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

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BACKGROUND

HPTN 067/ADAPT was a phase 2 randomized, open-label trial of oral tenofovir disoproxil fumarate/emtricitabine (TDF/FTC) pre-exposure prophylaxis (PrEP) in women (South Africa) and men who have sex with men (MSM; Thailand, US). The study included a directly observed therapy (DOT) phase followed by a self-administered therapy (SAT) phase with three dosing regimens. We characterized incident HIV infections in the trial. The analysis included HIV testing, HIV drug resistance testing, antiretroviral (ARV) drug testing, and review of self-reported PrEP use for participants who seroconverted in the trial.

METHODS

STUDY DESIGN

DOT phase → **SAT phase** → **Final visit**

Enrollment → week 5
5 once-weekly observed doses; no dose at week 5

Week 6/Randomization → Week 30
Study arms:
- Daily dosing
- Time-driven dosing
- Event-driven dosing

Week 34

STUDY COHORT

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).

LABORATORY TESTING

Samples from enrollment and follow-up visits

Two HIV rapid tests (study sites)

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Qualitative HIV RNA assay if one or both rapid tests was reactive (study sites)

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Retrospective testing at the HPTN Laboratory Center (LC):
- Two 4th generation assays
- Discriminatory assay
- Qualitative HIV RNA assay
- Western blot test
- Viral load assay
- Drug resistance testing:
  - ViroSeq HIV-1 Genotyping System
  - Next generation sequencing (NGS)

ARV drug testing by liquid chromatography tandem mass spectrometry (LC-MS/MS):
- Plasma samples (all three study sites)
- Peripheral blood mononuclear cell (PBMC) samples (South Africa, Thailand)
- Dried blood spot (DBS) samples (US)

RESULTS

Twelve participants seroconverted during the study (Table). Six were not randomized (5 were infected before randomization; 1 was pregnant); four did not receive the last observed dose. The remaining six participants were randomized, two in each study arm.

Table. Participants with HIV infection

Case	Study arm	HIV Infection detected		Resistance to study drug
		Retrospective testing (HPTN LC)	Study site	
1	Not randomized, Acute at enrollment	Enrollment	w4	K65R
2	Not randomized, Acute at enrollment	Enrollment	w4	M184I
3	Not randomized, Acute at enrollment	Enrollment	w4	Not detected
4	Not randomized, infected	w4	w5	Not detected
5	Not randomized, infected	w4	w4	Not detected
6	Not randomized, pregnant	w26	w26	Not detected
7	Daily arm	w6	w22	Not detected
8	Daily arm	w14	w18	Not detected
9	Time-driven arm	w22	w22	Not detected
10	Time-driven arm	w14	w18	K65R, M184I
11	Event-driven arm	w30	w34	Not detected
12	Event-driven arm	w18	w22	Not detected

DELAYED DETECTION OF HIV INFECTION

One or both HIV rapid tests was non-reactive at the 1st HIV-positive visit in 10/12 cases; in 9 cases, both rapid tests were non-reactive. In 4/12 cases, one or both HIV rapid tests was still non-reactive at the seroconversion visit. HIV RNA was detected at the first HIV positive visit in all 12 cases; the viral load was ≤400 copies/mL at the first HIV positive visit in 4/12 cases. In one case (Case 7, Figure A), both rapid tests were non-reactive at multiple study visits. Retrospective testing revealed that the participant had acute HIV infection at randomization (week 6), but HIV infection was not detected at the study site until week 22. The participant continued to use PrEP for 3-4 months after HIV infection. The participant reported taking >80% of the assigned daily doses; the concentration of drugs detected at some visits was consistent with less than daily PrEP use. HIV viral loads were persistently low. Fourth generation HIV assays were reactive after infection, but had low signal-to-cutoff ratios. A sensitive, qualitative HIV RNA assay was initially positive, but was negative at subsequent visits.

RESULTS

Figure. Test results for selected cases

(A) Prolonged PrEP use in a participant with false-negative rapid tests

Case	Site	Phase	Visit	Site rapid	Site RNA Qual	Arch 4th (S/C)	BioRad 4th (S/C)	RNA Qual	Disc	WB	VL	Resist VS	Resist NGS	Plasma TFV/FTC	PBMC 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

(B) Participants who acquired resistance to study drugs

Case	Site	Phase	Visit	Site rapid	Site RNA Qual	Arch 4th (S/C)	BioRad 4th (S/C)	RNA Qual	Disc	WB	VL	Resist VS	Resist NGS	Plasma TFV/FTC	PBMC 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	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 E138K	-/0.4	
			w5		R	R (14)					83,260	None		-/-	
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 M184I	1.4/13	8.3/0.1

Abbreviations: 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; Arch 4th: ARCHITECT HIV Ag/Ab Combo Assay; BioRad 4th; 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; TFV: tenofovir; FTC: emtricitabine; TFV-DP: tenofovir diphosphate; FTC-TP: emtricitabine triphosphate; ND: not determined.

Notes: The limits of detection/quantification for assays used in this report were: Viral load testing: 400 copies/mL; NGS: mutation frequency 2%; ARV drug testing: plasma: TFV and FTC 0.31 ng/mL; PBMC: TFV-DP 2.5 fmol/sample, FTC-TP 0.1 pmol/sample; DBS: TFV-DP 31.25 fmol/punch, FTC-TP 0.125 pmol/punch.

EMERGENCE OF HIV DRUG RESISTANCE

Mutations that confer resistance to TDF/FTC were detected in three cases (Figure B). Two participants (Cases 1 and 2) had acute HIV infection at enrollment and were not randomized. These participants received only four once-weekly observed PrEP doses. One participant was randomized to the time-driven arm (Case 10). All of the mutations were detected by NGS; only one mutation was identified using a standard genotyping assay (Case 10, M184I).

ARV TESTING AND SELF-REPORTED PREP USE

All 12 incident infections occurred in the setting of infrequent PrEP dosing or low/suboptimal adherence. Two infections occurred in participants who received only four once-weekly observed doses. Results for randomized participants are summarized here: Cases 7 and 8: low adherence to daily PrEP. Case 9: low adherence to time-driven PrEP. Case 10: adherent to time-driven PrEP with one missed dose resulting in a 7-day period with no drug a few days before acute infection was detected. Cases 11 and 12: Infrequent PrEP use in the event-driven arm; both reported infrequent sex events with low adherence to the event-driven regimen.

CONCLUSIONS

- All cases of HIV infection occurred in participants with infrequent PrEP dosing or low/suboptimal adherence.
- Two participants developed drug resistance with very limited exposure to PrEP.
- HIV infection was frequently missed by HIV rapid tests; in one case, this led to prolonged PrEP use in an HIV-infected participant.
- Use of sensitive assays improved detection of HIV infection and detection of HIV drug resistance.

ACKNOWLEGEMENTS

The authors thank the HPTN 067 study team, the study participants, and the laboratory staff at study sites and the HPTN Laboratory Center.

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The HIV Prevention Trials Network is sponsored by the U.S. National Institute of Allergy and Infectious Diseases, the U.S. National Institute of Mental Health, and the U.S. National Institute on Drug Abuse, all components of the U.S. National Institutes of Health.

Poster Number: 955
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