Plasma DTG Exposure Test as Objective Tool to Identify People With HIV at Highest Risk of Resistance

Interim Analysis of ITREMA-2

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Introduction

Second-generation integrase inhibitors are the cornerstone of antiretroviral therapy (ART) worldwide. Recently, higher levels of dolutegravir (DTG) resistance are reported in some countries compared to clinical trial data.

As resources are limited, we implemented a **prospective laboratory-based reflex testing strategy (ITREMA-2)** to prioritize resistance testing to those most likely to benefit.

Methods

- The **ITREMA-2 implementation pilot offers laboratory-based reflex drug exposure testing** to adults on DTG-based ART ≥12 weeks with detectable viral load. Only **in case of a detectable DTG drug level, reflex resistance testing was performed.**
- All adults from two hospitals in Johannesburg with VL≥400 c/ml and a remnant VL specimen were offered participation.
- Plasma DTG levels were assessed using a **semi-quantitative enzyme immunoassay** (ARK Diag. Inc.). Plasma DTG ≥20 ng/ml test results were classified as detectable.
- **Population based Sanger sequencing** was performed, and resistance profiles were assessed using Stanford HIVdb v9.6.
- To calculate **negative predictive value** (NPV) a random subset with undetectable plasma DTG was sequenced retrospectively.

Patient Characteristics

- Between April 2023 and September 2024, 208 out of 210 eligible individuals consented, contributing 288 samples for the interim analysis.

Table 1. Characteristics of included participants (n = 208)

Sex	*% female*	55.3% (115/208)
Age	*mean [SD]*	42 years [+/-13]
HIV VL	*median [IQR]*	4.1 $\log_{10}$ cp/ml [3.1-4.9]
Duration of ART	*median [IQR]*	89 months [46-155]
Duration of DTG treatment	*median [IQR]*	11 months [6-24]
ART at time of VL	TDF-3TC-DTG	81.7% (170/208)
	ABC-3TC-DTG	13.5% (28/208)
	AZT-3TC-DTG	1.9% (4/208)
	NRTI+DRV/r+DTG	2.9% (6/208)
Reported prior ART	No recorded prior ART	24.0% (50/208)
	Prior NNRTI	59.6% (124/208)
	Prior PI	46.2% (96/208)
	Prior RAL	0.5% (1/208)

- Prevalence of **DTG resistance** in individuals with VL≥400 c/ml and detectable plasma DTG levels was **13%;** higher than reported from unselected individuals in routine care.
- **Undetectable plasma DTG** is a strong predictor for the absence of DTG resistance **(NPV=97.7%)**
- **Laboratory-based reflex plasma DTG exposure testing** offers an objective and easily implementable measure to identify individuals with VL ≥400 c/ml and non-adherence, optimizing the allocation of limited resources for those most likely to benefit.

Plasma DTG detection

- **Plasma DTG** was **detectable** for **113/288 (39.2%)** of the samples, with a significantly lower proportion in samples with VL ≥1000 c/ml (33.0%) compared to samples with VL 400-999 cp/ml (64.0%, p<0.001) (Figure 1).
- Detectable plasma DTG levels were not associated with sex, age, regimen, facility, duration of DTG, total ART duration, prior INSTI, PI or NNRTI exposure.

DTG Resistance

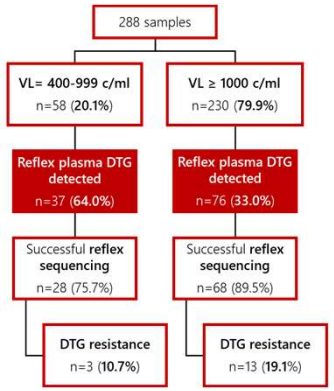
- 96 **sequences** (95 subtype C, 1 subtype A1) were generated from **75 individuals with detectable plasma DTG levels**.
- **DTG resistance** was detected in **13%** (10/75) of individuals, showing diverse mutation profiles.
- Accumulation of integrase mutations was seen in 3 individuals within 6 months of follow-up (Table 2).
- All 10 individuals with DTG resistance had prior NNRTI and/or PI exposure.

Table 2. Drug resistance profiles in those with positive DTG exposure test and DTG resistance

Accumulated INSTI mutations are indicated in bold.

Patient ID	VL (cp/mL)	Time on DTG	INSTI DRM	Other DRM
P017	45 900	36m	E138K, S147G, Q148QR, N155H, *S230R*	M184V K103N, E138K
P067	5 550 4 210	17m 19m	E138K, G140A, Q148R, *L74M* E138K, G140A, Q148R, *L74M*	A62V, K65R, M184V K101E,V106M,G190A
P071	6 850 3800 10 700 6 541	11m 12m 14m 26m	E138A, G140A, Q148R E138A, G140A, Q148R E138A, G140A, Q148R E138A, G140A, Q148R	M184V K103N, P225H
P091	7 990	11m	N155H, *H51HY*	M184V K103N, P225H
P105	875 703	10m 13m	G118R G118R, **T66I, E138K**	No amplification
P121	8 220 21 200 254 000	3m 5m 9m	G118R G118R G118R, **T66TI, E138K**	M46I, I50L, V82A, *L33F, Q58E, T74P* D67N, K70R, M184V, L210W, T215F, K219E K103N, P225H
P152	15 100 9 500	38m 46m	E138K, G140A, S147SG, Q148K E138K, G140A, **S147G**, Q148K	D67N, K70R, M184V, K219Q K103N, P225H
P157	129 000 55 700 27 700	9m 12m 15m	E138EK, S147G, N155H, *H51HY, T79TA* **E138K**, G140A, S147G, **Q148R, N155H** E138K, G140A, S147G, Q148R, N155H	D67N, K70R, M184V K103N, A98G, V108I
P2011	869	6m	T66I, G118R, E138A	No amplification
P413	328 000	13m	R263K	K65R, S68G A98G, E138A, G190E

Figure 1. Laboratory-based reflex testing strategy



Negative predictive value of plasma DTG drug-exposure testing

- 100 samples with **undetectable DTG exposure** test results were sequenced retrospectively. **DTG resistance** was **not** detected in **93.0%** (93/100).
- Throughout the study period, **consistent undetectable plasma DTG levels** predicted the absence of resistance in 84/86 individuals (**NPV=97.7%**)

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