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Deep Sequencing With Unique Molecular Identifiers for Evaluation of HIV-1 Drug Resistance in the DISCOVER Pre-Exposure Prophylaxis Trial

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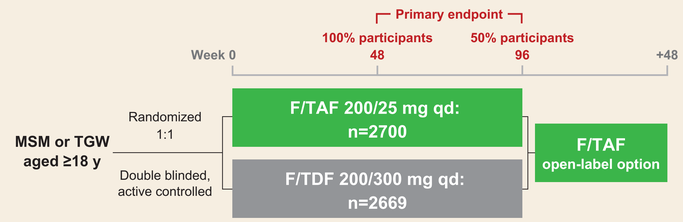
Introduction

DISCOVER: a Randomized, Double-blind, Noninferiority Trial[1]*

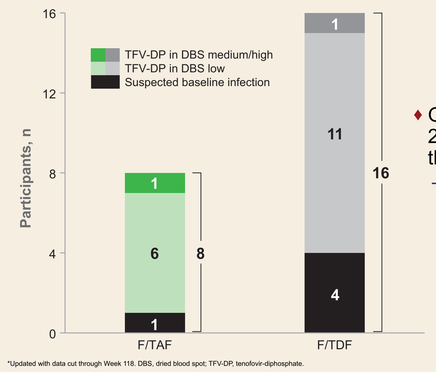


Eligibility criteria:

- High sexual risk of contracting HIV:
  - ≥2 episodes of condomless anal sex with ≥2 unique partners in 12 wk prior to enrollment
  - Diagnosis of rectal gonorrhea, chlamydia, or syphilis in 24 wk prior to enrollment
- HIV and HBV negative, prior use of PrEP allowed

*ClinicalTrials.gov NCT02842086. F/TAF, emtricitabine (FTC)/tenofovir alafenamide; F/TDF, FTC/tenofovir disoproxil fumarate; HBV, hepatitis B virus; MSM, men who have sex with men; PrEP, pre-exposure prophylaxis; TGW, transgender women.

Adherence and Resistance Analyses of HIV Infections[1]*



- Of 5335 analysis-set participants, 24 (0.4%) acquired HIV-1 infection through Week 118 on study
  - 5 participants had suspected baseline infections and 17 had low levels of TFV-DP found by DBS analysis

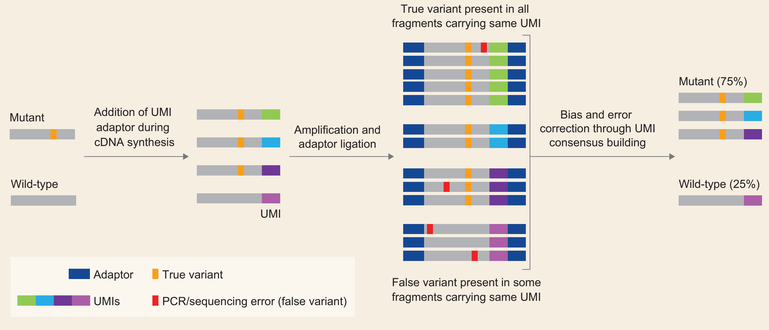
*Updated with data cut through Week 118. DBS, dried blood spot; TFV-DP, tenofovir-diphosphate.

Objective

- To characterize the resistance in the 24 participants in DISCOVER who acquired HIV-1 infection, using both standard and ultrasensitive sequencing

Methods

UMI-NGS: HIV Drug Resistance Assay*



*HIV gene-specific primers with unique molecular identifiers (UMIs) were used to synthesize complementary DNA (cDNA) from extracted HIV RNA from plasma samples; library preparation for next-generation sequencing (NGS) included 2 rounds of polymerase chain reaction (PCR) amplification of cDNA molecules with UMI and ligation of adaptor linkers; bioinformatics analysis was performed with in-house pipeline that builds template consensus sequences of reads sharing identical UMI sequences at 80% majority base calling; UMI-based consensus building allows for correction of PCR/sequencing error and accurate assessment of sampling depth/sample.

- Minor variant sequencing (≥1% of viral population) was performed on available plasma samples to analyze reverse-transcriptase (RT) codons 63–131 and 152–211
  - Library preparation with PCR and sticky-end linker ligation to amplify HIV-1 templates, and attach cDNA template-specific UMIs
  - NGS of HIV-1 RNA on MiSeq® System (Illumina®, Inc., San Diego, California, USA)
  - UMIs allowed for correction of PCR bias and error through bioinformatics analysis
  - UMIs enabled detection of minor variant mutations present down to 1% of the viral population, depending on the number of UMIs reported after sequencing
  - The assay was modified from a previously described technique[2]

Statistical Calculation for UMI-NGS Sensitivity[2-4]

- No. of UMIs required for levels of sensitivity ranging from 0.5% to 25% with 95% confidence was statistically determined by:

$$n = \frac{\log(1-P)}{\log(1-p)}$$

Where:
n = no. of clones
P = probability of detection
p = proportion of clones with variant

No. of UMIs Needed to Detect Various Proportions of Mutant Clones at *P* of 95% Detection

No. of UMIs	*p* (%)
298	0.01 (1)
58	0.05 (5)
28	0.10 (10)
13	0.20 (20)
10	0.25 (25)

Standard Sequencing

- NGS:
  - All plasma samples available were retrospectively tested with the deepType HIV assay (SEQ-IT GmbH & Co. KG, Kaiserslautern, Germany) using the Nextera XT DNA Library Preparation Kit (Illumina) and MiSeq
  - RT gene was analyzed for any known resistance mutations (>2% of viral population)
- Population sequencing:
  - Plasma samples from participants who acquired HIV-1 infection and had a viral load >400 copies/mL were tested with GenoSure® MG (Monogram Biosciences, South San Francisco, California), using Sanger sequencing to analyze the protease (PR) and RT genes for any known resistance mutations (at ≥15–20% of viral population)

Results

UMI-NGS

	Participant	Visit	HIV-1 RNA, Copies/mL	No. of UMIs	% Sample Sensitivity	NRTI-R Mutations (% mutant)*
F/TAF	1	Week 4	<20			Sample did not amplify
	2	Week 84	2900	658	1	—
	3	Week 60	199,000	7493	1	—
	4	Week 84	242,000	4260	1	**M184V** (2)
	5	Week 12	141,000	11,505	1	—
	6	Week 36	592,000	7979	1	—
	7	ESDD	2780			Sample did not amplify
	8	Week 96	21,700	1455	1	—
F/TDF	9	Week 4	1150	98	5	**M184V** (86); **M184I** (6)
	10	Week 108	359	11	>20	—
	11	Week 60	5340	47	10	—
	12	Week 60	211,000	5737	1	—
	13	Week 48	8610	1145	1	—
	14	Week 4	576	100	5	**M184V** (45); **M184I** (41)
	15	Week 24	5040	302	1	—
	16	Week 96	792	117	5	—
	17	Week 4	33,300	431	1	**M184V** (40); **M184I** (60)
	18	Week 72	ND†	14,991	1	—
	19	Week 4	18,700	90	5	T69N (100); **K65R** (1)‡; **M184V** (90); **M184I** (10)
	20	Week 36	176	34	10	—
	21	Week 12	8,070,000	6102	1	—
	22	Week 72	1,070,000	2664	1	—
	23	Week 36				No sample available for testing
	24	Week 108	15,900	410	1	—

*_Bold_ indicates nucleoside RT inhibitor-resistant (NRTI-R) mutations to study drug; †No viral load testing done for this sample; participant had testing done on later sample (5 d after this sample), which had viral load of 1,280,000 copies/mL; ‡K65R seen at 1.1% (below calculated 3% sensitivity of sample). —, no resistance mutations; ESDD, early study drug discontinuation; ND, not done.

- F/TAF arm: 1/8 participants had M184V at 2% detected by UMI-NGS
- F/TDF arm: 4/16 participants had M184V/I detected at 6–90% and 1/16 had K65R detected at 1% (K65R mutation cannot be called with confidence as depth of sampling only allows for 3% calculated sensitivity for this sample)

Standard Sequencing

	Participant	HIV-1 Subtype	Visit	Standard NGS: NRTI-R Mutations (% mutant)*	Population Sequencing: NRTI-R Mutations*	Population Sequencing: Genotypic Assessment†: FTC	Population Sequencing: Genotypic Assessment†: TDF
F/TAF	1	NA	Week 4	ND	ND	NA	NA
	2	B	Week 84	—	—	S	S
	3	B	Week 60	—	—	S	S
	4	B	Week 84	—	—	S	S
	5	F1	Week 12	—	—	S	S
	6	B	Week 36	—	—	S	S
	7	B	ESDD	—	—	S	S
	8	B	Week 96	—	—	S	S
F/TDF	9	B	Week 4	**M184V** (93)	**ND**	NA	NA
			Week 12	**M184V** (97)	**M184V**	R	S
	10	B	Week 108	—	ND	S	S
	11	B	Week 60	—	—	S	S
	12	B	Week 60	—	T215E	S	S
	13	B	Week 48	—	—	S	S
	14	B	Week 4	**M184V** (45); **M184I** (38)	ND	NA	NA
			Week 4	ND	**M184M/V/I**, T215T/S	R	S
	15	B	Week 24	—	—	S	S
	16	F1	Week 96	—	D67D/N	S	S
	17	B	Week 4	**M184V** (75); **M184I** (24)	**M184M/V/I**, T215T/I	R	S
	18	B	Week 72	—	—	S	S
	19	B	Week 4	T69N (>99); **M184V** (95.3); **M184I** (4.5); **K219E** (>99)	T69N, **M184V**, **K219E**	R	S
	20	B	Week 36	—	ND	S	S
	21	AG	Week 12	—	—	S	S
	22	B	Week 72	—	—	S	S
	23	B	Week 36	ND	—	S	S
	24	B	Week 108	—	—	S	S

*_Bold_ indicates NRTI-R mutations to study drug; †_Red shading_ indicates genotypic resistance to one of study drugs. NA, not available; R, resistant; S, sensitive.

- NGS:
  - F/TAF arm: 0/8 participants had resistance to study drugs
  - F/TDF arm: 1/15 participants had M184V and 3/15 had M184V/I
- Population sequencing:
  - F/TAF arm: 0/8 participants had resistance to study drugs
  - F/TDF arm: 2/14 participants had M184V and 2/14 had M184V/I

Participants With Resistance: ARV Regimens Initiated and Outcomes

Participant	Resistance Detected: UMI-NGS	Resistance Detected: Standard NGS	Resistance Detected: Population Sequencing	ARV Regimen	Suppressed	HIV-1 RNA, Copies/mL
4	M184V	—	—	DRV/C/F/TAF + RAL	Yes	VS*
9	M184V/I	M184V	M184V	E/C/F/TAF + DRV	Yes	No HIV-1 RNA detected
14	M184V/I	M184V/I	M184V/I	DTG + DRV/C	Yes	No HIV-1 RNA detected
17	M184V/I	M184V/I	M184V/I	B/F/TAF	Yes	No HIV-1 RNA detected
19	M184V, K65R‡	M184V/I	M184V	ABC/DTG/3TC	Yes	VS*

*Communication from investigator; ‡K65R seen at 1.1% (below 3% calculated sensitivity of sample based on no. of UMI reads). 3TC, lamivudine; ABC, abacavir; ARV, antiretroviral; B, bictegravir; C, cobicistat; DRV, darunavir; DTG, dolutegravir; E, elvitegravir; RAL, raltegravir; VS, virologically suppressed.

Conclusions

- Resistance data were similar between standard and ultrasensitive sequencing
- 4 participants in the F/TDF arm had M184V/I; all 4 had suspected baseline infections
- 1 participant in the F/TAF arm had M184V seen by UMI-NGS, but not by standard sequencing methods including traditional NGS; the participant had low drug levels by DBS
- All participants with resistant viruses were successfully treated with ARV regimens
- In DISCOVER, development of resistance was seen infrequently and most commonly with suspected baseline infections, as reported previously[5]

References: **1.** Hare CB, et al. CROI 2019, oral 104; **2.** Boltz VF, et al. *Retrovirology* 2016;13:87; **3.** Clarke L, Carbon J. *Cell* 1976;9:91-9; **4.** Hogg S. *Essential Microbiology*. Chichester, UK: Wiley, Blackwell; 2013; **5.** Parikh U, et al. *Curr Opin HIV AIDS* 2016;11:49-55.

Disclosures: **S. Cox**, **M. Das**, and **C. Callebaut**: Gilead; **U.M. Parikh**, **A.L. Heaps**, and **B.J. Goetz**: nothing to disclose; **J.W. Mellors**: Gilead; Abound Bio, Accelevir, Co-Crystal, Infectious Disease Connect, MSD.
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