Similar Risk of Resistance in Plasma and Genital Tract in Women who Seroconverted on PrEP in VOICE

Kerri J. Penrose[1], Rahil Sethi[1], Uma Chandran[1], Cliff Kelly[2], Zvavahera Mike Chirenje[3], Jeanne Marrazzo[4], John W. Mellors[1], and **Urvi M. Parikh[1]**, on behalf of the MTN 003 Protocol Team

[1]University of Pittsburgh, Pittsburgh, PA, USA; [2]SCHARP, Seattle, WA, USA; [3]University of Zimbabwe College of Health Science-Clinical Research Center, Harare, Zimbabwe; [4]University of Alabama at Birmingham School of Medicine, Birmingham, AL, USA

Background

- Few cases of tenofovir disproxil fumarate/emtricitabine (TDF/FTC) resistance HIV-1 have been found in seroconverters from oral PrEP trials. (Baeten et *al*., 2012; Van Damme et *al*., 2012; Grant et *al*., 2014; Marrazzo et *al*., 2015; Becker et *al*.,2018)

- VOICE was a Phase 2 safety and effectiveness study of tenofovir-based products for HIV prevention in women
  - Sanger sequencing of plasma samples in the VOICE study demonstrated:
    - ➢ No cases of resistance to oral tenofovir or tenofovir gel
    - ➢ 9 cases (2.5% overall) of transmitted resistance with major NNRTI mutations
    - ➢ 3 cases (0.8% overall) of FTC resistance ; all from TDF-FTC arm (3/71; 4.2%)

- HIV variants in the plasma of infected women can differ from variants found in her genital tract. (Kemal et. *al*, 2003)
- Higher viral genetic diversity can be found in the genital tract compared to plasma in early HIV-1 infection (Klein et. *al*, 2018)

Objective

To assess the frequency of HIV-1 drug resistance in the genital tract of women who seroconverted during use of oral TDF/FTC for HIV prevention in VOICE.

Sample Selection

Cryopreserved cervicovaginal swabs and plasma samples were selected from the VOICE trial with the following criteria:

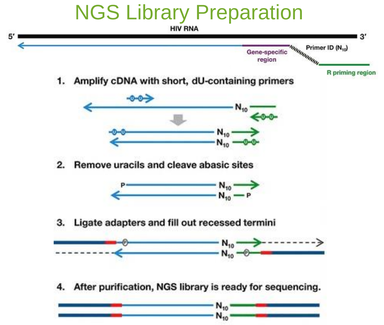
- Genital tract samples (i.e. cervicovaginal swab) collected within 60 days of seroconversion from participants:
  - In the oral TDF or TDF/FTC arm who had detectable plasma tenofovir at any study visit (n=17)
  - In the placebo arm matched to active arm swabs by time from seroconversion and geographical region (n=16)

- Plasma samples collected within 60 days of swab collection
  - Having detectable HIV RNA

Methods

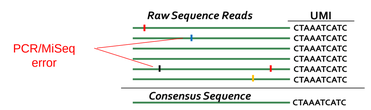
Illumina- based next generation sequencing (NGS) libraries were prepared from HIV RNA isolated from swab and plasma samples utilizing unique molecular identifiers (UMIs) to tag individual HIV templates.

NGS Library Preparation



Analysis

- Create consensus sequences from reads with identical UMI
  - ➢ Reduces PCR and sequencing errors
  - ➢ Allows depth of sequencing to be calculated

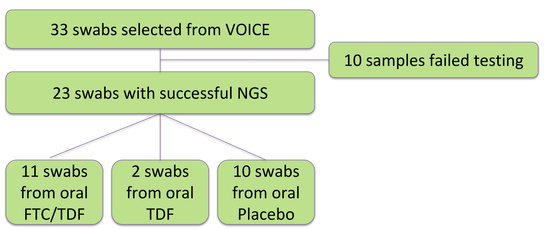


- Drug resistance mutation (DRM) frequency calculation per codon
- Hamming distance was calculated at the nucleotide level

Figures adapted from: Boltz et *al*.,2016; *https://tcs-dr-dept-tcs.cloudapps.unc.edu/*

Results

1. Success rate of genital tract swab testing



2. Sensitivity of DRM detection in NGS testing

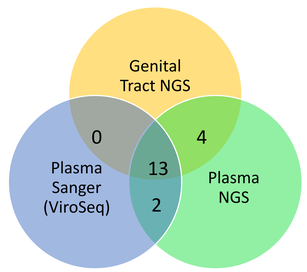
# of UMI needed (w/ 95% probability of detection)	Sensitivity of DRM detection	Genital tract swabs N (UMI range)	Plasma N (UMI Range)
298	1%	6(308-9536)	22 (398-15495)
58	5%	6 (64-294)	1 (134)
28	10%	6 (29-49)	0
13	20%	1 (19)	0
<13	>20%	4 (1-5)	0

3. Drug resistance

		Plasma				Genital Tract					
			NGS			NGS					
Sample Number	ARM	ViroSeq HIV DRM	% Sensitivity	# UMI	HIV DRM	% Sensitivity	# UMI	HIV DRM	Hamming distance (Plasma vs Genital Tract NGS)[3]	Closest VL	# of days of swab collection from seroconversion
1	FTC/TDF	None	1	1073	None	10	29	None	0	1531	0
2	FTC/TDF	None	1	1403	None	10	46	None	0	2019	39
3	FTC/TDF	None	1	2544	None	5	64	None	0	4160	9
4	FTC/TDF	None	1	6714	None	5	264	None	0	32214	0
5	FTC/TDF	M184V	1	529	73.3%M184V	20	15	100% M184V	1	33170	10
6	FTC/TDF	None	1	15495	None	5	124	None	0	53030	52
7	FTC/TDF	V90I	1	23241	100%V90I	10	39	100%V90I	0	59000	16
8	FTC/TDF	None	1	429	None	>20	2	None	0	63421	48
9	FTC/TDF	None	1	1754	None	>20	1	None	0	144372	30
10	FTC/TDF	None	1	10558	None	5	121	None	7	169183	-21
11	FTC/TDF	None	1	10595	None	1	9536	None	0	1419990	-25
12	TDF	None	1	1024	None	1	489	None	3	138039	-44
13	TDF	None	1	10882	None	1	1228	None	0	168608	-8
14	Placebo	None	5	134	None	1	308	None	0	1554	7
15	Placebo	L10I[2]	1	389	None	>20	5	None	0	3569	0
16	Placebo	None	1	11790	None	10	34	None	0	22303	15
17	Placebo	None	1	668	None	10	49	None	1	24169	0
18	Placebo	None	1	2464	None	5	100	None	0	66880	9
19	Placebo	None	1	4432	None	5	77	None	0	70185	0
20	Placebo	None	1	11553	None	1	407	None	6	90830	45
21	Placebo	None	1	577	None	>20	3	None	9	128211	21
22	Placebo	None	1	3528	None	1	497	1.6% A62V	0	398943	0
23	Placebo	None	1	4416	None	10	33	None	0	399956	30

1. HIV DRMs listed are based on the IAS-USA 2019 Resistance Mutations Update.
2. The NGS assay covers RT amino acids 63-131 and 152-211
3. Hamming distance was evaluated including all nucleotides in the amplified region of RT (not just DRMS)

4. Samples sharing identical sequence



- 6 of 23 samples show differences in genotype between plasma and genital tract NGS
- Hamming distance range from 1-9 of 394 nucleotides surveyed; ≥97.7% identity

Conclusions

- In 11 seroconverters tested from the TDF/FTC arm of VOICE, independent cases of FTC DRM M184V and NNRTI DRM V90I were found in both the genital tract and plasma.
- 98-100% genotypic identity between HIV-1 in the genital tract and circulating plasma was found in seroconverters from the VOICE trial.
- This limited dataset provides reassurance that women who seroconvert on PrEP do not have a greater risk of selecting resistant HIV-1 in their genital tract.

The Microbicide Trials Network is funded by the National Institute of Allergy and Infectious Diseases (UM1AI068633, UM1AI068615, UM1AI106707), with co-funding from the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development and the National Institute of Mental Health, all components of the U.S. National Institutes of Health.

GEMS is funded through the United States Agency for International Development (USAID) and the U.S. President's Emergency Plan for AIDS Relief (PEPFAR).