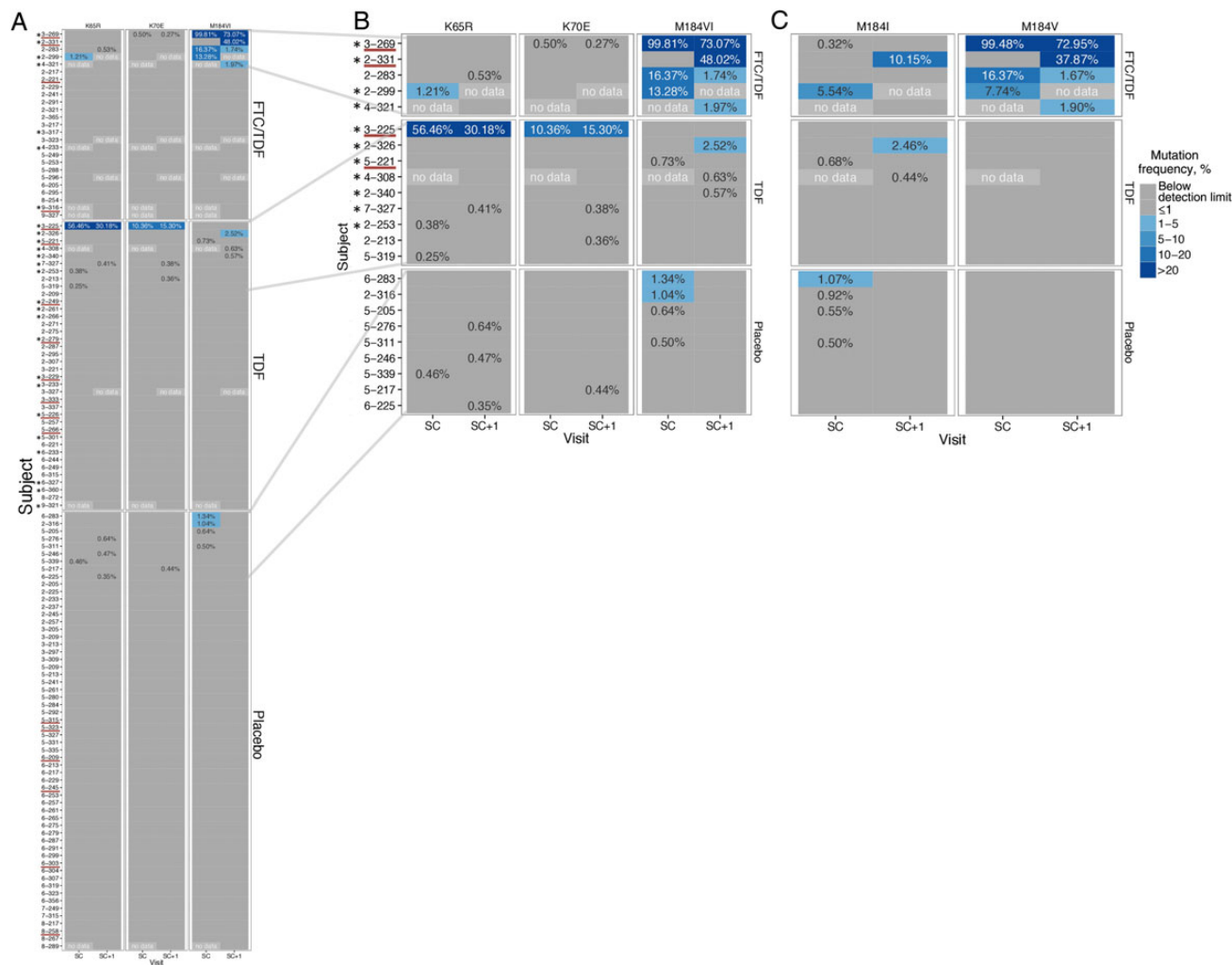


Figure 1. Levels of K65R, K70E, and M184IV in seroconverters in the Partners PrEP Study. *A*, Subjects are ordered by levels of resistance, from highest to lowest, within each treatment arm. Red underline denotes subjects with unrecognized acute infection at treatment assignment. An asterisk prior to the identification number indicates subjects with tenofovir detected in plasma during human immunodeficiency virus infection. Samples tested include the time that seroconversion was first detected (SC) and 1 month after seroconversion (SC + 1). Higher levels of resistance denoted by darker blue color. Grey cells with the percentage in black have levels of resistance of <1% that were detectable above the false-discovery rate. Solid grey cells indicate that levels were below the limit of detection. Cells are marked as “no data” if the sample could not be amplified. *B*, Levels of K65R, K70E, and M184IV in seroconverters with any resistance detectable by 454 sequencing. Table details are summarized in panel *A*. *C*, Levels of M184I or M184V. Table details are summarized in panel *A*. Abbreviations: FTC/TDF, emtricitabine plus tenofovir disoproxil fumarate; TDF, tenofovir disoproxil fumarate alone.

was discontinued) was a median of 45 days (interquartile range [IQR], 15–102 days). Based on partial *pol* sequences, 64 of 122 (52.5%) were infected with a subtype A virus; 7 (5.7%), with subtype C; 29 (23.8%), with subtype D; 16 (13.1%), with CRF01_AE; and 5 (4.1%), with rare subtypes; the *pol* sequence for 1 (0.8%) failed to amplify. The median HIV RNA level at seroconversion was 4.6 log₁₀ copies/mL (IQR, 3.8–5.2 copies/mL).

Low-Frequency Resistance in Seroconverters Randomly Assigned to Receive PrEP

To determine whether resistance developed during the period immediately after PrEP was withdrawn, 121 of 122 seroconverters

were tested for resistance at the visit seroconversion was first detected and/or 1 month after seroconversion (110 were tested at both time points); for 1 participant in the TDF arm, both samples failed to amplify. One or more PrEP-associated resistance mutations (K65R, K70E, and/or M184IV) were detectable in 23 of 121 seroconverters (19%; Figure 1A): 5 of 25 (20%) received FTC/TDF, 9 of 39 (23%) received TDF, and 9 of 58 (16%) received placebo. However, for 14 of these 23 (0 FTC/TDF recipients, 7 TDF recipients, and 7 placebo recipients), resistance was measured only at very low frequency, <1% of the viral population, and in many of those cases with frequencies <1%, resistance was detected only at one of the 2 time points tested

Table 1. Drug Resistance Present at Levels of >1%, According to Treatment Arm at the Time of Seroconversion

Group	Subjects With Resistance Frequencies >1%, Proportion (%), by Treatment Arm			
	FTC/TDF	TDF	Placebo	Overall
All patients	5/25 (20)	2/38 (5.3)	2/58 (3.5)	9/121 (7.4)
Among subjects retrospectively found to be HIV positive at enrollment or rerandomization ^a	2/4 (50)	1/8 (12.5)	0/6 (0)	3/18 (16.7)
Among subjects who acquired HIV after enrollment or rerandomization ^a	3/21 (14.3)	1/30 (3.3)	2/52 (3.8)	6/103 (5.8)

Abbreviations: FTC/TDF, emtricitabine plus tenofovir disoproxil fumarate; HIV, human immunodeficiency virus; PrEP, preexposure prophylaxis; TDF, tenofovir disoproxil fumarate alone.

^a Because of early demonstration of efficacy in the Partners PrEP Study, the placebo arm was discontinued in July 2011, and participants in the placebo arm were randomly assigned to receive FTC/TDF or TDF.

(Figure 1). Because resistance mutations detected at frequencies of <1% were observed across treatment arms (including placebo), false-positive rates in wild-type controls were all $\leq 1.05\%$ (Supplementary Figure 2), and resistance detected at levels <1% has not been associated with increased risk of viral failure during subsequent antiretroviral use [14–16], we focused on resistance frequencies of >1% for the remaining analyses. Nine seroconverters (7.4%) had ≥ 1 PrEP-related mutation at levels of >1% (Figure 1B and Table 1), of whom 7 had been assigned active PrEP. In the FTC/TDF arm, 5 of 25 (20%) had a resistance frequency of >1%, compared with 2 of 38 (5.3%) in the TDF arm and 2 of 58 (3.5%) receiving placebo (FTC/TDF vs placebo, $P = .024$; TDF vs placebo, $P = .65$; Table 2). All 5 cases with a resistance frequency of >1% in the FTC/TDF arm had M184I or M184V alone, while 1 also had K65R (Figure 1B and 1C). In the TDF arm, resistance was due to K65R/K70E in one individual and to a low level of M184I (without M184V) in the other. M184I (without M184V) was also present in 2 placebo arm participants at levels just above 1% (Figure 1C).

Table 2. Factors Associated With Resistance Detected at Frequencies of >1%

Variable	Resistance Frequency >1%		<i>P</i> Value ^a
	Proportion	Percentage	
Treatment arm at seroconversion			
FTC/TDF or TDF vs placebo	7/63 vs 2/58	11 vs 3.4	.17
FTC/TDF vs placebo	5/25 vs 2/58	20 vs 3.4	.024
TDF vs placebo	2/38 vs 2/58	5.2 vs 3.4	.65
FTC/TDF vs TDF	5/25 vs 2/38	20 vs 5.3	.1
Infected when randomly assigned to active PrEP arm			
Yes vs no	3/12 vs 4/51	25 vs 7.8	.12
PrEP use after HIV infection			
TDF detectable vs undetectable	6/26 vs 1/37	23 vs 2.7	.018

Abbreviations: FTC/TDF, emtricitabine plus tenofovir disoproxil fumarate; HIV, human immunodeficiency virus; PrEP, preexposure prophylaxis; TDF, tenofovir disoproxil fumarate alone.

^a By the Fisher exact test.

Resistance in Individuals Randomly Assigned to Receive Active PrEP During Unrecognized Acute Infection

Cases of resistance in PrEP trials have shown that resistance was predominately limited to those who initiated PrEP during unrecognized acute infection [1–3, 10–12]. In the present study, there were 12 individuals retrospectively determined to be HIV infected (ie, seronegative but RNA positive) when randomly assigned to receive active PrEP, and 3 (25%) had resistance frequencies of >1% (Figure 1A and 1B and Table 1). Thus, 3 of 7 cases of resistance detected among those assigned to the active PrEP arm occurred in individuals with unrecognized acute infection at the time of PrEP initiation. Of these, 2 were detected previously by standard consensus sequencing: 1 randomly assigned to receive FTC/TDF had M184V, and 1 randomly assigned to receive TDF had K65R/K70E (previously described as K65R only), neither of which had resistance detected by standard sequencing in the enrollment sample [2]. One additional case of resistance in an individual with unrecognized acute infection at PrEP start was detected by 454 sequencing in the FTC/TDF arm with M184IV. None of 6 individuals with acute infection at randomization who received placebo had resistance.

Resistance in Individuals Infected After Random Assignment to Receive Active PrEP

Among 51 seroconverters infected after enrollment and assigned to receive active PrEP, 4 (7.8%) had a resistance frequency of >1% (Figure 1 and Table 1): 3 FTC/TDF and 1 TDF. The case in the TDF arm (2–326) had only M184I, an FTC-associated mutation known to occur without drug exposure that was also observed in the placebo arm (Figure 1C), and thus we assume resistance in this case was not selected by PrEP exposure. The other 3 cases with >1% resistance frequencies that were infected after PrEP assignment were randomly assigned to receive FTC/TDF, of whom 2 had M184V alone and 1 had M184IV and K65R (Figure 1B and 1C).

Case 2–283 had M184V in 16% of virus variants at the seroconversion visit, which faded to 1.7% by 1 month after seroconversion. This individual was seronegative and HIV RNA

negative at enrollment, month 1, and month 3, after which she did not return to the clinic until month 21, when she tested positive for HIV RNA and HIV antibody. Plasma tenofovir was undetectable in samples from all clinic visits, suggesting that resistance may have been transmitted in the absence of drug. Interestingly, K103N (a non-PrEP-related resistance mutation) was also present in 18% of virus variants at the seroconversion visit, which faded below detection by 1 month after seroconversion. Consensus sequencing and phylogenetic analysis of her HIV-infected partner's virus revealed that the partner was not the source of this infection (data not shown). Thus, further information about the possibility of transmitted resistance is not available.

Case 2–299 had M184V, M184I, and K65R in 7.7%, 5.5%, and 1.2% of virus variants, respectively, at the seroconversion visit. No sample was available from 1 month after seroconversion. HIV RNA was first detected 3 months after enrollment, and seroconversion was detected at month 4. Plasma tenofovir levels revealed that levels of PrEP drug were undetectable at enrollment and month 3 but were 74 ng/mL at month 4, when seroconversion was detected. Consensus sequencing of the HIV-infected partner's virus did not detect resistance mutations, and phylogenetic analysis revealed that the HIV-infected partner was the source of infection, suggesting that transmitted resistance was unlikely.

Case 4–321 had 1.9% of virus variants with M184V 1 month after seroconversion, with no data available from the sample at seroconversion, which had an undetectable viral load (<80 copies/mL). This participant regularly attended clinic study visits, had HIV infection first detected 15 months after enrollment, and had consistently detectable tenofovir levels (range, 15–108 ng/mL) at months 1, 3, 6, 9, and 12, with a concentration of 66 ng/mL when HIV was first detected, at month 15. Resistance was not detected by consensus sequencing in this case's HIV-infected partner, who appeared to be the source of this infection, based on phylogenetic analysis.

Risk of Resistance and Association With PrEP Drug

The risk of detecting resistance in >1% of a participant's virus was highest in seroconverters who had evidence of PrEP use during or after HIV seroconversion, as determined by detectable tenofovir levels in samples that also had HIV RNA and/or HIV antibody detected ($P = .018$, compared with tenofovir levels below the detection limit; Table 2). Specifically, among participants with PrEP drug detectable in plasma after HIV acquisition, 5 of 26 had resistance mutations associated with their PrEP regimen at frequencies of >1%; 4 of 7 were in the FTC/TDF arm, and 1 of 19 were in the TDF arm (57% vs 5%; $P = .01$).

DISCUSSION

We used a highly sensitive assay to detect low-frequency resistance among HIV seroconverters participating in a randomized

trial of PrEP. Overall, resistance was detected in a minority of seroconverters, including those with objective evidence of PrEP use during HIV seroconversion. Nevertheless, we showed that resistance at frequencies of >1%, likely selected by PrEP use, was present in 5 of 26 individuals whose plasma tenofovir levels indicated PrEP use between HIV acquisition and PrEP discontinuation. Notably, 2 of these individuals (2–299 and 4–321) were infected after enrollment, had detectable plasma tenofovir levels after HIV acquisition, and had mutations known to be associated with the PrEP drugs they were assigned. Cases of resistance in individuals who acquired HIV while taking PrEP (ie, breakthrough infections) have rarely been reported [11], and it is uncertain whether the previously published cases were true cases of selected resistance in PrEP breakthrough infections, cases of transmitted drug resistance, or cases of resistance in individuals with undetected infection at enrollment [11]. Ours is the first study to indicate that, although antiretroviral resistance selected by PrEP is rare, resistance can occur both in settings of PrEP exposure during unrecognized acute infection and in breakthrough infections and that it may be more common with FTC/TDF than TDF alone.

The 5 cases (7.9%) of PrEP-selected resistance (3 infected at enrollment [3–269, 2–331 and 3–225] and 2 infected after enrollment [2–299 and 4–321]) among 63 seroconverters in the active PrEP arms suggest that resistance is likely to be rare as PrEP is scaled up. The observed rate of resistance is much lower than that assumed by multiple mathematical models, which estimated that acquired resistance due to PrEP could be 25%–44% [20]. As shown previously, high adherence and consistent PrEP use is associated with a relative risk reduction of approximately 90%, and drug level testing indicates that the majority of those who acquire HIV were likely not receiving PrEP during HIV seroconversion [17, 21], resulting in very low risk of resistance because of the lack of drug pressure. However, the risk of resistance may be higher in individuals receiving PrEP around the time of HIV infection and in those with prolonged PrEP use after HIV infection due to infrequent follow-up testing [3]. In this study, 9 individuals assigned to receive active PrEP who had unrecognized acute infection at randomization had evidence of PrEP use, of whom 3 had a resistance frequency of >1%. Of the 17 individuals who acquired HIV after randomization and also had evidence of PrEP use during HIV infection, 2 had a resistance frequency of >1% associated with their PrEP regimen. Importantly, the 5 cases that appear to be PrEP-related resistance should be weighed against the number of HIV infections averted, estimated at 74 through the end of placebo use and at 123 during the follow-up period (values were calculated as the HIV rate in the placebo arm minus the HIV rate in the active arms) [5, 21].

The rate of resistance in individuals treated with FTC/TDF was higher than that in individuals who received single-agent TDF, owing to the M184V mutation, which has lower fitness

costs and confers a higher level of resistance than K65R [22–24]. There was only 1 case of TDF-associated resistance among 38 seroconverters in the TDF arm, even though a higher proportion of seroconverters in the TDF arm (50%), compared with the FTC/TDF arm (28%), had tenofovir detected at some point after HIV acquisition. These findings, along with the combined data from all PrEP clinical trials, as well as studies of PrEP in macaques [25], in which M184IV is the predominant mutation detected, suggest that FTC resistance is more likely to emerge than TDF resistance [25–27] and that use of TDF-alone as PrEP may carry a lower risk of resistance. However the small risk of resistance observed with FTC/TDF must be balanced with the efficacy results, which showed a higher (although not statistically significant) risk reduction with FTC/TDF, compared with TDF alone, in this heterosexual population [21]. The relative balance of efficacy, safety (including resistance risk), cost, and other considerations for FTC/TDF versus TDF PrEP may be informed by additional clinical studies, mathematical modeling, and policy discussions.

There were 2 cases of PrEP-associated resistance in individuals who acquired HIV after enrollment and had evidence of PrEP use during infection. One of the cases (2–299) did not have detectable levels of tenofovir at the visit when HIV RNA was first detected (month 3) but had a tenofovir level consistent with daily dosing [17] at the next visit (month 4), when seroconversion was detected. This suggests that the individual did not take drug in the week prior to the month 3 visit and then took PrEP concurrent with or after HIV acquisition. While this case was infected with subtype C, which can result in a low frequency of K65R without drug selection [28], the presence of M184IV and detection of tenofovir during HIV infection suggests PrEP-selected resistance in this case.

Case 4–321 had high levels of tenofovir between months 3 and 15, when HIV antibody was first detected. This tenofovir pattern suggests consistent PrEP use, and the infection thus may be a true PrEP breakthrough infection. However, tenofovir levels were assessed only every 3 months, and thus missed doses and intermittent use cannot be ruled out. This case documents the possibility that infection that occurs during PrEP use can result in resistance below the level of detection of standard resistance testing.

We detected resistance at frequencies of >1% in 4 individuals who had mutations not likely associated with PrEP use. Three of these cases had only the FTC-associated mutation M184I but had no exposure to FTC, because they were randomly assigned to receive TDF or placebo. This result was not surprising because M184I is known to be polymorphic due to G to A hypermutation caused by Apobec3G and has been seen in antiretroviral-naïve populations [29]. We also observed a case of resistance (2–283) in the FTC/TDF arm that we cannot attribute to PrEP, because plasma tenofovir levels were undetectable in available samples. However, we do not have data from the

time between months 3 and 21, and it is unclear when this participant became infected. The fact that this individual's virus also carried K103N (a non-PrEP-related mutation) suggests that this is a likely case of transmitted resistance. However, we cannot rule out that the M184V mutation was selected by intermittent PrEP use.

One limitation to our study is that the objective measure of PrEP use was detectable levels of tenofovir in samples collected only every 3 months. Tenofovir has a half-life of 17 hours and, following a single dose, can be detected for only an average of 7 days with the assay used here [30]. Therefore, the lack of detectable tenofovir in a participant's quarterly samples does not rule out intermittent PrEP use. Additional limitations to our study include the fact that 454 sequencing error could yield false-positive results in our data, in particular at the K65R locus, which is homopolymeric. However, studies comparing 454 sequencing to Illumina (another next-generation sequencing assay) showed that these assays produce similar results, even for the low-frequency K65R mutation [13, 31]. Importantly, our use of genotype-specific RNA controls allowed us to determine the error rate in our 454 sequencing assay (Supplementary Materials) and to ensure that resistance mutations detected were statistically significantly above background error. We did detect samples with resistance frequencies of <1% that were distributed across treatment arms, including placebo, suggesting that resistance may be present at very low levels in both PrEP-exposed and PrEP-naïve individuals, as shown in other studies [32–35]. However, a small contribution from false-positive findings cannot be ruled out, because we saw a 3.1% false-positive rate among experiments testing wild-type templates (Supplementary Materials).

Another limitation is that only 7 of 25 seroconverters in the FTC/TDF arm and 19 of 38 seroconverters in the TDF arm had detectable levels of tenofovir during HIV infection. While these small numbers limit our ability to determine the prevalence of resistance that will occur in individuals who become infected during PrEP use when PrEP is implemented on a wider scale with less frequent monitoring of HIV infection, the details of each case of resistance observed here with tenofovir detected during HIV infection provides information that may inform the risk of resistance and the importance of regular monitoring for HIV infection during PrEP. In addition, the number of seroconverters with PrEP drug detected during HIV infection was considerably higher ($n = 26$) in our study than in any other PrEP clinical trial (which have had 4–7 subjects with detectable PrEP drug in samples with HIV RNA or HIV antibody also detected) [4, 10, 11, 13]. The fact that the majority of seroconverters did not have detectable tenofovir levels during HIV seroconversion reflects the high efficacy of PrEP among those who received it, resulting in low resistance risk overall.

The impact of low-frequency resistance on subsequent treatment has been studied more extensively in the context of

mother-to-child transmission, for which HIV prophylaxis has been in routine use for HIV prevention. Resistance present at frequencies undetected by standard resistance assays have been shown to compromise subsequent combination treatment in HIV-infected women and infants previously exposed to antiretrovirals through maternal or infant prophylaxis [14, 15, 33]. This suggests that some individuals with PrEP breakthrough infections may be at risk for treatment failure due to resistance not identified by standard resistance testing.

In conclusion, our highly sensitive resistance testing with 454 sequencing technology confirms previous findings with consensus sequencing that resistance occurred in a minority of seroconverters. The risk of resistance was highest in those with unrecognized acute infection at PrEP initiation, suggesting that careful screening is important in PrEP implementation. In addition, we document that resistance that arises in individuals who acquire HIV while taking PrEP is predominately due to the FTC-selected mutation M184V.

STUDY GROUP MEMBERS

University of Washington Coordinating Center and Central Laboratories (Seattle, Washington): Connie Celum (principal investigator, protocol cochair), Jared M. Baeten (medical director, protocol cochair), Deborah Donnell (protocol statistician), Robert W. Coombs, Lisa Frenkel, Craig W. Hendrix, Mark A. Marzinko, Jairam Lingappa, and M. Juliana McElrath.

Study sites (institutions) and site principal investigators: Eldoret, Kenya (Moi University and Indiana University): Kenneth Fife and Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye; Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase and Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira and Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute and University of California–San Francisco): Elizabeth Bukusi and Craig Cohen; Mbale, Uganda (The AIDS Support Organization and CDC-Uganda): Jonathan Wangisi, James Campbell, and Jordan Tappero; Nairobi, Kenya (University of Nairobi and University of Washington): James Kiarie, Carey Farquhar, and Grace John-Stewart; Thika, Kenya (University of Nairobi and University of Washington): Nelly Rwamba Mugo; and Tororo, Uganda (CDC-Uganda and The AIDS Support Organization): James Campbell, Jordan Tappero, and Jonathan Wangisi.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (<http://jid.oxfordjournals.org>). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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D. A. L., J. M. B., C. C., and J. O. designed the low-frequency resistance study. D. A. L., J. F. W., D. P., G. M., and J. O. designed or conducted the experiments. C. O. M. and F. A. M. designed and conducted the bioinformatic analysis of the 454 sequencing data. D. A. L., J. M. B., C. O. M., D. D., K. K. T., and F. A. M. developed and conducted the statistical analysis. C. W. H. and M. A. M. designed and conducted the pharmacological work. L. F. conducted the Sanger consensus sequencing. J. M. B., D. D., P. N., N. R. M., and C. C. designed and conducted the Partners PrEP Study. D. A. L., J. M. B., and J. O. wrote the first draft of the manuscript. All authors assisted with the writing of the manuscript.

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