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The Dynamics of Drug Resistance During Acute HIV Infection

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ABSTRACT

Background: HIV drug resistance detected during acute infection may be a result of transmission from an infected partner, selection by pre-exposure prophylaxis (PrEP), or error prone replication. Here we describe the long-term dynamics of resistance detected during early acute HIV infection.

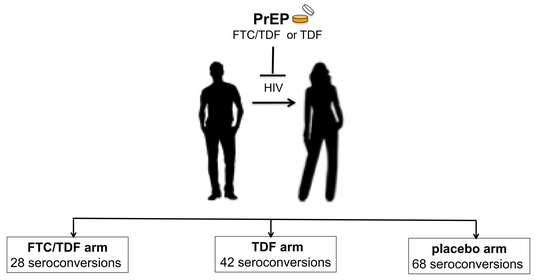
Methods: The Partners PrEP Study was a randomized trial in which serodiscordant partners were assigned to emtricitibine co-formulated with tenofovir (FTC/TDF) or TDF alone compared to placebo, and monitored for HIV infection monthly. In a previous study, baseline samples from 137 HIV serocovnverters were tested for drug resistance using 454 ultra deep sequencing at the time seroconversion was first detected. Here, we conducted resistance testing by 454 on plasma samples at 6, 12 and 24 months following seroconversion from individuals with resistance mutations detected during acute infection at frequencies ≥1% that had a Stanford score ≥30 and cause resistance to non-nucleoside or nucleoside reverse-transcriptase inhibitors (NNRTIs or NRTIs), including both PrEP and non-PrEP antiretrovirals.

Results: There were 35/137 (26%) individuals with resistance detected at frequencies ≥1% at the time seroconversion was first identified: 30 had resistance to non-PrEP antiretrovirals (not TDF or FTC) likely due to transmitted resistance or error-prone replication and 11 individuals had PrEP-related mutations (K65R, K70E and M184IV); of whom 6 had both PrEP-related and non-PrEP mutations. Of the 35, 31 individuals had resistance results available through 12-24 months. Resistance faded to frequencies <1% in 18/31 (58%) individuals by 6 months after seroconversion and in 23/31 (74%) by 12 or 24 months. PrEP-selected mutations did not persist. In 5 individuals, resistance persisted at low frequencies between 1-10% of the viral population and in 3 individuals, resistance mutations K103N (n=2) or Y181C (n=1) persisted at frequencies ≥ 99% throughout follow-up.

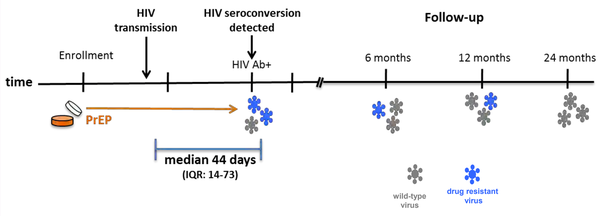
Conclusion: Among individuals who acquired HIV in a PrEP trial, non-PrEP (non TDF or FTC) mutations accounted for the majority of resistance detected during acute infection. Persistence of high frequency resistance was limited to 2 NNRTI mutations that were most likely transmitted. Most of the resistance present during acute infection faded below detection by 6 months regardless of whether it was selected or transmitted. These data suggest that in the absence of antiretroviral treatment, onward transmission of resistance is most likely to occur early after HIV infection.

STUDY DESIGN

The Partners PrEP Study



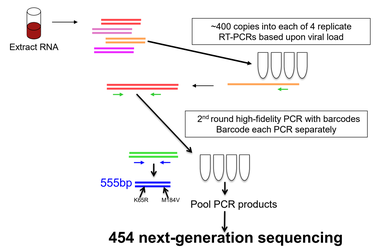
Does resistance detected during acute HIV infection persist or decay over time?



In the Partners PrEP Study, FTC/TDF combination, TDF alone or placebo was taken daily. Participants had monthly clinic visits to detect HIV infection. There were 137 seroconverters with a median 44 days (range 4-437) between the estimated date of HIV infection and detection of seroconversion. Resistance testing by 454 sequencing was performed in samples from visits when HIV seroconversion was first detected and 1, 6, 12 and 24 month(s) later. Mutations with Stanford resistance scores of ≥30 that are associated with resistance to PrEP drugs or other RT-inhibitors were assessed.

METHODS

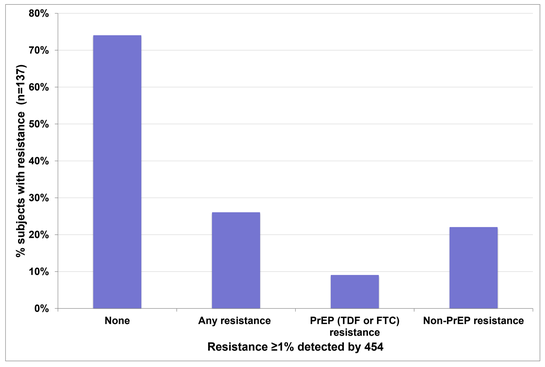
Low-frequency resistance testing

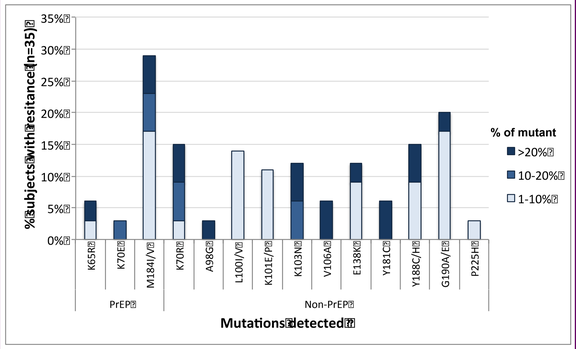


Viral RNA was extracted from 100 - 500ul of plasma using a guanidinium isothiocyanate extraction method optimized by the Coffin Lab. Four independent, parallel, high-fidelity RT-PCR reactions followed by a 2nd round PCR with 454-adaptors and 8bp barcodes were performed for each sample. PCR products were pooled and pyrosequenced on the 454 GS-Junior Platform. Resistance testing was conducted using 454 sequencing on plasma samples at 6, 12 and 24 months following seroconversion from individuals with resistance mutations detected during acute infection at frequencies ≥1% that had a Stanford score ≥30 and cause resistance to non-nucleoside or nucleoside reverse-transcriptase inhibitors (NNRTIs or NRTIs), including both PrEP and non-PrEP antiretrovirals.

RESULTS

During Acute HIV infection, resistance without evidence of drug selection is more prevalent than resistance due to PrEP exposure.





The graphs above show resistance detected at seroconversion in 137 subjects from the Partner's PrEP study. In the top graph there were 35/137 (26%) individuals with resistance detected at frequencies ≥1% at the time seroconversion was first identified: 30/137 (22%) had resistance to non-PrEP antiretrovirals (not TDF or FTC) and 11/137 (8%) individuals had PrEP-related mutations (K65R, K70E and M184IV); of whom 6/137 (4%) had both PrEP-related and non-PrEP mutations. In the bottom graph, prevalence of specific mutations is shown with each bar divided by the proportion of subjects in which the resistance mutation was present within the viral population at >20% (darkest blue), between 10-20% (blue), and between 1-10% (light blue)

RESULTS

Majority of resistance detected during acute infection decays rapidly

PrEP-related resistance

Mutation	PID	SC	1 month	6 months	12 months	24 months
K65R	*2-299	1.0%	-	-	-	-
	*3-225	56.0%	30.0%	bd	bd	bd
K70E	*3-225	10.0%	15.0%	bd	bd	bd
M184I	6-283	1.0%	bd	bd	bd	bd
	2-316	1.0%	bd	6.0%	bd	1.0%
	*2-326	bd	3.0%	bd	bd	bd
M184V	*4-321	-	2.0%	-	bd	bd
	2-283	16.0%	1.7%	-	bd	bd
	*3-269	99.0%	73.0%	bd	bd	bd
M184IV	11-298	7.0%	17.0%	bd	bd	bd
	*2-299	13.0%	-	-	-	-
	*2-331	bd	48.0%	bd	bd	bd

- sample not available
below detection (bd)
1-20%
20-40%
40-60%
60-80%
80-100%

Non-PrEP resistance

Mutation	PID	SC	1 month	6 months	12 months	24 months
K70R	5-301	bd	1.0%	bd	bd	bd
	6-265	2.0%	bd	bd	bd	4.0%
	2-253	3.0%	12.0%	bd	bd	bd
	7-327	86.0%	65.0%	bd	bd	bd
	2-249	98.0%	10.0%	78.0%	90.0%	9.0%
A98G	2-253	30.0%	85.0%	bd	bd	bd
L100I/V	5-246	1.0%	bd	bd	-	bd
	6-244	1.0%	bd	bd	-	-
	5-315	1.0%	bd	1.0%	2.0%	2.0%
	7-249	bd	1.0%	3.0%	1.0%	bd
	3-337	bd	2.0%	bd	bd	bd
K101E/P	5-323	bd	1.0%	bd	bd	bd
	4-321		2.0%	-	bd	bd
	6-249	4.0%	4.0%	bd	bd	bd
	11-298	6.0%	bd	3.0%	bd	bd
K103N	2-283	18.0%	bd	-	bd	bd
	6-221	19.0%	18.0%	15.0%	6.0%	-
	2-307	99.0%	99.0%	99.0%	99.0%	-
	6-307	100.0%	100.0%	99.0%	100.0%	100.0%
V106A	2-287	98.0%	96.0%	-	-	-
E138K	2-326	1.0%	3.0%	bd	bd	bd
	5-301	bd	2.0%	bd	bd	bd
	8-254	bd	5.0%	bd	bd	bd
	6-249	41.0%	51.0%	bd	bd	bd
Y181C	11-293	35.0%	bd	bd	bd	-
	11-298	100.0%	100.0%	100.0%	100.0%	100.0%
Y188C/H	2-261	2.0%	4.0%	bd	bd	-
	11-293	3.0%	bd	bd	bd	-
	2-266	6.0%	10.0%	bd	bd	bd
	5-301	33.0%	9.0%	bd	bd	bd
	7-327	92.0%	93.0%	bd	bd	bd
G190A/E	11-209	1.0%	bd	bd	bd	-
	2-326	1.0%	2.0%	bd	bd	bd
	5-288	2.0%	bd	bd	bd	bd
	10-360	2.0%	bd	-	-	-
	5-296	3.0%	-	-	bd	bd
	11-293	32.0%	bd	30.0%	bd	-
	6-221	68.0%	43.0%	bd	bd	-
P225H	3-225	bd	2.0%	bd	bd	bd

The chart on the left shows subjects with PrEP-related resistance over time, from detection of seroconversion (SC) through 24 months after seroconversion detection. Subjects are grouped by mutations. Higher frequencies of resitsance mutations in the viral population denoted by darker blue color. Stars have been placed next to PID of subjects who had tenofovir detected in the blood during HIV infection. PrEP-selected mutations decayed by 6 months. Subject 2-316, with mutation at M184I was in the placebo group of the Partners PrEP trial, and thus M184I was not selected by PrEP in this case.

The chart on the right shows subjects with non-PrEP resistance over time. Non-PrEP resistance decays in majority by 6 months.

Resistance is present in 8 individuals at the latest timepoint available (12 or 24 months of follow-up); low and high frequency resistance is seen in 5 and 3 individuals, respectively.

CONCLUSIONS

- Among individuals who acquired HIV in a PrEP trial, non-PrEP mutations accounted for the majority of resistance detected during acute infection.
- Persistence of high frequency resistance limited to 2 NNRTI mutations; most likely transmitted.
- Most resistance present during acute infection decayed below detection by 6 months.
- Data suggests, in the absence of antiretroviral therapy, onward transmission of resistance is most likely to occur early after HIV infection.

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