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Treatment options after virological failure of first line tenofovir-based regimens in South Africa: an analysis by deep sequencing

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Summary

In a South African cohort of participants living with HIV developing virological failure on first-line TDF-based regimens, at least 70% of participants demonstrated TDF resistance according to combined Sanger and MiSeq™ genotyping. Sanger sequencing missed the K65R mutation in 30% of samples. Unless HIV genotyping is available to closely monitor epidemiological HIV resistance to TDF, its efficacy as second line therapy will be greatly compromised.

Letter

Provision of antiretroviral therapy (ART) in resource-poor settings employing a public health approach has achieved major successes, saving thousands of lives and averting new HIV infections. Recently, ART initiation in all adults living with HIV disregarding CD4 cell count was recommended for the first time in World Health Organization (WHO) HIV treatment guidelines[1]. However, the ART arsenal available to most resource-poor settings remains limited, and treatment follow-up rarely includes virological monitoring. In this

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context, ARV resistance remains a major threat to the public health efforts to eradicate the HIV pandemic.

Tenofovir disoproxyl fumarate (TDF), in combination with lamivudine(3TC)/emtricitabine(FTC) and nevirapine(NVP)/efavirenz(EFV), remains an ARV of choice for first-line ART in Africa, being included in the South African national HIV/AIDS treatment plan for naïve patients[2]. Tenofovir has high antiviral potency, allows once-daily dosing (frequently co-formulated) and is well tolerated. However, its efficacy is diminished in the presence of the K65R mutation[3]. Subtype C, the most prevalent subtype in South Africa, selects for this mutation faster than other subtypes due to subtype-specific pathways [4,5]. This is an important concern because failure to TDF-containing regimens is often associated with additional resistance to nucleoside and non-nucleoside reverse transcriptase inhibitors (NRTI and NNRTI). Therefore, patients developing virological failure to TDF might potentially loose multiple 2nd-line ART options.

Using Sanger sequencing, previous studies reported the emergence of K65R mutation in 23 to 69.7% of participants developing virological failure to 1st-line TDF regimens [6–9]. The true prevalence of TDF resistance, however, might have been underestimated by the lack of sensitivity of standard Sanger-based genotyping methods. Achieving a precise estimate of TDF resistance after virological failure of first-line TDF regimens is also key to inform public policy as to whether TDF might be reused in second-line ART or subsequent regimens. Transmission of TDF resistance might also potentially compromise the efficacy of PrEP strategies [1]

To evaluate the prevalence of TDF resistance using ultrasensitive sequencing methods, we developed a retrospective reanalysis of participants developing virological failure to TDF within a larger cohort study conducted at the McCord Hospital, Durban, South Africa. All participants developing virological failure to first-line ART including TDF+3TC plus an NNRTI received a genotypic resistance test using a validated in-house Sanger-based sequencing assay in Durban, South Africa. Plasma samples from those with no K65R mutation by Sanger sequencing were reanalysed at the irsiCaixa AIDS Research Institute in Badalona, Spain using MiSeq™ Illumina (Illumina Inc. California).

In brief, the complete *pol* gene was amplified and sequenced in a MiSeq™ platform using a Nextera-XT shotgun approach. A 1% threshold level was chosen for detection of minority variants. Resistance mutations were defined according to the IAS-USA 2013 list. Drug susceptibility results were defined according to Stanford HIV Drug Resistance database, and were classified following the susceptible-intermediate-resistant (SIR) code.

Out of 158 participants included in the McCord cohort at the time of this analysis, 88 participants (55.7%) had developed virological failure to TDF-including regimens. PCR amplification failed in 9 samples (10.2%) leaving 79 evaluable subjects.

Sanger sequencing detected K65R mutation in 47 out of 79 samples (59.5%). Deep sequencing was attempted in the remaining 32 samples. However, 5 out of 32 samples had been depleted of volume and could not be further evaluated. K65R mutation was found in 8 of the 27 samples evaluable by MiSeq™ (29.6%) at frequencies in the virus population

ranging 1.3% to 32.5%. Considering Sanger and deep sequencing results together and assuming that none of the 5 subjects not evaluable by MiSeq™ had the K65R mutation, a conservative estimate of the overall prevalence of K65R mutation was 69.6%, a 10.1% increase in prevalence relative to Sanger sequencing. Prevalence was calculated using only TDF-failing and PCR-success subjects.

In addition, deep sequencing detected IAS-USA mutations missed by Sanger in 22 out of 27 subjects (81.4%) at frequencies ranging 1.1% to 35.7% in the virus population (Table 1). Such additional mutations changed the predicted drug susceptibility in 15 out of 27 subjects (55.5%), mostly affecting TDF, etravirine (ETR) and rilpivirine (RPV), although the predicted susceptibility to NVP or EFV was not affected(Table 1). According to deep sequencing data, 21/27 (77.7%), 25/27 (92.6%), 13/27 (48.1%) and 15/27 (55.5%) were resistant to 3TC/FTC, NVP/EFV, ETR and RPV, respectively, whereas only 3 participants (11.1%) had intermediate resistance to AZT –only 1 (3%) by Sanger sequencing.

Our findings confirm initial estimations that TDF might loose antiviral efficacy in virtually all patients infected with a subtype C HIV developing virological failure to this drug. Thereby, unless HIV genotyping is available to ensure that HIV remains susceptible to TDF, the use of this drug will be greatly compromised in efficacy for second line therapy, and should not be prescribed except if no other treatment options are available. Continued surveillance of primary resistance in Africa is key to survey transmission of TDF-resistant mutants to newly HIV-infected patients, which could impact the efficacy of both first-line ART and PrEP [10,11]. To date, rates of virological failure to first-line TDF regimens and transmission of K65R mutants have remained low according to Sanger sequencing estimates. [12,13] The fitness cost of the K65R mutation, however, makes K65R mutants wane and thus might be missed by Sanger methods.

Another remarkable finding of our study was that, in addition to identifying K65R, additional resistance mutations detected with MiSeq™ relative to Sanger mainly affected the predicted susceptibility to the second-generation NNRTIs ETR and RPV, but did not largely influence viral susceptibility to other ARVs, including AZT. On the one hand, this suggests that ETR and RPV might not be good options for second-line ART regimens following EFV or NVP failure. On the other hand, our findings support AZT as a second-line drug in South Africa, used in combination with 3TC and LPV or other PIs[2] or even integrase inhibitors. Whereas routine drug resistance testing may help decide which NRTIs to use in second-line therapy, the EARNEST trial recently showed that even without this information, second-line regimens including boosted PI plus two NRTIs retained better virological outcomes than PI monotherapy, even in the presence of high-level resistance to the NRTI backbone [14], suggesting residual NRTI activity may be sufficient when combined with highly potent boosted PI-based therapy.

Despite its limitations –including a small sample size, lack of adherence data and the inclusion of patients under clinical care which might not represent the general South Africa population– this study confirms the development of TDF resistance in most subjects developing TDF failure in South Africa, but also supports current public health algorithms for HIV clinical management.

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Table 1

Antiretroviral drug resistance at virological failure of TDF-containing 1st-line ART by Sanger and ultrasensitive HIV genotyping

Subject ID	IAS-2013 mutations detected by Sanger sequencing	Additional Mutations by Illumina (Frequency in the virus population, %)	Changes in Drug Susceptibility with MiSeq™ compared to Sanger
1	D67N; M184I; V90I; V179E; Y181C; H221Y	G190A (4.74); K70E (4.5); P225H (8); V106I (9.4)	TDF (S→I); ETR (I→R); RPV (I→R)
2	D67N; K70E; M184V; A98G; K103N; V106M	K65R (20.3); L100I (2.1); Y181C (16,1)	TDF (I→R); ETR (I→R); RPV (I→R)
3	M184V; V106M; G190A	D67N (1.3); K103N (16,7); K65R (27.8); M184I (29.4); M230L (28.1)	TDF (S→R); RPV (I→R)
4	M184V; T215Y; V106M; Y188L	D67N (1.9); G190A (13.6); K101E (12.5)	TDF (S→I); ETR (S→I)
5	M184V; K103N; V108I	A62V (2.8); D67N (4.2); P225H (2.4)	AZT (S→I)
6	M184V; K103N; V106M	D67N (1.3); M230L (31.8)	ETR (S→I); RPV (S→I)
7	M184I; V90I; Y181C; H221Y	A98G (3.2); G190A (9.5); M184I (14.6); V179D (11.6)	ETR (I→R); RPV (I→R)
8	K70E; M184V; V90I; K103N; E138G	K65R (1.3)	TDF (I→R)
9	M184V; V106M; V108I; E138A; G190A	H221Y (1.6); K219E (2.7); K70E (8.6); L74V (3.2); V90I (8.8); Y115F (35.7)	TDF (S→I)
10	M184V; K103N; V106M	K103S (2.4)	
11	M184I; V90I; Y181C; K101E	M184V (9.9)	
12	V106M	No additional mutations found	
13	M184V; V106M; V179D	No additional mutations found	
14	K103N; P225H	V90I (1.2)	
15	No mutations found	Y188C (27.7)	
16	No mutations found	No additional mutations found	
17	Y115F; V106M; Y188C	A62V (2.4)	
18	D67G; T69N; K101E; V106M; H221Y	K65R (4.9)	TDF (S→R); AZT (S→I); 3TC (S→I); FTC (S→I)
19	M184V; A98AG; K103RST; G190A	K65R (32.5); V108I (9.8); Y181C (12.5)	TDF (S→R); ETR (S→R); RPV (I→R);
20	M184I	No additional mutations found	
21	K103N; M184V; P225H	K65R (30.5); K70E (16.4); A98G (5.8); L100I (10.8); V108I (1.5); K219Q (5.1)	TDF (S→R); ETR (S→I); RPV (I→R)
22	M184I; V106M; V179D; M230L	K65R (17.5); K70E (16.7); L74V (15.9); H221Y (4.1); F227C (29.4)	
23	M184V; V106M; G190A; F227L	Y115F (1.4)	
24	M184V; K103N; V108IV; P225HP	No additional mutations found	
25	M184V; K103N; G190A	D67N (4.6); K103S (33.1); E138G (1.2)	
26	D67N; K70E; M184V; V90IV; K101E; V106M; G190A; F227L	K103N (3.8); V179D (9.2); H221Y (1.4)	RPV (I→R)
27	M184V; V106M; V179D	A62V (12.1); K65R (11.8)	TDF (S→R)

*TDF: Tenofovir Disoproxyl Fumarate; ETR: Etravirine; RPV: rilpivirine; AZT: zidovudine; 3TC: lamivudine; FTC: emtricitabine.