Published in final edited form as:

J Acquir Immune Defic Syndr. 2017 December 01; 76(4): e104–e106. doi:10.1097/QAI.0000000000001534.

Newly Acquired Infection with Multi-Drug Resistant HIV-1 in a Patient Adherent to Pre-Exposure Prophylaxis

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Antiretroviral agents as pre-exposure prophylaxis (PrEP) against HIV-1 infection is a recent and welcome addition to the prevention toolbox. Clinical trials have shown TDF/FTC as PrEP to be safe and associated with substantial reductions (44% to 86%) in the rates of HIV-1 acquisition in men who have sex with men (MSM) and transgender women (TGW),^{1–4} heterosexual HIV-discordant couples,⁵ and high-risk heterosexual men and women.⁶ In July 2012 the U.S. Food and Drug Administration approved the use of TDF/FTC as PrEP in combination with safer sex practices to reduce the risk of sexually acquired HIV-1.

Important lessons of PrEP use have emerged from clinical trials. Efficacy is highly dependent on adherence⁷. Self-reported adherence, however, is of limited positive-predictive utility. Indeed, objective measures of adherence (e.g. drug levels in plasma and PBMCs) proved critical to study interpretation. For instance, in clinical trials, individuals randomized to active treatment who acquired HIV-1 infection had low or undetectable tenofovir (TFV) levels in plasma, or TFV-diphosphate (TFV-DP) in peripheral blood mononuclear cells (PBMCs)^{2,8,9} indicating poor adherence. Because of the relatively short half-life of TFV in blood plasma, however, this metric indicates the timing of the last dose and not longer-term adherence.¹⁰ The measurement of TFV-DP in red blood cells using DBS technology,¹¹ and TFV in hair samples,¹² indicate cumulative dosing and longer-term use and have proven valuable in interpreting subsequent open-label studies.

Here we present a case report of a young MSM in an HIV-serodiscordant relationship who was prescribed PrEP and, despite reportedly high-level adherence, became infected with HIV-1.

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Conflicts of interest

There are no conflicts of interest for this manuscript.

This 26-year-old MSM had been tested for HIV-1 infection routinely and deemed to be uninfected in January 2013, October 2013, and March 2015. Immediately prior to being prescribed TDF/FTC as PrEP in December 2015 a 4th generation combination Ag/Ab test was non-reactive and an HIV-RNA level was undetectable, consistent with his uninfected status. He was circumcised and had a history of treated urethral gonorrhea in September 2014. Of note, his regular partner had consistent virologic suppression on 3-drug antiretroviral therapy that had been initiated 4 years previously during acute infection.

The patient was prescribed a 90-day supply of PrEP with one refill which was commenced on January 1, 2016. He was seen February 2, 2016 and was adherent by history and tolerating TDF/FTC. He presented on May 3, 2016, 5 months after PrEP initiation, for routine HIV-1 testing reporting excellent adherence to the prescribed regimen. He reported insertive and receptive condomless anal intercourse (ICAI and RAI) with his regular partner and 2 separate episodes of ICAI with 2 different partners of unknown HIV status, 11 and 5.5 weeks prior to presentation. Results of HIV-1 testing are shown in Table 1. The reactive 4th generation HIV test with a non-reactive HIV1/2 Multispot and reactive qualitative testing for HIV-1 RNA by nucleic acid amplification (NAAT) suggested recent HIV-1 infection. Supplemental tests were performed, as were tests for levels of plasma HIV-1 RNA and CD4+ T cells and results reported 2 weeks thereafter (Table 1). The results were consistent with extremely low levels of HIV-1 viremia and immune system preservation without seroconversion and the treatment regimen was intensified with the addition of dolutegravir 50 mg daily to TDF/FTC on May 26, 2016. On June 7th the patient was re-evaluated. To confirm the subject's self-reports of excellent adherence with his prescribed PrEP regimen, TFV and TFV-DP levels were measured in hair and DBS respectively (Table 1). Results were consistent with high (e.g. daily) level adherence over the preceding 6 to 8 weeks ^{11,13}, both prior and subsequent to the first indications of seroconversion ^{11,13}.

Since the patient's HIV-1 RNA level had been persistently below the level of detection, resistance testing on the virus in the plasma could not be performed. A GenoSure Archive test (Monogram), which derives HIV-1 sequences from cell associated DNA, was sent on June 7 and failed to provide a result. On June 9, 2016 re-testing revealed a reactive Bio-Rad GS HIV Combo Ag/Ab assay with both qualitative and quantitative HIV-1 RNA determinations being non-detectable. The Multispot HIV 1/2 Rapid test remained non-reactive (Table 1). By the current HIV testing algorithm, these results were reported as "no laboratory evidence of HIV infection" despite the persistently reactive combo assay.

In order to determine whether the patient's partner was the source of infection, we amplified and sequenced the V3 loop of env, reverse transcriptase, protease, and integrase coding regions from HIV-1 viral DNA extracted from isolated CD4+ T cells from blood drawn on both patient and partner on June 9th (supplemental Fig. 1). Of note, the viral burden was extremely low in the newly infected patient, approximately 33.0 copies/10⁶ CD4+ T cells on average that is orders of magnitude lower than typical viral loads among untreated individuals during early infection ¹⁴. Amplification of these coding regions proved challenging and few sequences were available for comparison to those of his partner. However, phylograms demonstrated conclusively that the 2 individuals were infected with

different viruses as viral populations clearly segregate from each other (Supplementary Fig. 1) and from additional viruses similarly analyzed.

Infection despite high-level adherence to TDF and FTC suggested acquisition of a virus resistant to the components of PrEP. Genotyping of the reverse transcriptase coding region on single genomes derived by limiting dilution PCR revealed linked mutations at K65R and M184V, resistance-conferring mutations to TDF and FTC, respectively ¹⁵, and additional mutations to non-nucleoside RT inhibitors (NNRTIs), specifically K103S, E138Q, and Y188L¹⁵ (Table 1, Supplementary Table 1). This individual was likely infected with a multi-drug resistant virus as the NNRTI-associated resistance mutations would not have evolved under pressure by TDF/FTC alone. However, given that sampling was not performed at the time of infection the possibility of evolution of resistance under pressure by TDF and FTC cannot be completely ruled out. Given that the patient harbored HIV-1 resistant to both TDF and FTC, co-formulated cobicistat-boosted darunavir was subsequently added to the patient's treatment regimen to ensure continued and prolonged virologic suppression.

This is the second reported case of the acquisition of a multidrug resistant HIV-1 variant in an apparently adherent individual on TDF/FTC-based PrEP ¹⁶. In both cases, the patients reported excellent adherence confirmed by objective measurements of TDF use. However, these measurements could not be performed at the exact time of infection in both cases, so we can only extrapolate that adherence around the time of HIV acquisition was adequate.

As the number and risk of sexual exposures was highest with the patient's regular partner, the question of transmissibility from an HIV-infected individual with an undetectable viral load was raised. Our patient's regular partner had achieved consistent virologic suppression on antiretroviral therapy for approximately 4 years. In this case, it was critical that we documented that the suppressed regular partner was not the source of the new HIV-1 infection in our index patient. The patient reported only ICAI with non-regular partners and although ICAI carries a much lower of HIV acquisition than RCAI (11 versus 138 HIV-1 infections per 10,000 exposures), this case does demonstrate the importance of counseling MSM as to the per-act probability of HIV-1 acquisition ¹⁷.

For our patient, repeated testing with the 4th generation Ag/Ab combination test was reactive. As HIV RNA levels were either barely or non-detectable, the presence of antibodies to HIV-1 was suggested, although the confirmatory immunoassay (Multispot HIV 1/2) remained repeatedly non-reactive over 5 weeks. Delayed seroconversion may be observed in those administered cART during acute infection ¹⁸ and in one other case report of an individual becoming HIV-infected during repeated courses of post-exposure prophylaxis with TDF/FTC ¹⁹. Essential to this case is that the combination of the reactive 4th generation combination Ag/Ab test and the qualitative NAAT test established the diagnosis of HIV infection. These findings highlight the importance of using sensitive HIV tests in the context of PrEP delivery²⁰.

In summary, newly acquired HIV-1 infection in patients being treated with PrEP and with evidence of adequate adherence as assessed via objective measures is rare. To date there have been two such cases of acquisition of MDR HIV-1 and a recent report of infection with

wild type HIV-1 in an MSM with repeated exposures via CAI²¹ despite more than 86,000 people using PrEP in the United States. Such cases may pose clinical and laboratory challenges. Although sensitive 4th generation tests were sufficient to detect infection in this case, subsequent recommended confirmatory testing following the 2014 CDC/APHL HIV testing algorithm proved challenging. The limitations of testing in the face of a low viral burden may complicate testing efficacy. Objective adherence testing in this case proved useful. However, despite the high effectiveness of TDF/FTC as PrEP in highly adherent individuals, the level of protection, if any, against infection by circulating viruses resistant to both medications is not known. Though the transmission of MDR HIV-1 has fallen over the past decade,^{22,23} this case, as well the “Toronto case”, highlight the importance of awareness of the potential risk of MDR HIV-1 viral transmission despite PrEP use. Surveillance for drug resistance among people who become infected after receiving PrEP is needed to evaluate whether these cases will remain rare.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sources of funding: Martin Markowitz received grant funding to his institution from Merck, Gilead, ViiV and GSK. He is a consultant to Merck and ViiV and is a member of the Gilead Speakers Bureau.

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Table 1

Summary of pertinent laboratory results

Test	Dec-18-2015	May-3-2016	May-17-2016	June 7, 2016	June-9-2016
4 th Generation HIV Combo Ag/Ab Assay	Non-reactive	Reactive	Reactive		Reactive
HIV Nucleic Acid Amplification Assay (qualitative)		Reactive	Reactive		Non-reactive
Multispot HIV1/2		Non-reactive	Non-reactive		Non-reactive
Roche Cobas TaqMan HIV-1 Test v2.0 (copies/mL)	<20 No signal detected	ND	<20 Signal detected		<20 No signal detected
CD4+ T cell count (cells/mm ³)			1123		
CD4/CD8 ratio			1.53		
TFV-DP level in DBS (fmol/punch)				1478 *	
TFV-DP level in hair (ng/mg)				0.0448 *	
Genosure Archive test				Failed	
Genotype of RT from patient proviral DNA					K65R, M184V K103S, E138Q, Y188L

* consistent with daily dosing.

RT= reverse transcriptase