Next Generation Sequencing of HIV Resistance on Dolutegravir (DTG) Failure under Variable Drug Pressure

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BACKGROUND

Next generation sequencing (NGS) detects HIV drug resistance mutations (DRMs) in minor variants. During ART failure, minor variants may signal early resistance and detection could enable timely interventions.

HYPOTHESIS: Minor/low-frequency DTG DRMs contribute to DTG-ART failure, and detection by NGS enhances DTG resistance monitoring.

APPROACH: We assessed the added value of a novel NGS HIVDR genotyping approach in characterizing low/high-frequency DTG DRMs in unsuppressed individuals on DTG-ART with/without objectively confirmed adherence, and compared results to conventional Sanger HIVDR genotyping.

STUDY DESIGN & METHODOLOGY

Fig. | 1

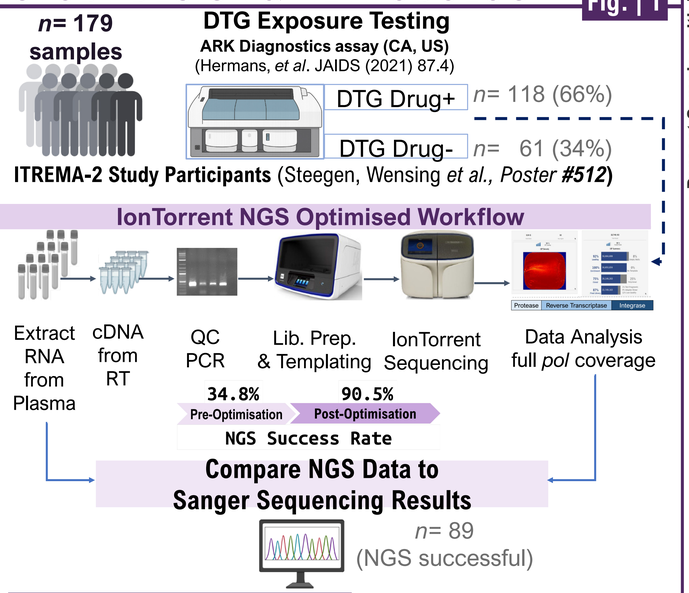
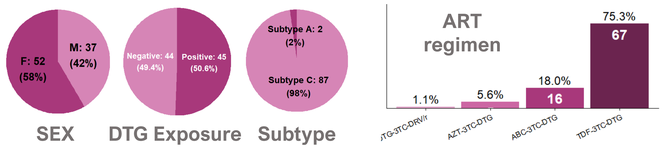


Fig. 2 | Cohort Summary



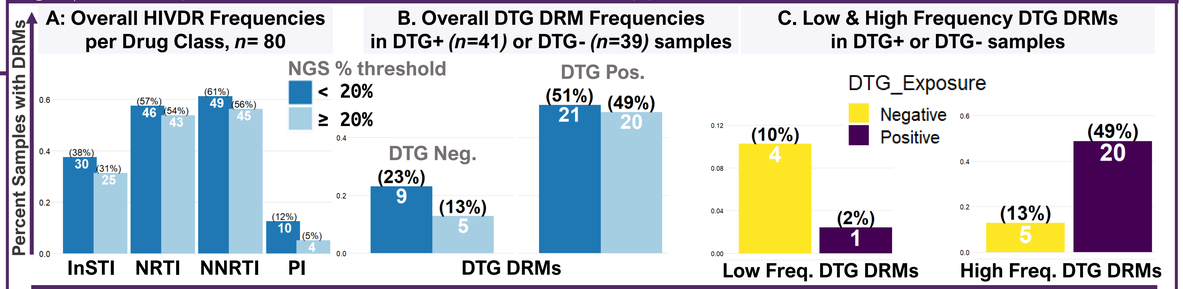
Variable	Mean	Median	IQR	SD
Age	41.8	43.0	10.0	11.0
Viral Load (log10)	4.3	4.4	1.5	0.9
Current ART Duration (months)	20.2	14.0	19.2	21.9
Total ART Duration (months)	102.3	91.0	91.5	64.0

89 successfully sequenced NGS samples' Variables : Top: categorical variables; Left: continuous variables

A novel next-generation-sequencing assay uncovers detailed low & high frequency DTG drug resistance profiles in HIV unsuppressed individuals receiving DTG-ART with or without confirmed ART adherence:

- *High frequency DTG-resistant mutations are common in individuals with measurable plasma-DTG (recently adherent);*
- *Low-frequency DTG-resistant mutations occur more in individuals without measurable plasma-DTG (non-adherent).*

Fig 3 | **Low** Freq. DTG DRMs are more common in unsuppressed individuals without detectable DTG

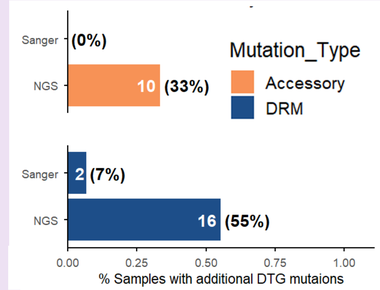


Cases	Samples	PID	SampleID	VL	DTG	Sanger DTG DRMs	NGS DTG DRMs	Sanger Acc.	NGS Acc.
1	1	P017	DTG0015	52500	Neg.	E138K,S147G,N155H	E138K, S147G, Q148R(4%), N155H	S230R	S230R
	2		DTG0209	45900	Pos.	E138K,S147G,Q148QR,N155H	E138K, S147G, Q148R(67%), N155H	S230R	H51Y(1%), S230R
2	3	P035	DTG0346	23100	Pos.	N155H	N155H(97%)	E157Q	E157Q
3	4	P036	LLV0016	498	Pos.	None	G140R	None	D25K, G52R, T97A(1%), G163R(12%)
4	5	P065	DTG0480	101000	Neg.	None	Q148R(1%)	None	None
5	6	P067	DTG0197	5550	Pos.	E138K,G140A,Q148R	E138A(22%), E138K(78%), G140A, Q148R	L74M	L74M(99%)
	7		DTG0254	4210	Pos.	E138K,G140A,Q148R	E138A(47%), E138K(52%), G140A, Q148R	L74M	L74M(98%)
6	8	P068	DTG0056	2090	Pos.	None	N155H	H51HY	H51Y(16%), T97A(1%)
7	9	P071	DTG0107	3800	Pos.	E138A,G140A,Q148R	E138A, G140A, Q148R	None	None
	10		DTG0059	6850	Pos.	E138A,G140A,Q148R	E138A, G140A, Q148R	None	None
	11		DTG0168	10700	Pos.	E138A,G140A,Q148R	E138A, G140A, Q148R	None	None
	12		DTG0515	6541	Pos.	E138A,G140A,Q148R	E138A, G140A, Q148R	None	L74I
8	13	P091	DTG0078	278000	Neg.	N155H	Q146R(22%), N155H(2%), R263K(4%)	None	H51Y(18%)
	14		DTG0223	7990	Pos.	N155H	N155H(79%), R263K(2%)	H51HY	H51Y(21%)
	15		DTG0463	46600	Neg.	None	N155H(5%)	H51HY	H51Y(63%)
9	16	P103	DTG0245	1660	Pos.	None	N155H	None	None
10	17	P104	LLV0024	706	Neg.	R263K	R263K	None	N155S(2%)
11	18	P105	LLV0025	875	Pos.	G118R	G118R	None	None
	19		LLV0053	703	Pos.	T66A,G118R,E138K	T66A(82%), G118R(65%), R263K(20%)	None	None
12	20	P121	DTG0099	8220	Pos.	G118R	G118R(88%), R263K(21%)	None	None
	21		DTG0287	109000	Neg.	T66I,G118R,E138AKT	T66I(90%), G118R(100%), E138A(28%), E138K(72%)	None	None
	22		DTG0361	254000	Pos.	T66TI,G118R,E138K	T66I(81%), G118R, E138K	None	None
13	23	P128	DTG0265	29500	Neg.	None	H51Y(1%)	None	None
14	24	P157	DTG0138	129000	Pos.	E138EK,S147G,N155H	E138K, G140A, S147G, Q148R, N155H	H51HY,T97TA	H51Y, T97A
	25		DTG0249	55700	Pos.	E138K,G140A,S147G,Q148R,N155H	E138K, G140A, S147G, Q148R, N155H	None	None
	26		DTG0374	27700	Pos.	E138K,G140A,S147G,Q148R,N155H	E138K, G140A, S147G, Q148R, N155H	None	None
15	27	P2019	DTG2-0017	123000	Neg.	R263K	R263K	None	None
16	28	P2224	DTG2-0181	3129	Neg.	None	E138K(4%)	None	None
17	29	P2302	DTG2-0230	34497	Pos.	T66I,G118R,E138K	T66I, G118R, E138K	E157Q	E157Q
18	30	P318	DTG0333	1290	Pos.	None	T66I(5%)	None	S147H(3%)

Fig 4 | NGS identifies DTG resistance and accessory (Acc.) mutations undetected by conventional genotyping in unsuppressed individuals on DTG-ART with or without detectable DTG exposure; *n*= 30 DTG-resistant samples.

Purple : discordant

(%) if not 100%



Frequency and number of samples with additional DTG resistance mutations exclusively detected by either NGS or Sanger among 30 samples with DTG resistance confirmed by NGS

Fig 5 | Additional DTG-DRM detection by sequencing method



Fig. 6 | Most Common DTG DRM Profiles detected by NGS

CONCLUSIONS

- We demonstrate the first implementation of an Ion Torrent NGS HIV drug resistance genotyping methodology in an African HIV cohort of unsuppressed individuals receiving DTG-based ART, with or without confirmed DTG exposure.
- Low-frequency DTG DRMs were predominantly identified in individuals negative for DTG exposure.
- DTG DRMs undetected by Sanger sequencing were identified by NGS in individuals with and without measured exposure.

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OPEN to COLLABORATE

HIV resistance testing by next-generation sequencing (NGS) identifies potentially relevant dolutegravir (DTG) resistance undetected by traditional testing in HIV unsuppressed persons receiving DTG-based ART with or without detectable DTG drug pressure. These results support the hypopthesis that HIV resistance testing by NGS can enhance DTG resistance management.