

Preexposure prophylaxis-selected drug resistance decays rapidly after drug cessation

Julie F. Weis^a, Jared M. Baeten^{b,c,d}, Connor O. McCoy^e, Chris Warth^e,
Deborah Donnell^{b,f}, Katherine K. Thomas^b, Craig W. Hendrix^{g,h,i},
Mark A. Marzinke^{g,j}, Nelly Mugo^{b,k}, Frederick A. Matsen IV^e,
Connie Celum^{b,c,d}, Dara A. Lehman^{a,b}, for Partners PrEP Study Team

Objective: Resistance to emtricitabine plus tenofovir disoproxil fumarate (FTC/TDF) or TDF alone used as preexposure prophylaxis (PrEP) has been detected in individuals who initiated PrEP during unrecognized acute HIV infection and, rarely, in PrEP breakthrough infections. PrEP-selected resistance could alter future treatment options, and therefore we sought to determine how long resistance persisted after PrEP cessation.

Methods: The Partners PrEP Study was a randomized placebo-controlled trial of FTC/TDF or TDF as PrEP for HIV prevention. We previously reported that PrEP-related mutations (K65R, K70E or M184IV) were detected by 454 sequencing following seroconversion in nine individuals who acquired HIV during the Partners PrEP Study. In the current study, we used 454 sequencing to detect and quantify PrEP-related mutations in HIV RNA-positive plasma samples prior to seroconversion, as well as in plasma from 6, 12, and 24 months after PrEP cessation from these nine individuals.

Results: HIV RNA-positive, antibody-negative samples were available prior to seroconversion for four of nine individuals with resistance detected at seroconversion. In all four cases, K65R, K70E and M184IV were not detected prior to seroconversion, suggesting PrEP-related resistance was selected and not transmitted. All PrEP-selected mutations were no longer detectable by 6 months after PrEP cessation and remained undetectable at 12 and 24 months in the absence of antiretroviral therapy.

Conclusion: Using highly sensitive assays, PrEP-selected resistance in plasma decays below detection by 6 months following drug cessation and remains undetectable for at least 24 months. Even high levels of resistance mutations during acute infection decay rapidly in the absence of ongoing PrEP exposure.

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^aDivision of Human Biology, Fred Hutchinson Cancer Research Center, ^bDepartment of Global Health, ^cDepartment of Medicine, ^dDepartment of Epidemiology, University of Washington, ^eDivision of Public Health Sciences, ^fVaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, ^gDepartment of Medicine, ^hDepartment of Pharmacology and Molecular Sciences, ⁱDepartment of Epidemiology, ^jDepartment of Pathology, Johns Hopkins University, Baltimore, Maryland, USA, and ^kKenyatta National Hospital, University of Nairobi, Kenya.

Correspondence to Dara A. Lehman, MHS, PhD, 1100 Fairview Ave N, C3–168, Seattle, WA 98109, USA.

Tel: +206 667 4148; e-mail: dlehman@fredhutch.org

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Introduction

Resistance to emtricitabine (FTC) or tenofovir disoproxil fumarate (TDF) used as preexposure prophylaxis (PrEP) has been detected in individuals who initiated PrEP during unrecognized acute HIV infection and, very rarely, in individuals that acquired HIV while taking PrEP [1–10]. When PrEP-related resistance does occur, it is important to understand whether it persists after PrEP cessation, which could impact subsequent antiretroviral treatment in these individuals and/or result in the transmission of drug resistance to their subsequent partners.

Standard clinical laboratory testing for HIV drug resistance relies on consensus sequencing that can only detect resistance mutations present at high frequencies (>20%) within the viral population. The use of highly sensitive assays, such as 454 ultra-deep sequencing, can detect resistance mutations present at very low frequencies (<1%). Previous studies used highly sensitive assays and revealed that resistance detected at frequencies as low as 1% following the use of antiretrovirals to prevent mother-to-child transmission can be associated with subsequent treatment failure [11–13] and that resistance may be transmitted even if only present at low frequencies of the viral population [14–18].

Among 121 seroconverters from the Partners PrEP Study, a randomized, placebo-controlled trial of FTC/TDF and TDF as PrEP, we previously reported detection of PrEP-related mutations (K65R, K70E and/or M184IV) by ultra-deep sequencing in nine individuals at the time HIV seroconversion was first detected [9]. Here we performed highly sensitive resistance testing in longitudinal samples from these nine seroconverters with PrEP-related resistance. HIV RNA-positive plasma samples available prior to HIV seroconversion, and thus as close as possible to the time of HIV acquisition, were tested to determine whether resistance was selected or transmitted. In addition, plasma samples from 6, 12 and 24 months following detection of seroconversion (when PrEP was withdrawn) were assessed for persistence of PrEP-related resistance mutations.

Methods

Study population

The Partners PrEP Study was a phase III randomized, double-blind, placebo-controlled trial of oral FTC/TDF and TDF-alone as PrEP for HIV prevention among HIV uninfected persons in HIV serodiscordant partnerships. Details and results from this trial have been reported previously [2,19]. Briefly, 4747 HIV-serodiscordant couples from Kenya and Uganda were enrolled and the HIV-uninfected partner was randomized to FTC/TDF, TDF or placebo. Clinic visits for safety assessments and HIV testing were monthly for up to 36 months. Plasma samples were stored quarterly throughout follow-up. In

addition, for those who seroconverted, plasma samples were also stored from the visit seroconversion was first detected and within 1 month after seroconversion.

HIV testing

HIV seroconversions were detected by rapid HIV tests conducted at monthly clinic visits and confirmed by western blot [2]. Reverse transcription (RT)-PCR was used to detect HIV RNA in quarterly archived pre-seroconversion plasma samples. Dates of infection were estimated as 17 days prior to the first HIV RNA-positive antibody-negative visit, or the midpoint between the date seroconversion was first detected and the prior HIV RNA-negative antibody-negative visit.

Antiretroviral resistance testing

Ultra-deep 454 sequencing was performed as described previously [9,13,20], with modifications as follows. Total RNA was extracted from 100 to 500 μ l plasma, as in Palmer 2005 [21]. For each sample, four replicate high-fidelity nested RT-PCR reactions were performed as previously described [9,13,20], with a unique 8 bp barcode for each. Input of 400 HIV copies into each replicate (1600 copies per sample) was based on Roche quantitative HIV RNA results. For samples with low virus levels ($n=5$), the maximum possible number of copies was added (range 17–1256 HIV RNA copies). Barcoded PCR products were purified with Agencourt AMPure XP beads (Beckman Coulter, Brea, California, USA), quantified by Qubit dsDNA HS (Invitrogen, Carlsbad, California, USA), mixed in equimolar concentrations and pyrosequenced in both directions on the 454 GS-Junior platform. Data were processed and analyzed using methods as described previously [9].

Results

No evidence of low-frequency preexposure prophylaxis-related resistance prior to seroconversion

Of the nine individuals in the Partners PrEP Study that had PrEP-related resistance at or within 1 month after detection of seroconversion [9], retrospective testing revealed that five individuals did not have HIV RNA detected in a prior archived sample. In four individuals, a plasma sample from a clinic visit prior to seroconversion was determined to be HIV RNA-positive. In three cases, these samples were from the time of enrollment (prior to randomization) in individuals assigned to active PrEP during unrecognized acute HIV infection (Fig. 1). In the fourth case, 2–299, the individual had been assigned to active PrEP and subsequently seroconverted at month 4 after enrollment. Retrospective testing of archived samples from case 2–299 revealed that HIV RNA was first detected at 3 months after enrollment, and that plasma tenofovir levels were undetectable at enrollment and month 3, but were 74 ng/ml at month 4.

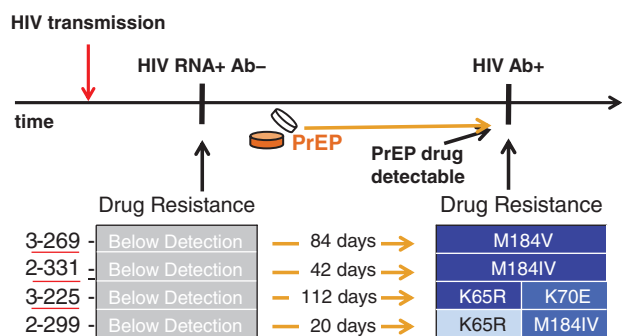


Fig. 1. Preseroconversion samples confirm resistance selected by PrEP. Timeline highlights four cases with HIV RNA-positive and HIV antibody-negative samples collected and archived prior to HIV seroconversion. Participants retrospectively found to be HIV infected at enrollment have ID numbers underlined. Three of four cases were unrecognized acute infection at study enrollment prior to PrEP drug administration, and the fourth had no evidence of PrEP drug prior to seroconversion. Resistance mutations were not detected in all four preseroconversion samples, shown on the left in gray. All four cases had resistance mutations at seroconversion detection, shown with darker blue denoting higher proportions of HIV variants harboring the noted resistance mutations. Time between the HIV RNA-positive antibody-negative sample and the HIV antibody-positive sample is noted between resistance results.

To determine whether the PrEP-related mutations detected in these individuals at seroconversion were present earlier in infection, prior to detection of PrEP drug, we performed 454 sequencing on the preseroconversion HIV RNA-positive plasma samples from these four individuals. In all four cases, resistance was below detection in HIV RNA from this preseroconversion visit, which ranged from 20 to 112 days prior to seroconversion when PrEP drug was detected (Fig. 1).

PrEP-related resistance decays rapidly after preexposure prophylaxis cessation

In individuals in the Partners PrEP Study, PrEP was discontinued immediately upon detection of HIV seroconversion. In the nine individuals that had PrEP-related mutations at frequencies greater than 1% when seroconversion was detected, we were interested to determine how long that resistance persisted in the absence of antiretroviral therapy. Thus, we performed 454 sequencing on plasma samples from months 6, 12, and 24 following seroconversion, when PrEP or placebo was withdrawn.

As reported previously, only five of the nine cases of resistance had both evidence of PrEP use (detectable levels of tenofovir) and resistance to the PrEP drugs they were taking (FTC selects for K65R and M184IV; TDF selects for K65R and K70E), suggesting that the resistance mutations detected in these five seroconverters were likely due to PrEP selection [9]. This included one

individual (3–225) randomized to TDF during unrecognized acute infection who had K65R and K70E detected in 56 and 10% of virus variants, respectively, when seroconversion was first detected. The other four cases of PrEP-selected resistance were individuals randomized to FTC/TDF (two individuals with unrecognized acute infection at randomization: 3–269 and 2–331; and two individuals who acquired HIV postrandomization: 2–299 and 4–321) who had M184V or M184IV at levels ranging from 1 to 99% at detection of seroconversion. Case 2–299, who also had 1.2% K65R, was lost to follow-up after seroconversion. In follow-up samples from these cases, resistance mutations decayed below detection by 6 months, and remained undetectable at 12 and 24 months after seroconversion (Fig. 2).

The remaining four cases of resistance were individuals who did not have detectable levels of PrEP drug in any of their HIV RNA or antibody-positive plasma samples (2–283, 6–283, 2–316, the latter two were assigned to the placebo arm), or whose resistance mutation was not associated with their assigned PrEP regimen (2–326 had M184I, which causes resistance to FTC, but this individual was assigned to TDF-alone as PrEP). Case 2–283 had M184V in 16% of virus variants when

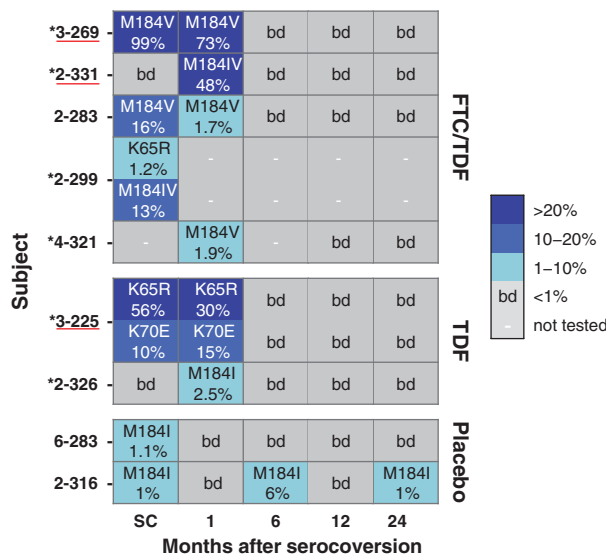


Fig. 2. Levels of K65R, K70E and M184IV after seroconversion and PrEP drug cessation. Subjects are ordered by levels of resistance from highest to lowest within each treatment arm. An asterisk prior to the identification number indicates subjects with tenofovir detected in plasma during HIV infection. Red underline denotes subjects with unrecognized acute HIV infection at treatment assignment. Samples tested include the time seroconversion was first detected (SC), and months 1, 6, 12 and 24 after seroconversion. Higher levels of resistance denoted by darker blue color. Gray cells with “bd” indicate below detection. Gray cells with “-” indicate sample not available or did not amplify. Abbreviations: FTC, emtricitabine; TDF, tenofovir disoproxil fumarate; bd, below detection.

seroconversion was detected and 1.7% M184V 1 month later, which faded below 1% by 6 months and remained undetectable through 24 months. This individual had no evidence of PrEP drug at clinic visits either before or after seroconversion, and the M184V mutation may have been a result of transmitted resistance [9]. The three other cases of resistance (one TDF: 2–326; two placebo: 6–283 and 2–316) had only low levels of M184I at seroconversion ranging from 1 to 2.5%. M184I, which is naturally polymorphic and known to occur without drug exposure, fluctuated at low levels between below detection and 6% throughout follow-up (Fig. 2).

Follow-up after initiation of combination antiretroviral therapy

Follow-up in these nine cases continued for a median of 40 months (range: 0–51 months) post seroconversion. Only one individual, case 2–331, initiated combination antiretroviral therapy (cART) during follow-up. In this case, cART was initiated at 33 months postseroconversion with a regimen of FTC/TDF/nevirapine. Viral load from the clinic visit prior to initiation of cART (month 30 postseroconversion) was 19 170 copies/ml, which dropped below detection (<40 copies/ml) at the clinic visit 3 months following initiation of cART (33 months postseroconversion).

Discussion

In this study we showed that PrEP-selected resistance decays rapidly after PrEP cessation. By 6 months after seroconversion (after PrEP was discontinued), resistance mutations K65R, K70E, and/or M184IV that were present at seroconversion were no longer detected, even with highly sensitive resistance testing. Although fitness assays were not performed on viral isolates from our study, previous studies that have shown that these mutations reduce viral replication fitness compared with wild-type HIV variants [22–25]. Evidence from other studies using highly sensitive resistance testing have suggested that in women and infants exposed to antiretrovirals as prophylaxis to prevent HIV mother-to-child transmission, resistance variants present at more than 1% of the virus population are associated with an increased risk of subsequent treatment failure [11–13]. Thus, our data suggest that in the rare cases in which resistance is selected by PrEP, resistance rapidly decays to levels that may no longer be clinically relevant.

Resistance mutations were selected (and not transmitted) in four individuals who initiated PrEP during unrecognized acute HIV infection. In these four cases, PrEP resistance mutations were not detected with highly sensitive resistance testing prior to evidence of PrEP drug exposure, but resistance was detected at the subsequent visit when both HIV seroconversion and PrEP drug in plasma were first detected (Fig. 1). Three of these cases

were individuals who tested HIV negative by rapid tests at study enrollment and randomization to FTC/TDF or TDF PrEP, but were retrospectively determined to be HIV RNA-positive at enrollment, indicating very early acute HIV infection. The fourth case was an individual who was both HIV antibody and HIV RNA-negative at enrollment but had undetectable tenofovir levels indicating no PrEP use until 4 months later, after HIV infection appears to have been acquired; thus, this case is analogous to the three individuals who had acute seronegative HIV infection at initial randomization to PrEP. These data help confirm that resistance in these cases was likely not transmitted, but was selected by PrEP drug exposure during acute infection, when HIV replication is high and errors in reverse transcription increase the probability of resistance.

Multiple studies have now shown that the risk of developing resistance from PrEP is very low, but is an important concern for those who initiate PrEP during unrecognized acute infection [1–10]. Our data show that resistance selected in these cases decays rapidly to levels below detection of even highly sensitive assays. However, whether resistance decays to levels that are no longer clinically relevant during cART remains to be determined. Case 2–331 supports this hypothesis, as this individual had M184IV in 48% of viral variants at 1 month following seroconversion and PrEP cessation, which decayed to below detection and remained below detection through 24 months of follow-up. Initiation of cART after month 30 with FTC/TDF/nevirapine resulted in complete viral suppression within 3 months. Future studies, that include follow-up of seroconverters who initiate cART after acquiring HIV when on PrEP, will allow us to determine whether the development of PrEP resistance early in infection followed by decay impacts later treatment options.

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Conflicts of interest

All authors report no conflict of interest.

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