Characterization of Mutations Outside the Integrase in HIV-1 Individuals in Whom Dolutegravir Failed

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BACKGROUND

- Virological failure (VF) to DTG, may occur without the selection of resistance in the integrase (IN) region;
- In vitro and in vivo studies, in subtypes B and C, have reported the selection of non-canonical (NC) mutations outside the IN region associated with IN inhibitor (INI) resistance;
- We aimed to investigate potential patterns of NC mutations among HIV-1 infected Cameroonians who failed a DTG-containing regimen.

METHODS

- Cross-sectional study, from 2020-2025 on individuals with DTG-VF at the Chantal Biya International Reference Centre (CIRCB) was carried out.
- HIV-1 whole genome sequencing (WGS) was performed with the ABL Deepcheck Assay (set at 5%) on Illumina Miseq platform.
- In addition to protease (PR), reverse transcriptase (RT) and IN, three extra target regions were analyzed: GAG (p1, p17, p24, p7), ENV (gp120, gp41) and 3'polypurin tract (3'PPT) with mutation frequency >5%.
- INI resistance was obtained by Sanger sequencing or WGS. HIV Drug Resistance was interpreted using Stanford HIVdb.v.9.8.
- Data were analyzed using Epi Info v7.2.7, Fisher's tests with $p<0.05$ were considered statistically significant.

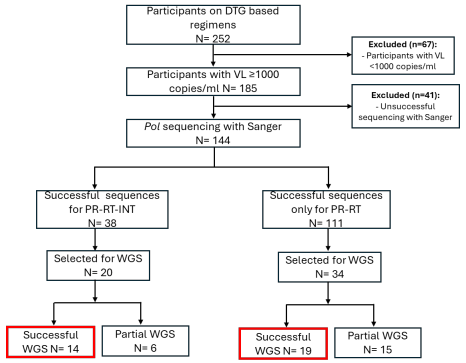


Figure 1. Study workflow

Our study confirms the presence of non-canonical extra-integrase mutations in DTG VFs in a Non-B subtype Cameroonian cohort. Specific gag and env novel mutations were significantly associated with INI major resistance, warranting further investigation.

RESULTS

- We successfully obtained 33 WGS from 33VFs with a median HIV-RNA of 70,195 cps/ml [IQR 7,361–141,611], 20 months [IQR 15–29]) duration of INI exposure, 51.5% with vertical transmission, 72.7% with poor adherence, and with 57.6% prevalence of CRF02_AG recombinant prevailing clade (Table 1).
- DTG resistance prevalence was 33.3% (11/33), and among those under DTG at GRT it was 100% (11/11).
- At least one mutation outside integrase was common with 25.9% in gag, 100% in env and 9.4% in 3'PPT.
- 12 previously reported NC mutations associated with DTG resistance were found in our population, but none was significantly associated with the presence of major INI resistance mutations (Fig. 2 Panel A).
- No known 3'PPT NC mutations were found in DTG VFs.
- 17 new NC mutations (3 in gag, 14 in env) prevailed in individuals with INI resistance and 2 (1 in gag, 1 in env) in those without INI resistance (Fig. 2 Panel B).

Table 1. Socio demographical, Clinical, Immunological and Virological Data

Sociodemographic characteristic	All participants, N = 33
Age in years, median [IQR]	21 [16-45]
- **Age ranges**	
0-18	14 (42.4%)
>18	19 (57.6%)
- **Sex, n (%)**	
Males	20 (60.6%)
Females	13 (39.4%)
Clinical, Immunological and Virological Data	**All participants, N = 33**
Time since diagnosis, median [IQR] years	9 [6-15]
- **Transmission, n (%)X**	
Horizontal	12/32 (37.5%)
Sexual	3/32 (9.4%)
MTCT	17/32 (53.1%)
- **WHO clinical stage at diagnosis, n(%)X**	
I	6/16 (37.5%)
II	0 (0%)
III	6/16 (37.5%)
IV	4/16 (25.0%)
- Pre-ART CD4 count, median [IQR] copies/mm3	64 [37-82]
- **Median [IQR] copies/ml viral load at GRT**	**70,195 [7361- 141 611]**
Ranges, n (%)	
50-1,000 copies/ml	0 (0%)
1,000-100,000	22 (66.7%)
≥10,0000	11 (33.3%)
- Viral load at virological failure, median [IQR] copies/ml	72,435 [18,146 – 39,862]
Time between first virological failure and current GRT, median [IQR] months	24 [9 – 37]
- **Viral clade, n (%)**	
CRF02_AG	19 (57.6%)
Non-CRF02_AG	14 (43.4%)
- **Number of regimens at DTG failure , n (%)**	
First	1 (3.0%)
Second	11 (33.3%)
Third or more	19 (57.6%)
Unknown	2 (6.1%)
- **Adherence**	
Good	9 (27.3%)
Poor	24 (72.7%)
- **INSTI Resistance**	
Yes	11 (33.3%)
No	22 (66.7%)

X: reported for those with available information

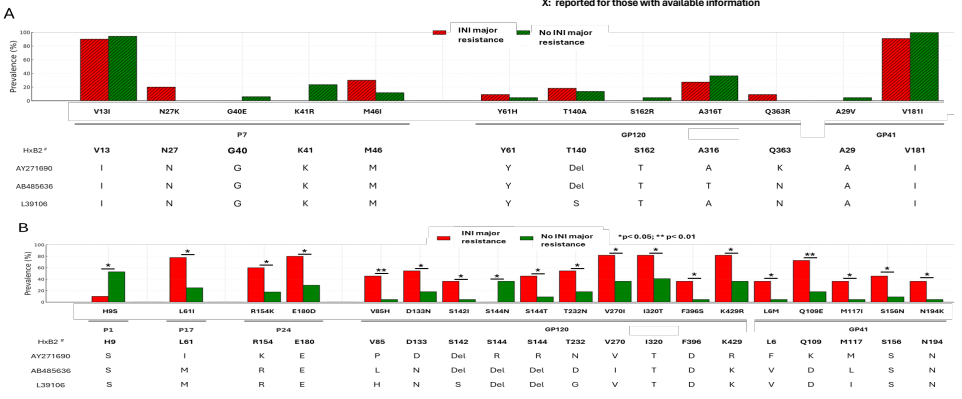


Figure 2. Prevalence of non canonical mutations associated with INI resistance in extra target regions. Panel A: known mutations. Panel B: Novel mutations.
#Individual positions are shown in HXB2 (as reference for Subtype B) and in AY271690, AB485636, L39106 (as references from Los Alamos DB for WGS of HIV-1 CRF02_AG).

CONCLUSIONS

- DTG resistance was found in one third of VF individuals, and 100% among those on DTG treatment at GRT (11/11).
- NC mutations previously associated with DTG resistance were found in our population, but none was significantly associated with major INI resistance.
- We identified novel NC mutations in both gag and env, significantly associated with major INI resistance.
- The newly identified NC mutations especially in env are strong candidates for compensatory fitness effects, altering entry/fusion dynamics and replication enhancement under DTG pressure, though all these need additional investigation.

LIMITATIONS

- WGS was performed only in DTG failure cases so far.

PERSPECTIVES

- Increase sample size, perform WGS in both DTG-VF and naïve patients and characterize the associations among INI and NC mutations.

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PLAIN LANGUAGE SUMMARY

After studying the entire HIV-1 genome in non-B subtypes in people from Cameroon, who were not responding to a Dolutegravir-based treatment, we found new, unusual changes in parts of the virus that are generally not targeted by medications. These changes were associated to resistance against the treatment. These findings warrant further investigation.