HIV-1 Drug Resistance in the iPrEx Preexposure Prophylaxis Trial

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Background. The iPrEx study demonstrated that combination oral emtricitabine and tenofovir disoproxil fumarate (FTC/TDF) as preexposure prophylaxis (PrEP) protects against HIV acquisition in men who have sex with men and transgender women. Selection for drug resistance could offset PrEP benefits.

Methods. Phenotypic and genotypic clinical resistance assays characterized major drug resistant mutations. Minor variants with FTC/TDF mutations K65R, K70E, M184V/I were measured using 454 deep sequencing and a novel allele-specific polymerase chain reaction (AS-PCR) diagnostic tolerant to sequence heterogeneity.

Results. Control of primer-binding site heterogeneity resulted in improved accuracy of minor variant measurements by AS-PCR. Of the 48 on-study infections randomized to FTC/TDF, none showed FTC/TDF mutations by clinical assays despite detectable drug levels in 8 participants. Two randomized to FTC/TDF had minor variant M184I detected at 0.53% by AS-PCR or 0.75% by deep sequencing, only 1 of which had low but detectable drug levels. Among those with acute infection at randomization to FTC/TDF, M184V or I mutations that were predominant at seroconversion waned to background levels within 24 weeks after discontinuing drug.

Conclusions. Drug resistance was rare in iPrEx on-study FTC/TDF-randomized seroconverters, and only as low-frequency minor variants. FTC resistance among those initiating PrEP with acute infection waned rapidly after drug discontinuation.

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Antiretroviral drugs have demonstrated efficacy when used as preexposure prophylaxis (PrEP) for preventing human immunodeficiency virus (HIV) acquisition through sexual or intravenous exposure in uninfected men, women, and transgendered women [1–5]. A consistent finding among reported randomized, placebo-controlled trials testing oral and topical PrEP strategies is that efficacy is associated with drug exposure [6, 7]. In the iPrEx study, where the safety and efficacy of daily oral dosing of combination nucleoside/nucleotide reverse-transcriptase inhibitors (nRTI) emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) was tested in men and transgender women who have sex with men, a 44% reduction in infection incidence was seen in participants randomized to FTC/TDF compared with placebo [3], and a 99% reduction among those with blood FTC/TDF levels commensurate with daily dosing [8]. In contrast, in the Fem-PrEP and
VOICE trials where product adherence was low, the use of oral TDF, TDF/FTC, or tenofovir (TFV) 1% vaginal gel did not significantly reduce HIV infections [9].

Decades of experience with therapeutic use of antiretroviral drugs have accumulated an extensive understanding of the causes and consequences of HIV drug resistance (DR), which emerges with suboptimal antiretroviral therapy (ART), including the dual nRTI regimens such as that recommended for PrEP [10–12]. If HIV infection occurred during suboptimal PrEP use, DR could be selected early in infection [13, 14]. In rhesus macaque studies investigating oral FTC/TDF PrEP, breakthrough infections following rectal exposure showed FTC-selected DR mutations M184V/I [15]. In people with unrecognized infection, antiretroviral use intended as PrEP is functionally postexposure prophylaxis (PEP), where DR can occur in breakthrough systemic infection [2–4, 16, 17]. Additionally, initial efforts administering single-dose nevirapine to mothers at birth for prevention of mother-to-child-transmission (MTCT) resulted in rapid emergence of drug-selected mutations in infected infants measured by population sequencing [18–22]. Ultra sensitive diagnostic assays revealed a substantially higher frequency of mothers and infants with minor variant DR [23–26]. These findings demonstrate that the use of antiretrovirals to prevent infection can select for DR when breakthrough infections occur, and warrant thorough and sensitive monitoring in PrEP studies.

We report the results from comprehensive viral DR testing and drug exposure measurements in iPrEx seroconverters in a clinical trial setting. Clinical genotype and phenotype assays were performed to detect resistance in viral populations. We quantified minor variant FTC/TDF-selected mutations by deep sequencing and a novel allele-specific polymerase chain reaction (AS-PCR)–based assay that controls for target sequence polymorphisms and yields improved performance when testing isolates from diverse geographic locations [27–29]. The nature and frequency of DR mutations are presented in the context of concurrent systemic FTC/TDF exposure.

MATERIALS AND METHODS

Study Design and Sample Selection
The iPrEx study design, conduct, and primary analysis results through 1 May 2010 are described [3]. The protocol was approved by institutional review boards at all participating sites. All study participants provided written informed consent. Participants were monitored for HIV infection by antibody testing at monthly visits. Study drug was discontinued with confirmed seroconversion (SC) and subjects monitored prospectively for plasma HIV-1 RNA levels. Retrospective HIV-1 RNA testing was performed on archived specimens from visits at and prior to SC to define the infection window (last RNA negative to first RNA positive). Plasma HIV RNA was tested for DR at the first SC visit or if unavailable, a pre-SC RNA positive, or proximal post-SC sample. Participants randomized to FTC/TDF with any drug-associated resistance mutation by genotype were monitored for DR longitudinally.

Sample Preparation and HIV Resistance Testing
Blood plasma HIV RNA was assessed for DR by HIV-1 pol population sequencing with mixed base detection at approximately 20% (TRUGENE, Siemens Healthcare Diagnostics, Inc, Tarrytown, NY); the Phenosense assay (Monogram Biosciences, Inc. South San Francisco CA) for drug susceptibility profiles; an AS-PCR-based quantitative minor variant assay (qMVA) as the primary analysis targeting rt K65R, K70E, M184V, and M184I; and 454 GS-FLX Titanium single-amplicon deep sequencing (Roche Applied Sciences, Pleasanton, CA) for confirmatory analysis. Population and 454 sequences are available in GenBank (Accession numbers KJ585857-KJ586124 and SRP037739, respectively). Cell-free virions were concentrated from 1 mL ethylenediaminetetraacetic acid blood plasma by centrifugation at 52 800 × g for 60 minutes at 4°C. RNA was extracted using the QIAamp viral RNA kit (QIAGEN, Inc, Valencia, CA). For nonsubtype C viruses, minor variant DR was quantified directly from the 1.3 kilobase (kb) sequencing amplicon generated by 1-step reverse transcription (RT)–PCR. For subtype C viruses, a 692 base-pair (bp) amplicon (homebrew amplicon, HBA) was generated using high-fidelity conditions to minimize base misincorporation in homopolymer regions adjacent to and within the rt 65 codon during template generation [29–32], which decreased the background mutation frequency measured by both qMVA and deep-sequencing assays (Supplementary Methods).

Quantitative Minor Variant Assay
The qMVA combines an initial “cure” PCR of the rt amplicon followed by AS-PCR using sybr-green master mix (AnaSpec, Fremont, CA) in parallel reactions with primers specific for wild-type (WT) and mutant (Mut) codons. The cure step is an 8-cycle PCR that normalizes AS-PCR primer target sites to consensus sequences, while maintaining the single-nucleotide polymorphism (SNP) conferring resistance. The percent minor variant was determined by extrapolating the participant sample ΔCt (Ct [Mut primers] minus Ct [WT primers]) from AS-PCR reactions against a 6-point standard curve. In samples where Mut or WT/Mut mixtures were detected by population sequencing, primers designated as major or minor variant were adjusted accordingly. Detailed assay conditions, primers, and assay performance characteristics are described in Supplementary Methods.

454 Sequencing
The 1.3 kb or 692 bp HIV rt amplicon containing FTC/TDF DR sites were further amplified to generate a 517 or 535 bp
amplicon, including 25-base Titanium adapter and 10-base multiplex identifier sequences. Following purification and quantification, amplicons were pooled, diluted to 1.0 × 10⁶ molecules/µL, and further amplified by emulsion PCR using the Roche 454 emPCR Kit (Lib A) (Roche Applied Sciences, Indianapolis, IN) according to the manufacturer’s instructions. Recovered DNA beads were sequenced using a 454 GS FLX+ with Titanium sequencing chemistry (Supplementary Methods). The Roche Amplicon Variant Analysis software (version 2.6) was used for determining codon variant frequencies by employing predefined codon changes at resistance sites. Subtype-matched consensus sequences (http://www.hiv.lanl.gov) were used as alignment references.

Biological Cut-off Values for Minor Variant Frequencies

The biological cut-off (BCO) was determined to define the natural frequency of DR mutations in the absence of drug exposure, and above which a sample was considered positive. We defined the BCO for each DR site, template (TRUGENE or HBA), and assay primer set (qMVA and 454 sequencing) using panels of 12 subtype B and 9 subtype C samples from individuals infected prior to the general availability of antiretroviral drugs, selecting for K65R, K70E, and M184V/I (Supplementary Methods). The BCO was defined as the highest value measured plus 3 SD, or 0.50%, whichever was higher. BCO values ranged from 0.50% to 0.60% for the qMVA, and 0.50% to 1.70% for 454 assays.

HIV-1 Subtype and Surveillance Drug Resistance Mutation Assignment

HIV-1 subtypes were assigned from population sequences of HIV-1 protease codons 10–99 and reverse transcriptase codons 40–247 using the Recombinant Identification Program (RIP, http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html) with consensus alignment, 300 nucleotide (nt) window size, 90% confidence threshold in regions of significant match between query and consensus sequence. Surveillance DR mutations (SDRM) were derived from a consensus genotypic definition for identifying transmitted mutations [33, 34] and were assigned from population sequences by the Stanford HIV DR Database CPR tool (v6.0) (http://cpr.stanford.edu) [35].

Statistical Methods

Between-group comparisons were performed using a 2-sample t test with unequal variances.

RESULTS

Seroconversions by Randomization Arm and Viral Subtypes

Of the 2499 randomized iPrEx participants, 2451 (98%) were followed at 1 or more visits (1226 FTC/TDF, 1225 placebo). There were 141 infections, including 10 subjects (2 FTC/TDF, 8 placebo) with acute infection at study entry unrecognized by serology-based screening (HIV RNA positive, seronegative). Postrandomization infections included 48 FTC/TDF and 83 placebo (Figure 1). Virus subtypes were predominately subtype B, reflecting recruitment predominance in North and South America (1% A1, 85% B, 5% B/C or B/F mosaic, 2% C, 3% CRF01_AE, 2% F and 2% G).

Accurate Minor Variant Quantification With Tolerance to Sequence Polymorphisms

Viral sequence diversity presents challenges for quantitative target sequence-based diagnostics, especially in homopolymer regions adjacent to DR codons such as subtype C K65R [29, 30, 32]. To increase the specificity and accuracy of AS-PCR strategies for minor variant quantification across multiple subtypes, we developed the quantitative minor variant assay (qMVA), which includes a “cure” PCR step before the AS-PCR to normalize the primer target sites adjacent to the SNP conferring resistance to defined, consensus-based sequences (Figure 2).

To evaluate the diagnostic impact conferred by viral sequence polymorphisms in minor variant quantification by AS-PCR in the presence and absence of a cure-PCR, we measured mixtures of K65R (aga vs aaa for WT and Mut, respectively) at 0%, 0.5%, 1.0%, and 10% Mut in a consensus B background template, with or without 4 polymorphic bases in both discriminatory and universal primer-binding regions. The percent mutant was measured by the qMVA using the matched and mismatched panels in the presence and absence of a cure-PCR step (Supplementary Table 1). In the absence of a cure-PCR, the homologous AS-PCR primer:template accurately quantified minor variant DR mixtures, yielding values of 0%, 0.48%, 1.02%, and 10.38% Mut for the panel. However, when the same primers were used with a mismatched template, mutant frequencies at levels <1.0% were overestimated, while those at 10% target levels were underestimated. Addition of a cure step prior to the AS-PCR reaction restored the accurate measurement of mutant frequencies with mismatched AS-PCR primer and template (0 = 0.04%, 0.5 = 0.44%, 1 = 0.94%, and 10 = 9.5%). These results demonstrate the potentially deleterious effects of sequence polymorphisms when present within the AS-PCR primer targets, resulting in inaccurate minor variant frequency estimates.

Genotypic, Phenotypic, and Minor Variant Drug Resistance

Among the 131 participants with incident infections, none had FTC- or TDF-selected mutations or reduced phenotypic susceptibility (Figure 1). As previously reported, DR was detected exclusively in those initiating PrEP during unrecognized acute infection at study entry. Of these, genotypic and phenotypic resistance to FTC was seen in 2 of 2 participants randomized to FTC/TDF and 1 of 8 randomized to placebo with mutations M184V and I, with maximally increased fold change half maximal inhibitory concentration (IC₅₀) values for FTC [3]. Both FTC-resistant viruses from participants randomized to FTC/TDF were hypersusceptible to TDF and zidovudine (ZDV;
Figure 1. Genotype, phenotype, and minor variant drug resistance in iPrEx seroconverters by randomization arm. Genotype and phenotype results from a subset of iPrEx participants were described previously in the interim analysis report [3] and are included here for a cumulative assessment. Of the 131 participants with incident infection (48 FTC/TDF, 83 placebo), drug resistance assays were performed on specimens collected at first evidence of seroconversion (n = 128) or if unavailable, the HIV RNA-positive visit prior to (n = 2) or following (n = 1) seroconversion. Minor variant drug resistance testing by qMVA was performed on all participants at 1 or more drug resistance mutations. The number of mutation sites ineligible for testing based on preestablished criteria regarding cure primer 3’ end match with viral target sequence among the 131 samples possible are as follows: K65R: n = 2 (1.5%); K70E: n = 11 (8.4%); M184V: n = 5 (3.8%); and M184I: n = 4 (3.0%). Plasma virus from 1 participant was not available for analysis by 454 sequencing (FTC/TDF arm). Abbreviations: 3TC, lamivudine; BCO, biological cut-off; FC IC50, fold change half maximal inhibitory concentration; FTC, emtricitabine; HIV, human immunodeficiency virus; MAX, maximal FC IC50; PCR, polymerase chain reaction; qMVA, quantitative minor variant assay; TDF, tenofovir disoproxil fumarate; ZDV, zidovudine.
Figure 2. Schematic of the qMVA for low-level drug resistance. A, FTC/TDF selected mutations measured by the qMVA. DNA amplicons spanning TDF-associated mutations K65R, K70E, and FTC-associated mutations M184V/I are generated by nested RT-PCR from plasma HIV-1 virions, representing the predominant and minor quasispecies. The example shows a hypothetical viral mixture at rt 65 with 99% WT (consensus B codon AAA, Lys) and 1.0% Mut (consensus B codon AGA, Arg) where the mutant sequence is undetectable by population sequencing.

B, Cure PCR step. The cure PCR step is performed prior to the AS-PCR step to normalize possible sequence heterogeneity in the AS-PCR primer target sites that can reduce PCR efficiency and result in inaccurate quantification of the minor variant population. The example portrays a virus that differs from the discriminatory AS-PCR primer at 2 sites upstream from the SNP conferring resistance. A low-stringency PCR with limited cycle number is performed on the mixed WT (99%) and Mut (1%) DNA amplicons, using a consensus sequence-based primer pair covering the AS-PCR target sites adjacent to but not including the SNP conferring resistance. When paired with another consensus primer, AS-PCR target amplicons are generated representing the original WT-Mut SNP mixtures, but with AS-PCR target sequences...
M184V virus showed fold change IC_{50} of 0.46 and 0.36; M184I showed fold change IC_{50} of 0.44 and 0.22, respectively. Viruses from 6 participants (3 FTC/TDF, 3 placebo) had other single nucleoside reverse-transcriptase inhibitor (NNRTI; M41L), non-nucleoside reverse-transcriptase inhibitor (NNRTI; K103N/E), or protease inhibitor (PI)—selected (185V) SDRM [33, 34] (Table 1). The participant with acute infection who was randomized to placebo had multiclass resistance mutations M184V, T215Y, K103N, and P225H. These mutations, appearing in the absence of drug exposure, likely result from acquisition of resistant virus from a treatment-experienced source.

Minor variant DR at FTC/TDF-selected codons was measured in participants with incident infections. Of those randomized to FTC/TDF, plasma virus from 48/48 (100%) was assayed by qMVA and 47/48 (98%) by deep sequencing. Plasma virions from all subjects randomized to placebo (83/83) were assayed by both ultrasensitive tests (Figure 1). The viral load (95% confidence interval [CI]) for samples tested was sufficiently high for detecting mutants at 0.5% in both randomization arms (mean, 95% CI, FTC/TDF 5.24 log_{10} cps/mL, 5.00–5.48; placebo 5.13 log_{10} cps/mL, 4.98–5.29; P = .44).

Of the seroconverters randomized to FTC/TDF, none showed minor variant mutations above the BCO at K65R or K70E. Two subjects showed the FTC-associated resistance mutation M184I at <1.0%, one by 454 sequencing exclusively (0.75%), and another by qMVA (0.53%). Repeat testing by qMVA using an independent amplification product yielded a value just below the BCO of 0.5% (0.43%), most likely reflecting normal variation bracketing the established cutoff rather than selected resistance. Among those randomized to placebo, 1 participant had 0.69% K65R by qMVA and 1.64% by deep sequencing. Another subject showed minor variant M184V by both qMVA (1.26%) and 454 sequencing (2.75%). In summary, minor variant DR mutations were rare, and when detected, were measured at very low frequencies.

**Longitudinal Monitoring of FTC-resistance Mutations**

The proportion of contemporaneous Mut:WT species was monitored over time in both participants with unrecognized infection prior to FTC/TDF randomization (Figure 3). Minor variant frequencies by qMVA and 454 sequencing were highly concordant. At study entry, the subject in Figure 3, panel A, showed high viral load consistent with acute infection, WT genotype, and no detectable minor variant DR. At first evidence of seroconversion (study week 4), there was partial virologic suppression with detectable study drug in peripheral blood mononuclear cells (PBMCs) (TFV-diphosphate [DP] = 18.7 fmol/10^6 cells, FTC-triphosphate [TP] = 0.944 pmol/10^6 cells). The genotype revealed mixed M184VM/V, with predominance of the Mut codon (84% by qMVA, 85% by 454 sequencing), consistent with selection by FTC. Following study drug withdrawal at the SC visit, plasma viral load increased to 5.42 log_{10} cps/mL, and M184V waned to <0.5% within 14 weeks, remaining at background levels through 52 weeks of follow-up.

The second subject (Figure 3, panel B) had insufficient plasma viremia for bulk or minor variant assays at entry (1.68 log_{10} cps/mL). By the next visit, plasma viremia had increased with nonsuppressive drug exposure (TFV-DP = 1.47 fmol/10^6 cells, FTC-TP = 0.073 pmol/10^6 cells) and M184I was evident by genotype. The mutation M184I remained predominant at week 15 (99.5% by qMVA, 98.1% by 454 sequencing), dropping to <0.5% by week 29, and remaining at residual levels.

**Table 1. Surveillance Drug Resistance Mutations**

<table>
<thead>
<tr>
<th>No.</th>
<th>NRTI</th>
<th>NNRTI</th>
<th>PI</th>
<th>Treatment Group</th>
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</thead>
<tbody>
<tr>
<td>3a</td>
<td>None</td>
<td>None</td>
<td>185V</td>
<td>FTC/TDF</td>
</tr>
<tr>
<td>3b</td>
<td>M41L</td>
<td>None</td>
<td>None</td>
<td>Placebo</td>
</tr>
<tr>
<td>3c</td>
<td>M41L</td>
<td>None</td>
<td>None</td>
<td>Placebo</td>
</tr>
<tr>
<td>4</td>
<td>None</td>
<td>K103N</td>
<td>None</td>
<td>FTC/TDF</td>
</tr>
<tr>
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<td>K103N</td>
<td>None</td>
<td>FTC/TDF</td>
</tr>
<tr>
<td>6</td>
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<td>K101E</td>
<td>None</td>
<td>Placebo</td>
</tr>
<tr>
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<td>K103N, P225H</td>
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<td>Placebo</td>
</tr>
<tr>
<td>8</td>
<td>M184MV</td>
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<td>None</td>
<td>FTC/TDF</td>
</tr>
<tr>
<td>9</td>
<td>M184I</td>
<td>None</td>
<td>None</td>
<td>FTC/TDF</td>
</tr>
</tbody>
</table>

FTC-associated drug resistance mutations in bold. Genotypic drug resistance mutations are from samples collected at first evidence of seroconversion with sufficient plasma viral load.

Abbreviations: FTC/TDF, emtricitabine + tenofovir disoproxil fumarate; NNRTI, nonnucleoside reverse-transcriptase; NRTI, nucleoside reverse-transcriptase inhibitor; PI, protease inhibitor.  

- Subjects 1–6 infected postrandomization.  
- Subject 7 had unrecognized infection at study entry with NRTI mutations M184V, T215Y and NNRTI mutations K103N, P225H by genotype.  
- Subject 8 had unrecognized infection at study entry with wild-type genotype.  
- Subject 9 had unrecognized infection at study entry with indeterminate genotype due to insufficient plasma viral load.

**Figure 2 continued.** normalized to HIV-1 consensus sequences. C, AS-PCR reaction. Amplicons generated by the cave PCR are diluted appropriately and duplicate PCR reactions are performed with primers specific for the WT or Mut SNP. The discriminatory capability of the AS-PCR primers is enhanced by incorporating a 3′, 2 base mismatch into the AS-PCR primer [36]. D, Determining percent mutant by ΔCt. The percent minor variant in a mixture of WT:Mut amplicons is determined by ΔCt measurements from real-time PCR using WT- and Mut-specific discriminatory primers. When extrapolated by linear regression against a 6-point standard curve run simultaneously (0.1% to 24.3% Mut input in WT background), the percent minor variant (65R) can be derived. Abbreviations: AS-PCR, allele-specific polymerase chain reaction; FTC, emtricitabine; HIV, human immunodeficiency virus; Mut, mutant; PCR, polymerase chain reaction; qMVA, quantitative minor variant assay; RT-PCR, reverse-transcriptase PCR; SNP, single nucleotide polymorphism; TDF, tenofovir disoproxil fumarate; WT, wild-type.
Drug Exposure Levels Among Seroconverters

The absence of PrEP-selected DR in subjects with postrandomization infection, and the rare occurrence of minor variant DR may reflect the low adherence reported in iPrEx [3, 8]. To address drug resistance in the context of measured drug exposure, we examined the plasma FTC and TFV and intracellular PBMC

![Figure 3](https://academic.oup.com/jid/article/210/8/1217/2911872)

**Figure 3.** Longitudinal measurements of FTC-associated drug resistance over time. HIV-1 Log10 viral load vs the relative proportion of FTC-associated drug resistance mutations M184V (A) and M184I (B) measured by the qMVA (blue, solid lines) and 454 deep sequencing (black, dashed lines) are shown for each of 2 iPrEx participants with unrecognized infection at baseline, and randomized to FTC/TDF. The dashed green line represents the lowest measured BCO value between the qMVA and 454 sequencing assays (0.5%), above which values are considered positive. Shaded areas represent the potential FTC/TDF exposure window from the entry/randomization visit to first evidence of seroconversion, when study drug was terminated. Abbreviations: BCO, biological cut-off; Cps, copies; FTC, emtricitabine; HIV, human immunodeficiency virus; qMVA, quantitative minor variant assay; TDF, tenofovir disoproxil fumarate.
FTC-TP and TFV-DP levels in all participants with incident infection where at least 1 measurement was detectable within 90 days prior to or including the seroconversion visit (Figure 4). Eight of 48 (17%) subjects had detectable drug within 90 days of seroconversion. Four of 48 (8.3%) participants had detectable drug prior to, but not at the SC visit, and 3/48 (6.3%) had detectable drug only at SC, indicating intermittent dosing. One subject (Figure 4, orange symbols) had detectable drug prior to and at the SC visit. The participant with minor variant M184I (0.53% by qMVA, open symbols) had undetectable plasma drug levels, and low but detectable PBMC drug levels prior to SC (FTC-TP 2.62 pmol/10⁶ cells; TFV-DP 3.58 fmol/10⁶ cells). Intracellular FTC-TP concentrations ranged from 0.15 to 8.55 pmol/10⁶ cells, and TFV-DP concentrations ranged from 3.58 to 34.0 fmol/10⁶ cells, values consistent with nondaily oral dosing [8].

**DISCUSSION**

We have performed a comprehensive analysis of FTC/TDF-selected DR in 131 participants who seroconverted postrandomization in the iPrEx study, and in 2 participants with exposure to FTC/TDF during unrecognized acute infection. Overall, detection of PrEP-selected DR mutations was infrequent. Bulk genotypic and phenotypic resistance assays revealed FTC-associated mutations (M184V or I) exclusively in participants exposed to FTC/TDF during acute infection, of which 1 had confirmed WT virus at entry [3]. TDF-selected mutations were not observed in PrEP recipients. Of the 48 infected participants randomized to FTC/TDF, minor variant FTC-selected DR (M184I) was detected in 2 participants, 1 by qMVA (0.53%) and another by deep sequencing (0.75%), of which only the former had detectable FTC within the estimated infection window. Taken together, clinically significant DR selected by PrEP was limited to those who initiated drug after established infection, and was associated with FTC exposure.

The emergence of PrEP-associated viral mutations where PrEP was initiated during acute infection was also reported for men and women in the TDF2 study and HIV serodiscordant couples in Partners PrEP, respectively [2, 4]. Consistent with the findings in iPrEx, and the Bangkok Tenofovir study, PrEP-selected mutations were absent among seroconverters infected postrandomization. This may reflect drug concentrations or exposure duration that were insufficient to prevent infection and select for drug resistance. In all PrEP study reports, efficacy significantly increases when measured drug exposure is taken into account [6]. By contrast, in the Fem-PrEP trial, PrEP-associated DR in SC randomized to the FTC/TDF arm was noted exclusively in those infected postrandomization [9]. Seroconversion in 3 of 4 women occurred within 8 weeks of study entry, raising the possibility of emergent infection during PrEP initiation. Taken together, these results underscore the importance of recognizing the rare individual with acute seronegative infection, through sensitive HIV RNA testing, or 4th generation antigen-antibody combination enzyme immunosays. Other potential strategies where such diagnostic testing is impractical.
include delaying PrEP initiation in individuals with symptoms consistent with acute viral infection syndromes.

In the absence of continued drug selection, the FTC-resistant viruses proportionately waned within the infected host over time, reaching background levels by 24 weeks. This is consistent with our findings measuring the time-course of transmitted M184V reversion and WT outgrowth in ARV-naive subjects [37, 38], and reflects the impaired replication capacity in viruses carrying FTC-selected mutations [39–41]. The contribution of archived, low-frequency M184V/I in the proviral DNA reservoir to treatment response is not known. Long-term persistence of K103N is detected as minor variants in untreated infants exposed to single-dose nevirapine at birth [24]. In a meta-analysis [42], there is increased risk of treatment failure with preexisting NNRTI minor variant resistance, but reduced risk is noted with minor variant NRTI resistance comparable to frequencies seen in our study (approximately 1%).

The FTC-resistant viruses associated with PrEP exposure had phenotypic susceptibility profiles predicting durable suppression with current second-line regimens based on World Health Organization recommendations [43]. The response to regimens containing FTC or lamivudine (3TC) for viruses harboring isolated M184VI mutations is difficult to predict, although use of a boosted PI is an option to minimize the risk of virological failure. The mutations M184VI confer hypersusceptibility to ZDV and TDF [40, 44], raising the possibility of successful ZDV- or TDF-based dual NRTI regimens combined with a fully active NNRTI or integrase inhibitor, thus sparing 2nd-line boosted PI-based regimens.

In iPrEx, antiretrovirals were withdrawn at first evidence of infection, with clinical follow-up consistent with the in-country standard of care. However, recent insights into the potential clinical benefit of very early treatment initiation warrants reconsideration of this strategy, as therapy intensification during hyperacute infection might attenuate the course of HIV spread and decrease viral reservoir size [45, 46]. Indeed, the decreased frequency of seroconversions in subjects undergoing acute infection at randomization detected in the active arms compared with placebo in iPrEx (2 vs 8) and CAPRISA 004 (1 vs 7) may reflect attenuated infection when antiretrovirals are started during the Fiebig 1 infection stage or earlier [47].

A better understanding of the FTC/TDF dose and dosing patterns that are associated with prophylactic effects and selection for drug resistance is important for evaluating risk-benefit ratios of PrEP. In a case-control substudy of iPrEx participants, the frequency of drug detection in infected participants within a 90-day infection window was significantly lower (11%) than that seen in uninfected controls (51%), but equivalent at time points farther from infection, indicating increased risk of infection during periods of low drug exposure [8]. We find that such periods of low drug exposure were not sufficient for selection of drug resistance. One subject who had detectable HIV-1 RNA when starting PrEP rapidly developed FTC resistance; his drug concentrations were TFV-DP 18.7 fmol/10^6 PBMCs and FTC-TP 0.95 pmol/10^6 PBMCs at the seroconversion visit 4 weeks later, by which time M184V was selected. This indicates that these concentrations of drug were sufficient to select for resistance, while being in the prophylactic range for those who are uninfected when starting PrEP [8, 48]. This case, and the lack of FTC or TDF resistance among those with incident infections, suggests that concentrations of drug required to overcome viral fitness barriers to DR selection are also sufficient to prevent HIV infection.

There are limitations in directly extrapolating our findings to other settings of PrEP use. The iPrEx study design included monthly serologic monitoring of PrEP recipients and termination of PrEP at seroconversion. Current guidelines for PrEP in clinical settings indicate HIV-1 monitoring at least 3-month intervals [49], potentially increasing drug exposure duration and risk of DR if infection occurs. The absence K65R or K70E in iPrEx may be due to insufficient duration of TDF exposure. Among PrEP users with preexisting infection in other trials, K65R appeared after 4 weeks of TDF PrEP in 1 person [2], and after 7 months of FTC/TDF PrEP in another [4]. In GS-934, a randomized study of FTC/TDF/efavirenz (EFV) versus 3TC/TDF/EFV in infected ART-naive subjects, 2/19 developed M184V or 1 while none developed K65R or K70E after treatment for a median of 16 weeks postvirologic failure [50]. In clinical practice, PrEP may be used intermittently, started, and stopped as people change their sexual practices; the frequency and nature of DR in these or other settings may differ from that reported here.

In summary, we found DR in iPrEx SC was limited to those initiating PrEP with unrecognized infection. In 8 participants with measurable systemic drug levels near the infection window, PrEP-selected DR with a potential clinical impact was absent, due to insufficient levels or exposure duration. Continued surveillance of PrEP exposure and DR in seroconverters from ongoing PrEP demonstration projects will extend these findings from randomized controlled trials.

**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 copyrighted. 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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