PrEP Exposure and the Risk of Low-frequency Drug Resistance

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Dara Lehman[1], Jared Baeten[2], Connor McCoy[1], Julie Weis[1], Dylan Peterson[1], Connie Celum[2], Nelly Mugo[3], Julie Overbaugh[1], Frederick Matsen[1], and The Partners PrEP Study Team

[1]Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [2]University of Washington, Seattle, WA, USA; [3]University of Nairobi, Nairobi, Kenya.

Dara Lehman, PhD
FHCRC, Seattle, WA
dlehman@fhcrc.org
Phone: 206-667-4148

ABSTRACT

Background
Pre-exposure prophylaxis (PrEP) reduces HIV acquisition. However, for those who acquire HIV while receiving PrEP there is the potential for drug resistance to arise as a result of partial HIV suppression through PrEP medication exposure before HIV seroconversion is detected. The level of resistance selected by PrEP may not be detectable with standard sequencing methods, which only detect resistance present at frequencies above 20% of the viral population. Studies of antiretroviral treatment failure have suggested that resistance as low as 1% may be clinically significant during subsequent antiretroviral use. 454 ultra-deep sequencing can detect resistance at frequencies below 1%.

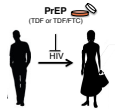
Methods
In the Partners PrEP Study, a randomized trial of daily oral tenofovir disoproxil fumarate (TDF) or tenofovir plus emtricitabine (FTC), plasma samples from the time of HIV seroconversion were tested for predefined mutations that could be selected by TDF (K65R and K70E) and FTC (K65R and M184IV) using 454 ultra-deep sequencing. Samples were considered resistant if the proportion of mutant sequences was significantly above that observed in wild-type genotype-matched controls using Fisher's exact test with a 5% false-discovery rate. Plasma levels of tenofovir were measured among seroconverters in the active PrEP arms at the time of seroconversion. Fisher's exact test was used to determine associations with resistance.

Results
A total of 121 seroconverters were tested: 38 had received TDF, 25 FTC-TDF, and 58 placebo. PrEP-related resistance mutations (K65R, K70E and/or M184IV) were detected in 9 (7.4%) at levels >1%, 2 of which had previously been detected by standard sequencing. In the TDF arm, 2 of 38 (5.3%, p=0.65 vs placebo) had resistance >1%: 1 had K65R/K70E and 1 had M184IV. In the FTC-TDF arm, resistance was detected in 5 of 25 (20%, p=0.024 vs placebo): 4 had M184IV alone and 1 had M184IV/K65R. Two of the 58 (3.5%) placebo participants had M184IV resistance. Resistance was detected in 3 individuals retrospectively determined to be HIV infected (seronegative but RNA positive) when PrEP was initiated, and in 6 individuals infected after PrEP start. In seroconverters in the active PrEP arms, PrEP use was associated with higher risk of resistance: 6 of 23 (26%) with measurable drug levels versus only 1 of 39 (2.6%) without evidence of PrEP use had resistance >1% (p=0.009).

Conclusions
High-sensitivity resistance testing detected more resistance than standard sequencing in persons acquiring HIV who were exposed to PrEP, although resistance still occurred in a minority of subjects. More resistance was selected by FTC than TDF, as evidenced by the prevalence of M184IV compared to K65R.

STUDY POPULATION

The Partners PrEP Study

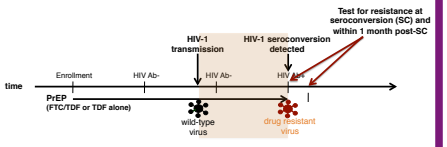


4758 serodiscordant couples enrolled in Partners PrEP

The HIV-negative partner was randomized

FTC/TDF arm	TDF arm	placebo arm
25 seroconversions	39 seroconversions	58 seroconversions
21 infected after enrollment	31 infected after enrollment	52 infected after enrollment
3 were HIV+ at enrollment	5 were HIV+ at enrollment	6 were HIV+ at enrollment
1 was HIV+ at re-randomization	3 were HIV+ at re-randomization	

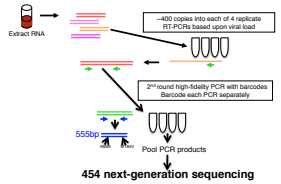
What is the risk of resistance in individuals who become infected while taking PrEP?



In the Partners PrEP Study, FTC/TDF combination or TDF alone was taken daily. Participants had monthly clinic visits to detect HIV infection. When transmission occurred there was often a delay in detection, resulting in the potential for a period of time in which HIV was replicating in the presence of non-suppressive drug treatment and resistance could have been selected.

METHODS

Low-frequency resistance testing



454 next-generation sequencing

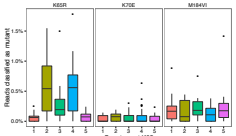
Between 1000 to 1600 input virus copies per sample
2000 bidirectional reads per sample = 1000 reads in each direction

Viral RNA was extracted from 100 - 500ul of plasma using a guanidinium isothiocyanate extraction method optimized by the Coffin Lab. Four independent, parallel, high-fidelity RT-PCR reactions followed by a 2nd round PCR with 454-adaptors and 8bp barcodes were performed for each sample. PCR products were pooled and pyrosequenced on the 454 GS-Junior Platform.

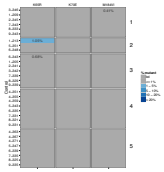
K65 genotype	Sequence surrounding K65	% of cohort
1	TAAA**G**AAAAAG	73%
2	TAAAAAA**G**AAAG	0%
3	TAAA**G**AA**G**AAAG	3%
4	TAAAAAA**G**AA**G**G	6%
5	TAAA**G**AAAAA**G**G	18%

The sequence surrounding K65R is homopolymeric and varies across HIV subtypes. 454 sequencing is known to be error-prone in homopolymeric regions, and thus we created a panel of 5 engineered RNA controls which include the variation at K65 present in the Partners PrEP cohort. The wild-type controls are isogenic other than the noted sequence variation (highlighted in bold) surrounding K65. The K65R mutation results from an A to G change at the position highlighted in orange.

Error rates in wild-type controls

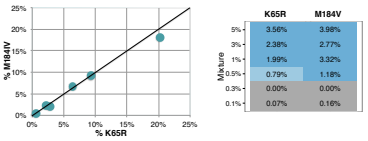


False-positive rates in wild-type controls



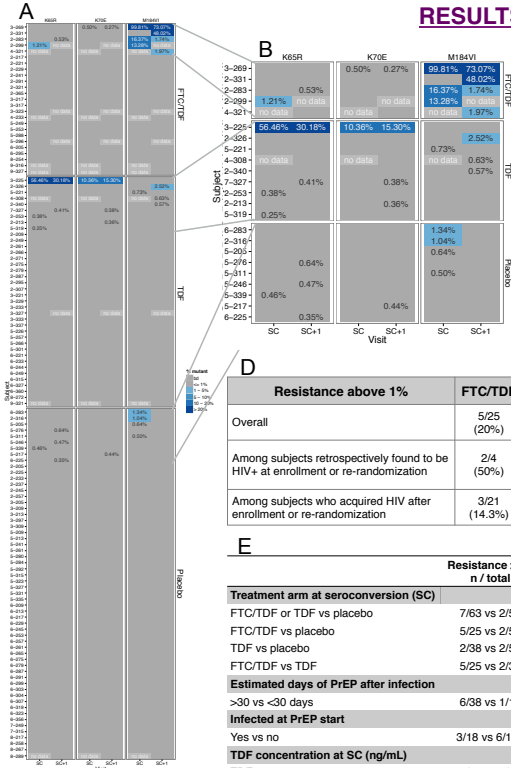
Wild-type controls with variation at the K65-locus were RT-PCR amplified, processed and sequenced in parallel with patient samples in up to 9 Junior 454 runs. **Left:** Box plots show the percent of sequence reads classified as mutant at each of the 3 sites of interest in the 5 different K65 genotype wild-type controls. **Right:** Each row of the table shows the % K65R, K70E and M184IV detected in a wild-type control sample of the K65 genotype specified on the right vertical axis. Samples are considered resistant if the proportion of mutant sequences was significantly above that observed in the wild-type genotype-matched controls using Fisher's exact test with a 5% false discovery rate. Solid grey cells are below detection (bd), grey cells with % in black have detectable levels of resistance below 1%, blue cells have resistance detectable above 1%. A false-positive rate of 3.12% was seen in 96 wild-type control tests.

Low-frequency K65R/M184V controls



Low-frequency controls were made by quantifying and mixing in-vitro RNA controls that contain both K65R and M184V with RNA controls that are wild-type at both sites. Concordance between quantitative measures of both K65R and M184V show the ability to detect both mutations at low frequencies.

RESULTS



D

Resistance above 1%	FTC/TDF	TDF	Placebo	All
Overall	5/25 (20%)	2/38 (5.3%)	2/58 (3.5%)	9/121 (7.4%)
Among subjects retrospectively found to be HIV+ at enrollment or re-randomization	2/4 (50%)	1/8 (12.5%)	0/6 (0%)	3/18 (16.7%)
Among subjects who acquired HIV after enrollment or re-randomization	3/21 (14.3%)	1/30 (3.3%)	2/52 (3.8%)	6/103 (5.8%)

E

	Resistance >1% n / total	Resistance >1% %	Fisher's exact p-value
Treatment arm at seroconversion (SC)			
FTC/TDF or TDF vs placebo	7/63 vs 2/58	11% vs 3.45%	0.167
FTC/TDF vs placebo	5/25 vs 2/58	20% vs 3.45%	0.024
TDF vs placebo	2/38 vs 2/58	5.2% vs 3.45%	0.65
FTC/TDF vs TDF	5/25 vs 2/38	20% vs 5.2%	0.1
Estimated days of PrEP after infection			
>30 vs <30 days	6/38 vs 1/19	16% vs 5.3%	0.4
Infected at PrEP start			
Yes vs no	3/18 vs 6/103	16.7% vs 5.8%	0.13
TDF concentration at SC (ng/mL)			
TDF >0.31 vs <0.31	6/23 vs 1/39	26% vs 2.6%	0.009

A: Levels of K65R, K70E, and M184IV in all seroconverters in the Partners PrEP Study. Subjects are ordered by levels of resistance from highest to lowest within each treatment arm. Samples tested include the time that seroconversion was first detected (SC) and one month after seroconversion (SC+1). Higher levels of resistance denoted by darker blue color. Grey cells with % in black have detectable levels of resistance below 1%. Solid grey cells are below detection. Cells are marked as 'no data' if the sample could not be amplified. **B**: Levels of K65R, K70E, and M184IV in seroconverters with any resistance detectable by 454 sequencing. Table details as in A. **C**: Levels of M184I or M184V. Table details as in A. **D**: A summary table of resistance detected at levels above 1% across treatment arms in all seroconverters, in seroconverters who were retrospectively found to be infected at the time they started PrEP or placebo, and those who seroconverted after PrEP start. **E**: Factors associated with resistance at levels >1%. SC is the visit seroconversion was first detected.

CONCLUSIONS

- Resistance, including low-frequency resistance, selected by PrEP is rare.
- Resistance was detected in 3 individuals retrospectively found to be HIV-positive at enrollment, and 6 individuals infected after PrEP start.
- Detection of PrEP drug in blood plasma was associated with an increased risk of resistance.
- Risk of resistance was highest in the FTC/TDF arm due to M184IV mutations.

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Partners PrEP Study Team
University of Washington Coordinating Center and Central Laboratories, Seattle, USA: Connie Celum (principal investigator, protocol co-chair), Jared M. Baeten (medical director, protocol co-chair), Deborah Donnell (protocol statistician), Robert W. Coombs, Jairam Lingappa, M. Juliana McElrath.
Study sites and site principal investigators:
Eldoret, Kenya (Moi University, Indiana University): Kenneth Fife, Edwin Were; Kabwohe, Uganda (Kabwohe Clinical Research Center): Elioda Tumwesigye, Jinja, Uganda (Makerere University, University of Washington): Patrick Ndase, Elly Katabira; Kampala, Uganda (Makerere University): Elly Katabira, Allan Ronald; Kisumu, Kenya (Kenya Medical Research Institute, University of California San Francisco): Elizabeth Bukusi, Craig Cohen; Mbale, Uganda (The AIDS Support Organization, CDC-Uganda): Jonathan Wangisi, James Campbell, Jordan Tappero; Nairobi, Kenya (University of Nairobi, University of Washington): James Kiarie, Carey Farquhar, Grace John-Stewart; Thika, Kenya (University of Nairobi, University of Washington): Nelly Rwamba Mugo; Tororo, Uganda (CDC-Uganda, The AIDS Support Organization): James Campbell, Jordan Tappero, Jonathan Wangisi.
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