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Characterization of HIV seroconverters in a TDF/FTC PrEP study: HPTN 067/ADAPT

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All authors contributed to manuscript preparation. Additional author roles are listed below.

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Abstract

Background—HPTN 067/ADAPT evaluated tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) pre-exposure prophylaxis (PrEP) in women (South Africa) and men who have sex with men (Thailand, US). Participants received once-weekly directly observed TDF/FTC (DOT), and were then randomized to daily, time-driven, or event-driven PrEP. This report describes characterization of 12 HIV seroconversion events in this trial.

Methods—HIV rapid testing was performed at study sites. Retrospective testing included: 4th generation assays; HIV RNA testing; Western blot; an HIV-1/2 discriminatory assay; resistance testing; and antiretroviral (ARV) drug testing.

Results—Six of the 12 seroconverters received TDF/FTC in the DOT phase, but were not randomized (3 were acutely infected at enrollment; 2 were infected during the DOT phase; one was not randomized due to pregnancy). One of the six randomized participants had acute infection at randomization but was not diagnosed for 3–4 months because HIV rapid tests were non-reactive; continued daily PrEP use was associated with false-negative antibody tests and low HIV RNA levels. The five participants infected after randomization included four with low adherence to the PrEP regimen, and one who reported a 7-day period without dosing prior to infection. Three

participants had TDF/FTC resistance (M184I, K65R), including two who received only four once-weekly TDF/FTC doses; most TDF/FTC mutations were detected by next generation sequencing only.

Conclusions—In HPTN 067/ADAPT, participants who acquired HIV infection had infrequent PrEP dosing or low/suboptimal adherence. Sensitive assays improved detection of HIV infection and drug resistance. Drug resistance was observed with limited PrEP exposure.

Keywords

Pre-exposure prophylaxis; HIV seroconverter; antiretroviral drugs; resistance; tenofovir; emtricitabine

INTRODUCTION

A combined formulation of oral tenofovir disoproxil fumarate (TDF) and emtricitabine (FTC) is a component of first-line regimens for antiretroviral therapy (ART) in HIV-infected individuals.[1] TDF/FTC can also be used for pre-exposure prophylaxis (PrEP).[2–4] Daily oral TDF/FTC was approved by the United States (US) Food and Drug Administration (FDA) for PrEP in individuals at high risk of HIV acquisition in 2012,[5] and is now recommended by the US Centers for Disease Control (CDC) and World Health Organization (WHO) for prevention of HIV infection in diverse risk groups.[6,7] The IPERGAY[8] study demonstrated that an event-driven non-daily TDF/FTC regimen can also reduce the risk of HIV infection in men who have sex with men (MSM).

A meta-analysis of randomized controlled trials and observational studies showed that high adherence was significantly associated with a protective effect of TDF and TDF/FTC PrEP.[4] In the iPrEx study, participants in the TDF/FTC arm who had detectable levels of study drugs had a >92% risk reduction (95% CI: 40–99%) compared to those without detectable drug.[9] In other trials, such as Fem-PrEP[10] and VOICE,[11] low PrEP efficacy was attributed to low adherence to the study regimens.

HIV drug resistance can arise in individuals who become infected while using PrEP. However, most studies show that resistance to tenofovir (TFV) and FTC arises infrequently in the setting of TDF/FTC PrEP use.[4,12] In a report that included results from eight randomized clinical trials, resistance emerged in 5.9% of 305 seroconverters.[3] Higher rates of TDF/FTC resistance were observed in those who had undiagnosed acute HIV infection at the time of PrEP initiation.[4,12] M184I/V drug resistance mutations, which confer resistance to FTC, are seen more commonly in the setting of PrEP than the K65R mutation, which confers resistance to TDF.[12] Emergence of resistance in individuals who become infected while using PrEP may limit options for subsequent ART.

Detailed characterization of seroconversion events in PrEP trials can help distinguish between infections due to infrequent dosing/non-adherence and true breakthrough infections, and can provide information on the relationship between PrEP exposure and emergence of drug resistance. In this study, we characterized incident HIV infections in the HIV Prevention Trials Network (HPTN) 067/ADAPT trial.[13–15] The trial included a once-

weekly directly observed treatment (DOT) phase followed by a period of self-administered treatment (SAT) that included three study arms with different TDF/FTC PrEP regimens (daily, time-driven, and event-driven). The trial was designed to compare the coverage of sexual events, number of doses needed for coverage, and self-reported side-effects/symptoms associated with daily vs. non-daily PrEP use. The DOT phase of the HPTN 067/ADAPT trial was implemented prior to randomization to establish individual pharmacokinetic (PK) parameters to help interpret PK-based adherence assessments performed during the SAT phase of the study. Unfortunately, a priori estimates of the half-life of tenofovir diphosphate (TFV-DP) in peripheral blood mononuclear cells (PBMCs) were too long, and weekly DOT dosing was too infrequent to achieve consistently measurable drug concentrations one week after dosing. While inclusion of the DOT phase did not provide the desired information (PK parameters in individual study participants), it did provide an opportunity to evaluate infections that occurred in the setting of infrequent (once-weekly) observed drug dosing. Infections that occurred during the SAT phase of the trial were also analyzed. Data analyzed in this report includes self-reported PrEP use and results obtained with a panel of HIV diagnostic tests, HIV viral load testing, antiretroviral (ARV) drug testing, and HIV drug resistance testing.

METHODS

Study cohort

Samples and data were obtained from the HPTN 067/ADAPT study, a Phase 2 randomized, open-label trial of the use of oral TDF/FTC (300 mg TDF/200 mg FTC tablet) PrEP among HIV-uninfected individuals (NCT: 01327651, 2011–2014). The study enrolled 622 participants in Cape Town, South Africa (women who have sex with men, N=191), Bangkok, Thailand (MSM and transgender women, N=193), and New York, USA (MSM and transgender women, N=238). The study included a 6-week lead-in period with five once-weekly directly observed TDF/FTC doses (DOT phase; doses were administered at enrollment and weeks 1–4 with no dosing at week 5). At the 6-week visit, participants were randomized to one of three PrEP regimens: daily (once daily dosing); time-driven (twice weekly dosing with an additional dose following sexual intercourse); and event-driven (24–48 hours before and within 2 hours after sexual intercourse). Study drug was dispensed and participants were tested for HIV infection at monthly study visits during the SAT phase (weeks 6–30). The final study visit was at week 34. All participants were instructed not to take more than two pills per 24-hour period, or more than seven pills per week.

Self-reported dosing

After randomization, weekly interviews were conducted by phone or in person with an interviewer who was not involved in other study activities. Data from electronic dose monitoring (WisePill) was discussed to determine which device opening events were reflective of dosing and which were not; the date and time of sex events were also recorded.

HIV testing

HIV testing was performed at enrollment, at weeks 4 and 6, and at monthly follow-up visits. Two HIV rapid tests were performed in parallel at study sites; tests used included: the Uni-

gold Recombigen HIV Test (Trinity Biotech PLC, Bray, County Wicklow, Ireland); the Determine HIV-1/2 Test (Abbott Laboratories, Abbott Park, IL); and the OraQuick Advance Rapid HIV-1/2 Antibody Test (Orasure Technologies Inc., Bethlehem, PA). PrEP was discontinued if one or both of the HIV rapid tests was reactive. In these cases, HIV infection was confirmed at study sites using a qualitative HIV RNA assay (APTIMA HIV-1 RNA Qualitative Assay, Hologic Gen-Probe INC., San Diego, CA). Additional HIV testing was performed retrospectively at the HPTN Laboratory Center (Johns Hopkins University, Baltimore, MD) using a panel of assays (Figure 1).

Drug resistance testing

Drug resistance testing was performed retrospectively at the HPTN Laboratory Center for plasma samples with viral loads >400 copies/mL. Two methods were used for drug resistance testing: the ViroSeq HIV-1 Genotyping System (Abbott Molecular, Des Plains, IL) and next generation sequencing (NGS). HIV subtyping was performed using HIV *pol* sequences obtained from the ViroSeq system, as described.[16] NGS was performed using viral RNA that was extracted from plasma samples using the ViroSeq system; methods used for NGS are described in Supplemental Digital Content 1. HIV drug resistance reports were generated using the Stanford University HIV drug resistance database.[17]

ARV drug testing

ARV drug testing was performed retrospectively by the HPTN Laboratory Center by liquid chromatography tandem mass spectrometry (LC-MS/MS) using plasma samples (all three sites), PBMC samples (South Africa and Thailand), and dried blood spots (DBS) samples (US).[18–20] The lower limit of quantification (LLOQ) for these assays are: plasma: tenofovir (TFV) and FTC 0.31 ng/mL,[18] PBMC: TFV-DP 2.5 fmol/sample, emtricitabine triphosphate (FTC-TP) 0.1 pmol/sample, the average LLOQ based on cells assayed per sample is 0.57 fmol/10^6 cells for TFV-DP and 0.014 pmol/10^6 for FTC-TP,[20] DBS: TFV-DP 31.25 fmol/punch, FTC-TP 0.125 pmol/punch.[21] Plasma and PBMC drug concentrations were interpreted based on results from a dose ranging PK study with directly observed TDF/FTC dosing.[20]

Ethics statement

All study participants provided written informed consent for participation in the HPTN 067/ADAPT study. The study was approved by the participating academic institutions and ethics committees for each study site.

RESULTS

Twelve study participants acquired HIV infection (8/191 in South Africa; 2/193 in Thailand; 2/238 in the US, Table 1). HIV subtypes were consistent with subtypes prevalent at each study site (South Africa: subtype C; Thailand: CRF01_AE; US: subtype B). All 12 participants received once-weekly observed TDF/FTC doses in the DOT phase. Six participants were not randomized due to HIV infection or pregnancy (Figure 1A) and six were randomized at the 6-week study visit (2 in each study arm; Figures 1B–D).

Detection of HIV infection using third generation HIV rapid tests

Participants were tested with two 3rd generation HIV rapid tests at each study visit. Retrospective testing using 4th generation HIV tests, HIV RNA tests, and other assays revealed that the rapid tests often missed HIV infection. In 9/12 cases, both of the rapid tests were non-reactive at the first HIV positive visit. In these cases, HIV-infected participants continued to use PrEP until their infection was detected at the study site. A qualitative HIV RNA assay was positive in all 12 cases at the first HIV-positive visit; the HIV viral load was ≤400 in four cases. In 8/12 cases, retrospective testing revealed that participants had acute HIV infection at the first HIV-positive visit; in five of these cases, positive tests results were obtained for HIV RNA assays only; in the other three cases, one or both of the 4th generation tests was also reactive. In two other cases where HIV infection was missed by one or both of the rapid tests, other HIV tests indicated the presence of anti-HIV antibodies. In one case, the Western blot was indeterminate and the discriminatory assay was negative (Case 4); in the other case, the Western blot was indeterminate and the discriminatory test was positive (Case 5). In 4/12 cases, one or both of the rapid tests was still non-reactive at the seroconversion visit (Cases 1, 2, 5, and 7); in one case (Case 7), both rapid tests were non-reactive at multiple study visits.

Analysis of HIV infection in participants who were not randomized to a PrEP regimen

Six participants were not randomized (Figure 1A); these participants received only once-weekly TDF/FTC dosing in the DOT phase of the trial. Three had acute HIV infection at enrollment that was not detected at the study sites (Cases 1–3), and two acquired HIV infection during the DOT phase (Cases 4 and 5). HIV infection was diagnosed at the study site at the 4-week visit in four cases; those participants did not receive TDF/FTC at week 4. In the fifth case (Case 4), HIV infection was diagnosed at the 5-week visit; that participant received all five DOT doses. One participant was not randomized to a PrEP regimen due to pregnancy (Case 6); this participant acquired HIV infection between the 22-week and 26-week study visits.

ARV drug testing was performed using plasma samples collected at week 4 (prior to dosing) and week 5. TFV and/or FTC was detected in plasma at one or both visits in five of the six cases; FTC was detected at week 6 in one of two participants who received TDF/FTC at week 4 (Case 6). PBMC testing was performed for the five participants. TFV-DP and/or FTC-TP was detected at week 4, 5, or 6 in all five cases. Plasma testing was performed at weeks 22 and 26 for the participant who was not randomized due to pregnancy (Case 6); TFV and FTC were not detected at those visits.

Analysis of HIV infection in participants who were randomized to a PrEP regimen

The remaining six participants acquired HIV infection after randomization (Figure 1B–D). PrEP was discontinued when one or both of the HIV rapid tests was reactive. Two participants were randomized to the daily PrEP study arm (Figure 1B). In Case 7, a female participant had undiagnosed acute infection at the 6-week randomization visit; HIV testing was negative at week 5, indicating that this participant was infected 1–2 weeks after receiving 5 once-weekly DOT doses. This participant continued to use PrEP for 3–4 months after infection (from week 6 to week 22), until the infection was detected by HIV rapid

testing at the study site; the participant reported taking 84.4% of the assigned doses during this period. Study drugs were detected in plasma and PBMCs at all visits tested during the SAT phase (weeks 10–22), but the concentration of drugs detected at some visits were consistent with less than daily PrEP use (TFV concentration corresponded to 7 doses/week at week 10, ≥4 doses/week at week 14, 7 doses/week at week 18, and 7 doses/week at week 22). At the visit before the participant was diagnosed with HIV infection at the study site (week 18), the concentration of TFV-DP in PBMC was consistent with 1 dose/week (based on comparison to her data from the DOT phase); the discrepancy between plasma and PBMC drug concentrations at week 18 suggests that this participant may have taken the drug shortly before the study visit ("white coat effect"). HIV viral loads were persistently low in this case (<400 copies/mL at all but one visit; 650 copies/mL at week 18). Fourth generation HIV assays were reactive after infection, but had low signal-to-cutoff ratios (<2.5 for the ARCHITECT assay; <9 for the Bio-Rad assay). The APTIMA qualitative HIV RNA assay was initially positive, but was negative at subsequent visits; this assay has a limit of detection of 40 copies/mL HIV RNA. Resistance testing results were not obtained in this case because the viral load was low at all visits following HIV infection. In Case 8, the participant acquired HIV infection between weeks 10 and 14 (4–8 weeks after randomization). Study drugs were detected in only one plasma sample and one DBS sample collected during the SAT phase. These results suggest that the participant was not adherent to the daily PrEP regimen.

Two participants were randomized to the time-driven study arm (Figure 1C). In Case 9, the participant was infected between weeks 18 and 22 (12–16 weeks after randomization). Study drugs were detected in only two of four plasma samples collected during the SAT phase (weeks 10–22); low levels of study drugs were detected in PBMC samples at two of these visits. These results suggest that the participant was not adherent to the time-driven study regimen. In Case 10, HIV infection was diagnosed at the study site at week 18. Retrospective testing revealed that the participant had acute HIV infection at the prior visit (week 14, 8 weeks after randomization); PrEP was continued in the 4-week interval between these two visits. Study drugs were detected in plasma and PBMCs at all visits during the SAT phase (weeks 10–18); the drug concentrations were higher than expected for the time-driven regimen (TFV concentration corresponded to 7 doses/week at week 10, multiple doses/day at week 14, and 7 doses/week at week 18). The concentration of TFV-DP in PBMC at week 18 was also higher than expected (consistent with 7 doses/week). Self-reported data indicated that this participant took a dose every 3–4 days for the first 6 weeks after randomization. She continued to take 2 pills/week over the next two weeks, but took those doses at irregular intervals. She reported taking a pill 4 days prior to the acute infection visit, after taking no pills for 7 days.

Two participants were randomized to the event-driven study arm (Figure 1D). In Case 11, HIV infection was diagnosed at the study site at week 34, 4 weeks after the end of the SAT phase. Retrospective testing revealed that the participant had acute HIV infection at the prior visit (week 30, 24 weeks after randomization). Study drugs were detected in only one of seven plasma samples and two of three PBMC samples collected during the SAT phase (at weeks 10–30). In Case 12, HIV infection was diagnosed at the study site week 22. Retrospective testing revealed that the participant had acute HIV infection at the prior visit

(week 18, 12 weeks after randomization); PrEP was continued in the 4-week interval between these two visits. Study drugs were detected in only two of four plasma samples and one of two PBMC samples collected during the SAT phase (at weeks 10–22), indicating infrequent PrEP use. Both participants reported infrequent sex events with low adherence to the event-driven regimen (42% of assigned doses taken in Case 11; 67% of assigned doses taken in Case 12).

HIV drug resistance

Samples from the 12 seroconverters were tested for HIV drug resistance. Mutations associated with resistance to the study drugs were detected in three cases using NGS. This included two cases where participants had acute HIV infection at enrollment (Case 1: K65R was detected in 24.7% of sequences; Case 2: M184I was detected in 3.5% of sequences), and one case where the participant was randomized to the time-driven arm (Case 10: K65R was detected in 3.9% of sequences; M184I was detected in 62.3% of sequences). In the first two cases (Cases 1 and 2), participants received only four once-weekly DOT doses of PrEP (at study enrollment and at weeks 1–3) before resistance was detected. Using a genotyping assay based on population sequencing (ViroSeq), resistance to study drugs was detected in only one of the three cases (Case 10); in that case, only one of the two drug resistance mutations was detected (M184I). Resistance to other ARV drugs was detected in two cases (resistance to non-nucleoside reverse transcriptase inhibitors (NNRTIs, Cases 2 and 8).

DISCUSSION

This report presents analysis of 12 cases of HIV infection in the HPTN 067/ADAPT trial. This included three cases where participants were acutely infected at enrollment, two cases where participants were infected during the once-weekly DOT phase, and six cases where participants were infected after randomization to one of three self-administered PrEP regimens. In the last case, a participant was not randomized due to pregnancy and was infected during the follow-up period. In the seven of eight cases that occurred in the context of PrEP use, participants had infrequent PrEP dosing (e.g., once-weekly DOT only) or low/suboptimal adherence. In one case, adherence was high during most of the follow-up period; infection followed a 7-day period with no dosing (one missed dose in the time-driven arm).

The findings in this report highlight the higher diagnostic yield of sensitive assays for HIV diagnosis in the setting of PrEP use. In 9/12 cases, HIV infection was missed by two 3rd generation HIV rapid tests at the first HIV-positive visit; in eight of these cases, rapid tests were non-reactive at the first HIV-positive visit because the participant had acute HIV infection. Frequent detection of acute HIV infection in this cohort likely reflected the short intervals between study visits. In seven cases, one or both of the 4th generation assays was also non-reactive at the first HIV-positive visit. In three cases, one or both of the rapid tests also missed infection at subsequent study visits where both 4th generation tests were reactive. Failure to detect HIV infection using 3rd generation rapid tests resulted in continued PrEP use in eight cases. In one case, PrEP use was continued for 3–4 months after infection. In three of these cases, participants developed resistance to the study drugs (see below). In the iPrEx study, most HIV-infected individuals who had non-reactive HIV rapid

tests had a positive HIV RNA test.[22] In HPTN 067/ADAPT, four of eight participants who had acute infection at the first HIV-positive visit had a very low viral load (≤400 copies/mL); in these cases, infection was only detected using a sensitive, FDA-cleared qualitative HIV RNA assay. In the case where PrEP use was continued for 3–4 months after HIV infection, HIV RNA levels dropped below the level of detection for this sensitive assay, and the signal-to-cutoff ratios for both 4th generation tests remained low. This case illustrates that prolonged daily PrEP use in some individuals with undiagnosed HIV infection may be associated with low-level antibody production and sustained viral suppression.

In a study in rhesus macaques, PrEP was associated with delayed antibody maturation and low viral load.[23] In the Partners PrEP study, TDF or TDF/FTC PrEP was associated with delayed anti-HIV antibody formation (delayed time to develop a positive Western blot).[24] In the CAPRISA study, antibody maturation was delayed in women receiving vaginal TFV gel for PrEP.[25] In that study, antibody maturation was evaluated using assays developed for cross-sectional HIV incidence estimation.[25] In contrast, detection of HIV seroconversion was not delayed in two other PrEP trials.[9,26] These findings suggest that it may be difficult to diagnose HIV infection in some individuals who are taking TDF-based PrEP, particularly if less sensitive assays are used for HIV screening, and that PrEP use in cohorts and populations could also impact estimation of HIV incidence using cross-sectional surveys.[27]

In HPTN 067/ADAPT, resistance to study drugs was detected in three of 12 seroconverters. In two cases, resistance mutations were detected by NGS only; in the third case, one mutation was detected by routine HIV genotyping (M184I) and one was detected by NGS only (K65R). This demonstrates the additional diagnostic yield in this setting when more sensitive methods are used for analysis of HIV drug resistance. In two of these cases, participants with undiagnosed acute HIV infection were only exposed to four once-weekly doses of TDF/FTC; this indicates that very limited exposure to PrEP is sufficient to induce resistance in individuals with early/acute HIV infection.

Detailed characterization of seroconversion events in this study revealed that all 12 incident infections occurred in the setting of infrequent PrEP dosing or low/suboptimal adherence, and that drug resistance can arise with minimal exposure to PrEP. This report also highlights the importance of using sensitive assays for HIV diagnosis and resistance testing in the setting of PrEP. Identification of HIV infections before starting PrEP and prompt discontinuation of PrEP in those who become infected after starting PrEP should reduce the risk of HIV drug resistance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A. HIV-infected participants who were not randomized to a study arm.

Case	Site	Phase	Visit	Site rapid	Site RNA Qual	LC Arc (S/C)	LC BR (S/C)	LC RNA Qual	LC Disc	LC WB	LC VL	Resist VS	Resist NGS	Plasma TFV/FTC	TFV-DP/ FTC-TP
1	SA	DOT	En	NR/NR		NR	NR	P			400	Fail		-/-	
			w4	NR/R	R	R (4.1)	R (11)	P	P	IND	3,460	None	K65R=24.7%	0.4/0.6	8.4/0.3
			w5		R	R (9.2)				IND	5,732,050	None			3.4/-
2	US	DOT	En	NR/NR		NR	NR	P			<400	None	Fail	-/-	
			w4	NR/R	R	R (5.9)	R (13)	P	P	P	40,900	None	M184I=3.5% E138K=35.1%	-/0.4	
			w5		R	R (14)					83,260	None		-/-	
3	TH	DOT	En	NR/NR		NR	R (1.3)	P	N*		78,450	None	None	-/-	
			w4	R/R	R	R (8.2)	R (8.0)	P	P*	IND	710	None	None	-/1.4	6.6/0.3
			w5		R	R (9.8)				P	1,570	None		-/-	
4	SA	DOT	En	NR/NR		NR	NR	N						-/-	
			w4	NR/NR		R (45)	R (13)	P	N*	IND	3,667,690	None	None	-/-	8.5/0.1
			w5	R/R	R	R (31)	R (12)	P	P*	P	3,938,670	None	None	-/-	2.6/0.02
			w6		R	R (26)					1,341,050	None		-/-	2.7/-
5	TH	DOT	En	NR/NR		NR	NR	N						-/-	
			w4	NR/R	R	R (9.0)	R (11)	P	P*	IND	749,590	None	None	-/0.8	12/0.2
			w6		R	R (17)				P	92,960	None		-/-	6.3/-
6	SA	DOT	En	NR/NR		NR	NR							-/-	
			w4	NR/NR										1.2/2.2	2.4/0.1
			w5											0.6/3.2	7.6/0.2
		SAT	w6	NR/NR										-/1.7	0.8/-
			w22	NR/NR		NR	NR	N						-/-	
			w26	R/R	R	R (13)	R (13)	P	P*	P	503,950	None	None	-/-	
			w27		R	R (50)					227,820	None			-/-

B. HIV-infected participants who were randomized to the daily study arm.

Case	Site	Phase	Visit	Site rapid	Site RNA Qual	LC Arc (S/C)	LC BR (S/C)	LC RNA Qual	LC Disc	LC WB	LC VL	Resist VS	Resist NGS	Plasma TFV/FTC	TFV-DP/ FTC-TP
7	**SA**	**DOT**	**En**	NR/NR		NR	NR							-/-	
			w4	NR/NR										0.4/0.9	5.3/0.3
			w5			NR	NR	N						0.5/4.1	8.1/0.2
		SAT	**w6**	NR/NR		NR	NR	**P**			<400	Fail		-/-	0.8/-
			w10	NR/NR		**R (2.1)**	**R (8.7)**	**P**	IND*	**P**	<400	Fail		316/2700	38/9.4
			w14	NR/NR		**R (1.2)**	**R (3.4)**	**P**	**P**		<400	None	Fail	17/18	
			w18	NR/NR		**R (1.1)**	**R (7.2)**	**P**			**650**	Fail		194/2760	5.9/3.6
			w22	**R/R**	**R**	**R (2.3)**	**R (7.3)**	N			<400	None	Fail	69/184	
			w23		N	**R (2.2)**	**R (8.0)**	N			<400	Fail		0.8/2.6	3.9/0.1
8	**US**	**DOT**	**En**	NR/NR		NR	NR							-/-	
			w4	NR/NR		NR								-/0.5	
			w5			NR								-/0.5	
		SAT	**w6**	NR/NR		NR								-/-	
			w10	NR/NR		NR	NR	N						270/2460	
			w14	NR/NR		**R (25)**	**R (12)**	**P**	N*	N	**1,567,040**	K103N	K103N=98.6%	-/-	
			w18	**R/R**	**R**	**R (31)**	**R (31)**		**P**	**P**	**73,030**	K103N	K103N=98.6%	-/-	DBS: 35/-
			w19		**R**	**R (39)**					**33,020**	K103N	K103N=98.7%	-/-	

C. HIV-infected participants who were randomized to the time-driven study arm.

Case	Site	Phase	Visit	Site rapid	Site RNA Qual	LC Arc (S/C)	LC BR (S/C)	LC RNA Qual	LC Disc	LC WB	LC VL	Resist VS	Resist NGS	Plasma TFV/FTC	TFV-DP/ FTC-TP
9	**SA**	**DOT**	**En**	NR/NR										-/-	
			w4	NR/NR										-/0.47	5.88/0.18
			w5											0.6/0.8	9.1/0.4
		SAT	**w6**	NR/NR										-/-	1.3/0.02
			w10	NR/NR										-/-	0.9/-
			w14	NR/NR										15/9.3	
			w18	NR/NR		NR	NR	N						-/-	1.1/-
			w22	**R/R**	**R**	**R (53)**	**R (ND)**	**P**	N*	N	**5,887,760**	None	None	-/0.5	
			w23		**R**	**R (58)**			**P**	**P**	**4,503,500**	None		-/-	0.7/-
10	**SA**	**DOT**	**En**	NR/NR		NR	NR							-/-	
			w4	NR/NR										0.7/2.1	5.1/0.1
			w5											0.9/6.4	6.4/0.1
		SAT	**w6**	NR/NR										0.4/0.7	2.1/-
			w10	NR/NR		NR	NR	N						31/42	36/2.88
			w14	NR/NR		NR	NR	**P**			**2,100**	None	None	577/2100	
			w18	**R/R**	**R**	**R (31)**	**R (12)**	**P**	**P***	**P**	**3,710**	**M184I**	None	120/1470	37/5.6
			w19		**R**	**R (48)**					**5,360**	**M184I**	**K65R=3.9% M184I=62.3%**	1.4/13	8.3/0.1

D. HIV-infected participants who were randomized to the event-driven study arm.

Case	Site	Phase	Visit	Site rapid	Site RNA Qual	LC Arc (S/C)	LC BR (S/C)	LC RNA Qual	LC Disc	LC WB	LC VL	Resist VS	Resist NGS	Plasma TFV/FTC	TFV-DP/ FTC-TP
11	SA	DOT	En	NR/NR		NR	NR							-/-	
			w4	NR/NR										0.4/0.4	6.8/0.1
			w5											0.3/0.4	3.4/0.03
		SAT	w6	NR/NR										-/-	2.7/-
			w10	NR/NR										-/-	2.8/0.01
			w14	NR/NR										2.4/1.6	
			w18	NR/NR										-/-	1.5/-
			w22	NR/NR										-/-	
			w26	NR/NR		NR	NR	N						-/-	
			w30	NR/NR		NR	NR	**P**			<400	Fail		-/-	-/-
		F/U	w34	**R/R**	**R**	**R (5.0)**	**R (12)**	**P**		**P**	**19,790**	None	None	-/-	
			w35		**R**	**R (42)**			**P**		**13,580**	None			-/-
12	SA	DOT	En	NR/NR		NR	NR							-/-	
			w4	NR/NR										0.6/0.7	6.9/0.2
			w5											3.3/1.7	9.8/1.0
		SAT	w6	NR/NR										-/-	1.0/0.04
			w10	NR/NR										1.4/1.3	6.5/0.4
			w14	NR/NR		NR	NR	N						-/-	
			w18	NR/NR		NR	**R (1.3)**	**P**	N*		**83,010**	None	None	-/-	-/-
			w22	**R/R**	**R**	**R (58)**	**R (12)**	**P**	**P***	**P**	**136,310**	None	None	-/2.2	
			w23		**R**	**R (104)**					**65,230**	None		-/-	-/-

Figure 1. Laboratory test results for HIV-infected participants

Laboratory test results are shown for 6 participants who were not randomized (Figure 1A, Cases 1–6) and 6 participants who were randomized to the daily, time-driven, or event-driven study arms (Figure 1B–D, Cases 7–12). The shaded area indicates the period when participants received once-weekly directly observed therapy (DOT); four participants did not receive DOT at the week 4 visit because one or both of the HIV rapid tests was positive (Cases 1, 2, 3, and 5). One participant was not randomized due to pregnancy (Case 6); this participant was followed during the study, but did not receive PrEP in the SAT (self-administered therapy) phase. The remaining six participants were randomized to one of three PrEP regimens at week 6 (SAT). PrEP was discontinued when one or both of the HIV rapid tests was reactive; in one case, the participant stopped PrEP at the end of the SAT phase (week 30) and had reactive rapid tests at the next visit in the follow-up phase (at week 34, Case 11). Reactive/positive test results are shown in bold font. Two HIV rapid tests were performed in parallel at study sites (Site rapid); results are shown as reactive (R) or non-reactive (NR). HIV infection was confirmed at study sites using the APTIMA HIV-1 RNA Qualitative Assay (Site RNA Qual; limit of detection: <40 copies/mL, Hologic, Marlborough, Massachusetts). Additional testing was performed retrospectively at the

HPTN Laboratory Center (LC). This included two 4th generation tests (LC Arc: ARCHITECT HIV Ag/Ab Combo Assay, Abbott Diagnostics, Weisbaden, Germany; LC BR: GS HIV Combo Ag/Ab Enzyme Immunoassay, Bio-Rad Laboratories, Redmond, WA). The signal to cut-off ratios (S/C) for the 4th generation tests are shown in parenthesis; a ratio <1 is considered to be nonreactive. HIV infection was confirmed using a Western blot assay (LC WB, Genetics System HIV-1 Western blot test, Bio-Rad Laboratories) and a discriminatory assay (LC Disc, the Multispot HIV-1/HIV-1 Rapid test or Geenius HIV 1/2 Supplemental Assay, Bio-Rad Laboratories]); an asterisk indicates that the Geenius assay was used; other samples were tested using the Multispot assay. Western blot results were reported as positive (P), indeterminate (IND), or negative (N). Viral load testing (LC VL) was performed using a modified version of the COBAS AMPLICOR HIV-1 MONITOR test (Roche Diagnostics, Branchburg, NJ) with a lower limit of detection of 400 HIV RNA copies/mL. HIV drug resistance testing was performed using the ViroSeq HIV-1 Genotyping System (Resist VS) and a next generation sequencing assay with a mutation cut-off of 2% (Resist NGS). Mutations associated with resistance to tenofovir (TFV) and emtricitabine (FTC) are shown in bold font; other major drug resistance mutations are shown in regular font. TFV and FTC testing was performed using plasma samples (Plasma TFV/FTC, reported as ng/mL). TFV-diphosphate (TFV-DP) and FTC-triphosphate (FTV-TP) testing was performed using peripheral blood mononuclear cell PBMC samples (PBMC TFV-DP, reported as fmol/10^6 cells; PBMC FTC-TP, reported as pmol/10^6 cells), with one exception: testing for TFV-DP and FTC-TP was performed using DBS samples in Case 8 (reported as fmol/punch). Antiretroviral (ARV) test results below the limit of detection for each assay are shown with a dash (−). The s/c values for the 4th generation tests and the study drug concentrations values less than 10 are rounded to one decimal place; the values greater than 10 are rounded to the integer.

Abbreviations: SA; South Africa; US: United States; TH: Thailand; En: enrollment; w: week; NR: nonreactive; R: reactive; P: positive; N: negative; IND, indeterminate; RNA Qual: qualitative HIV RNA testing; S/C: signal to cut-off ratio; LC, HPTN Laboratory Center; Arc: ARCHITECT HIV Ag/Ab Combo Assay; BR: GS HIV Combo Ag/Ab Enzyme Immunoassay; Disc: Multispot HIV-1/HIV-1 Rapid test or Geenius HIV 1/2 Supplemental Discriminatory Assay; WB: Western Blot; VL: viral load; Resist: Resistance; VS: the ViroSeq HIV-1 Genotyping System; NGS: next generation sequencing; DBS: dried blood spot; TFV: tenofovir; FTC: emtricitabine; TFV-DP: tenofovir-diphosphate; FTC-TP: emtricitabine-triphosphate; DOT: directly observed therapy; SAT, self-administered therapy; F/U: follow-up; ND: not determined.

Table 1

Participants who seroconverted at the HPTN 067/ADAPT trial

Twelve participants seroconverted in the HPTN 067/ADAPT trial. The table shows the randomization status and study arm for each participant. The table also shows the first HIV-positive study visit, which was determined by retrospective testing performed at the HPTN Laboratory Center (LC), and the study visit where HIV infection was first detected at the study site using HIV rapid tests. An asterisk (*) indicates that the participant had acute HIV infection at the first HIV-positive study visit. All participants were exposed to once-weekly directly observed therapy (DOT). The six randomized participants were also exposed to self-administered pre-exposure prophylaxis (PrEP) with one of three dosing regimens (daily, time-driven, event-driven). Information on PrEP exposure during the self-administered phase of the trial was obtained by retrospective antiretroviral (ARV) drug testing and review of self-reported data on dosing that was collected during the trial. HIV mutations that confer resistance to tenofovir disoproxil fumarate/emtricitabine (TDF/FTC, study drug) were detected in three participants (at the week 4 study visit in Cases 1 and 2, and at the week 19 study visit in Case 10).

Case	Study Arm	HIV infection detected		PrEP exposure	Resistance tostudy drug
		Retrospective testing (HPTN LC)	Study site		
1	Not randomized Acute at enrollment	Enrollment*	w4	Once-weekly DOT only (4 doses)	K65R
2	Not randomized Acute at enrollment	Enrollment*	w4	Once-weekly DOT only (4 doses)	M184I
3	Not randomized Acute at enrollment	Enrollment*	w4	Once-weekly DOT only (4 doses)	Not detected
4	Not randomized Infected by 6 weeks	w4	w5	Once-weekly DOT only (5 doses)	Not detected
5	Not randomized Infected by 6 weeks	w4	w4	Once-weekly DOT only (4 doses)	Not detected
6	Not randomized, Pregnant	w26	w26	Once-weekly DOT only (5 doses)	Not detected
7	Daily arm	w6*	w22	DOT plus daily PrEP; low adherence	Not detected
8	Daily arm	w14*	w18	DOT plus daily PrEP; low adherence	Not detected
9	Time-driven arm	w22	w22	DOT plus time-driven PrEP; low adherence	Not detected
10	Time-driven arm	w14*	w18	DOT plus time-driven PrEP; adherent with a missing dose (7-day period with no drug before acute infection)	K65R, M184I
11	Event-driven arm	w30*	w34	DOT plus infrequent event-driving dosing	Not detected
12	Event-driven arm	w18*	w22	DOT plus infrequent event-driving dosing	Not detected

Abbreviations: HPTN: HIV Prevention and Treatment Network; LC: Laboratory Center; PrEP; Pre-exposure prophylaxis; DOT: directly observed therapy; w: week.