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DOI: <https://doi.org/10.1097/QAD.0000000000001920>

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**Four class drug resistant HIV-1 subtype C in a treatment experienced individual  
on dolutegravir based antiretroviral therapy in Botswana**

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**Short title;** Multidrug HIV-1C resistance in Botswana

**Conflicts of Interest and Source of Funding;**

*This work was supported through the Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE), a DELTAS Africa Initiative [grant # DEL-15-006]. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust [grant # 107752/Z/15/Z] and the UK government. The views expressed in this publication are those of the author(s) and not necessarily those of AAS, NEPAD Agency, Wellcome Trust or the UK government.*

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**Conflicts of interests**

All authors have no conflicts of interests to declare.

## ABSTRACT

There is limited data on the effectiveness of Dolutegravir (DTG) based combination antiretroviral therapy (ART) in real life settings in southern Africa where HIV-1C predominates.

We report here a patient infected with HIV-1C on DTG based ART previously exposed to raltegravir who developed multidrug resistance mutations to four ARV classes. There is need for drug resistance monitoring and clinical vigilance to ensure effectiveness of HIV treatment programmes even in the era of DTG based ART.

**Keywords:** dolutegravir, drug resistance, HIV-1, treatment-experienced, adherence, Botswana

## **INTRODUCTION**

Maintaining viral load (VL) suppression in people living with HIV (PLWHIV) is critical to ensure both the health of PLWHIV, and prevent onward transmission of HIV to sexual partners [1–3]. Despite major advances in the development of antiretroviral (ARV) drugs and antiretroviral therapy (ART) treatment guidelines [4–6], low and middle income countries (LMICs) continue to face challenges such as poor ART adherence, limited HIV care specialists, drug stock-outs, lack of ancillary health care services, etc [7–9]. These barriers to effective ART may lead to development of extensive HIV-1 drug resistance.

Dolutegravir (DTG) has recently been introduced as part of the first-line ART regimen in Botswana, and may soon be adopted by other countries in sub-Saharan Africa (SSA) [10],[11]. Therefore, there is a need to monitor the development of integrase strand transfer inhibitor (INSTI) resistance mutations. We report a case of a four-class drug resistant HIV-1 subtype C in a 51-year-old male currently on DTG-based ART with persistent viremia.

## **METHODS**

Chart reviews of treatment-experienced patients not virologically suppressed while on salvage ART therapy were conducted at a local tertiary hospital as part of the Botswana Epidemiological ART Treatment (BEAT) cohort study. A case-file reported herein was identified and an analysis of the patient's medical record was conducted with information gathered from September 2003 to November 2017. The patient provided written informed consent to complete the analysis for publication, and the BEAT Study is approved by the human research and development council of Botswana.

Genotypic resistance testing (GRT) was performed using Sanger sequencing of the *pol* and envelope genes. Sequences obtained were assessed for drug resistance mutations using the Stanford University HIV drug resistance database (<https://hivdb.stanford.edu/>), International Antiviral Society USA (IAS-USA) 2017 mutational list [12] and coreceptor usage was determined using with geno2pheno<sub>[coreceptor]</sub> [13]. All sequences generated were submitted to GenBank under accession numbers MG989439 - MG989443, MH004049 and MH004050.

## RESULTS AND DISCUSSION

### Case Report

This patient was initiated on zidovudine (AZT), lamivudine (3TC) and nevirapine (NVP) on September 2003 as per the 2002 Botswana National ART guidelines. Apart from persistent viremias, his 14-year follow-up history has been clinically uneventful and devoid of opportunistic infections.

He intermittently achieved virological suppression between September 2003 to April 2014 (6 HIV-1 RNA levels <400 copies/ml out of 67 tests) (Figure 1). His ART regimens while virologically suppressed were AZT / tenofovir (TDF) / ritonavir-boosted lopinavir (LPV\|r) from April 2007 and TDF / emtricitabine (FTC) / ritonavir-boosted darunavir (DRV\|r) / raltegravir (RAL) for the remaining aviremic episodes from February 2010. The majority of VL revealed virological failure (VF) with VL ranging between 2.61 log<sub>10</sub> and 5.88 log<sub>10</sub> copies/ml (Figure 1). Nine switches in ARV medications were made from September 2003 to November 2017 (Figure 1). Adherence to clinic appointments and medications was suboptimal and he experienced one episode of drug stock-out of ARVs (darunavir) for approximately 2 weeks in March 2012. His social circumstances remained challenging, making it difficult for him to be adherent to his

medications despite multiple counseling and adherence support sessions from health care providers.

The patient is infected with HIV-1C clade and during the period under review had a median HIV-1 VL of 3.48 log<sub>10</sub> (IQR: 3.27-3.68) copies/mL and absolute CD4+T cell count of 595 cells/μL (IQR: 501-692) respectively. As of November 2017, VL and CD4+T cell count were 3.57 log<sub>10</sub> cps/mL and 670 cells/μL respectively (Figure 1).

### **Resistance Testing**

GRT analysis revealed major drug resistance mutations conferring resistance to: nucleoside (nucleotide) reverse transcriptase inhibitors [N (t) RTIs] (K65R, D67N, K70R, M184V and K219N), non-nucleotide reverse-transcriptase inhibitors (NNRTIs) (Y181C), protease inhibitors (PIs) (V32I, I47V, 154L, I84V) and INSTIs (E138K, G140A, S147G, Q148R, T97A). The virus was chemokine receptor 5 (CCR5) tropic and did not have any resistance mutations to enfuvirtide (T-20).

To our knowledge, this is the first report in SSA of a patient with a virus which has developed drug resistance mutations to all the standard ARVs belonging to four classes including INSTIs. Multidrug resistant mutations likely developed as a result of multiple factors including, suboptimal adherence, inadequate psychosocial support and limited HIV specialist care – all issues encountered frequently in LMICs.

While *invitro* and *invivo* clinical studies have shown DTG to retain some activity against a virus with INSTI mutations [14],[15], this is not always the case in real-life settings as highlighted by this case report. An interesting observation made was the isolated emergence of the INSTI accessory resistance mutation T97A between May 2015 and November 2017 and concurrent rise

in VL from 2,888cps/mL to 3,690 cps/mL between the same time period. Similar observations of viral rebound after sole emergence of T97A mutations have recently been reported [16]. Phenotypic testing, ARV drug tracing levels and directly observed therapy would have been ideal but often these are not available or feasible in LMICS.

This patient may benefit from a regimen that includes two new agents expected to be fully active, such as maraviroc (MVC) or enfuvirtide (T-20) with an optimized background regimen. Currently, MVC and T-20 are not available under the Botswana National HIV Clinical Care Guidelines.

As this patient remains sexually active with significant psychosocial issues, this raises important public health issues including the possible introduction of a multidrug resistant HIV-1C strain into the circulating pool.

## **CONCLUSIONS**

The case highlights the need for continued monitoring of HIV-1 VL and the development of robust HIV drug resistance surveillance systems for failing and highly treatment-experienced patients. Managing treatment-experienced patients in LMICs, where there is often inadequate psychosocial support, lack of clinical HIV care expertise, limited ARV options and drug resistance testing capacity, will remain an ongoing challenge that will need to become a public health priority to ensure the effectiveness of national HIV programmes.



## ACKNOWLEDGEMENTS

We thank our patients and staff of Princess Marina Hospital Infectious Disease Care Clinic (PMH IDCC). We thank the Botswana Harvard HIV Reference Laboratory staff for their hard work and dedication, Botswana Harvard Partnership and Ministry of Health and Wellness for their collaboration. We thank Thongbotho Mphoyakgosi, Tshenolo Ntsipe, Segomotso Maphorisa, Mompoti Mogwele and Wonderful Chonga for their outstanding laboratory assistance.

### Authors' contributions

KKS wrote the first manuscript, is involved in the clinical care of the patient and performed most of the drug resistance experiments. AA, MM, TG, JNJ, KKS were and are still involved in the clinical care of the patient.

TD, SM, DR, CFR, MM, JNJ, IK, SG performed the drug resistance testing and/or provided supervision and guidance.

All authors helped to edit and approved the final manuscript version for publication.

*This work was supported through the Sub-Saharan African Network for TB/HIV Research Excellence (SANTHE), a DELTAS Africa Initiative [grant # DEL-15-006]. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership*

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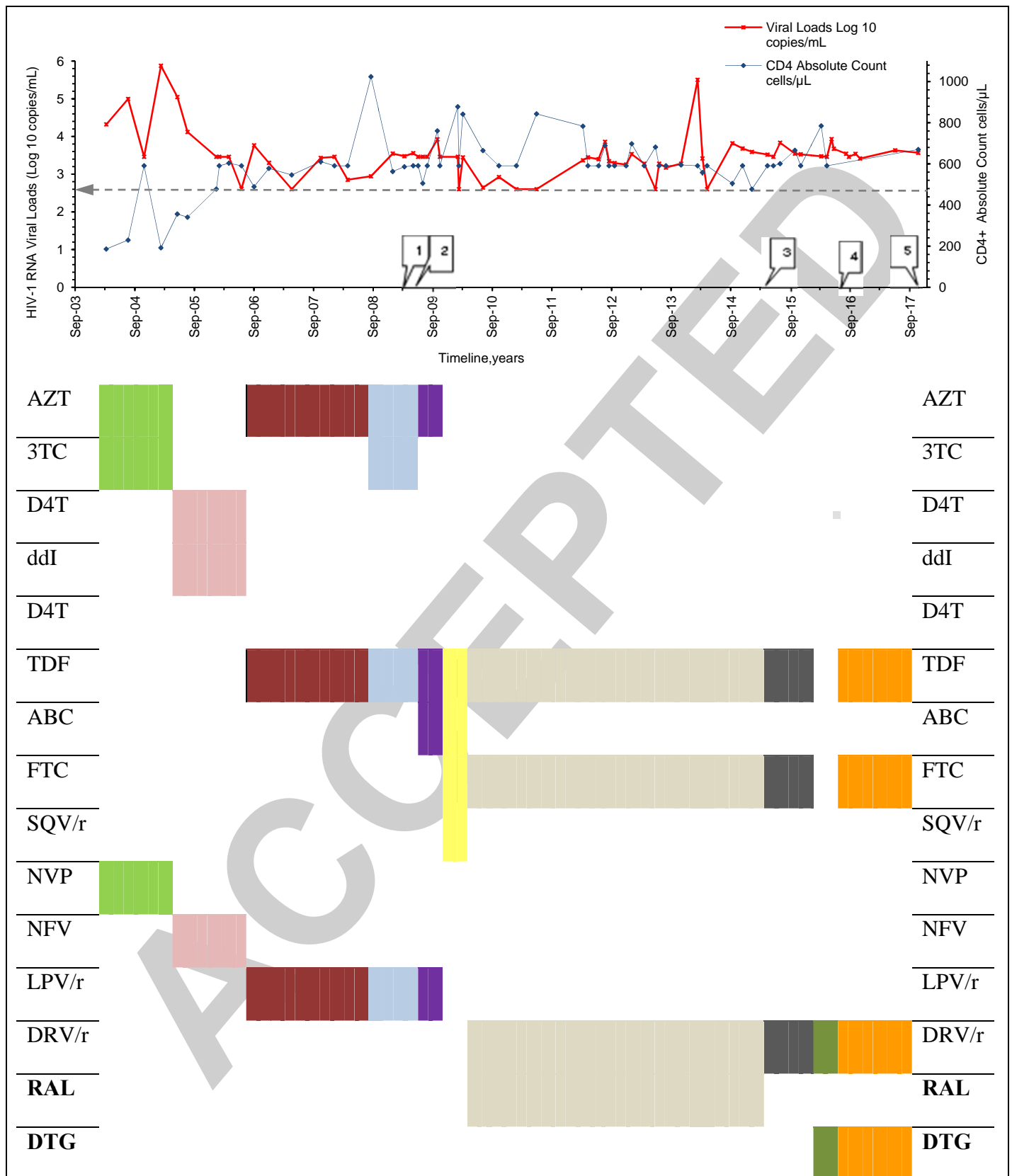
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**Figure 1. Plasma Viral Loads, CD4+T cell count and HAART regimens at different time points over a 14 year period for the patient.**

(Source: modified version from;

<http://bioafrica.mrc.ac.za/workshops/PDFs/deOliveiraRegaDB.pdf>)

Horizontal line within chart depicts VL of 400copies/ml.

Numbered rectangular callouts depict when drug resistance testing was done

1. Mar-2009; †NRTIs, D67N, K70R, M184V; NNRTIs, Y181C; PIs Major R.M, V32I, I47V, I54L, I84V, †PIs Accessory R.M, L33F, G73V, L89T; INSTIs, Not tested

2. Apr-2009; † NRTIs, D67N, K70R, M184V, K219N; †NNRTIs, Y181C; †PIs Major R.M, V32I, I47V, I54L, I84V, PIs Accessory R.M, L33F, G73V, L89T; INSTIs, Not tested

3. May-2015; †NRTIs, K65KR, D67DN, K70KR, M184MV, K219KHNQ; †NNRTIs None; †PIs Major R.M, V32VI, I54IL, I84IV, †PIs Accessory R.M, L33LF, G73GV, L89IMT;

†INSTIs Major R.M, E138K, G140A, Q148R, INSTIs Accessory R.M, None;

4. Aug-2016; †NRTIs, D67N, K70R, M184V; † NNRTIs, None; †PIs Major R.M, V32I, I47V, I54L, I84V, †PIs Accessory R.M, L33F, G73V, L89T; INSTIs, Not tested.

5. Nov-2017; † INSTIs Major R.M, E138K, G140A, S147G, Q148R, INSTIs Accessory R.M, T97A; †FIs, none; EIs, §R5 Tropic; NRTIs, Not tested; NNRTIs, Not tested.

NRTIs, nucleoside/nucleotide reverse transcriptase inhibitors; NNRTIs, non nucleotide reverse transcriptase inhibitors; PIs, protease inhibitors; INSTIs, integrase strand transfer inhibitors; RM, Resistance Mutations; FIs, Fusion Inhibitors; EIs, entry inhibitors; AZT, zidovudine; 3TC, lamivudine; NVP, nevirapine; d4T, didanosine; ddI, didanosine; NFV, nelfinavir; TDF, tenofovir; LPV/r, ritonavir boosted lopinavir; ABC, abacavir; FTC, emtricitabine; SQV/r, ritonavir boosted saquinavir; DRV/r, ritonavir boosted darunavir; RAL, raltegravir; DTG, dolutegravir; VF, virological failure

Drug resistance mutations interpreted using;

†, Stanford University HIV Drug resistance database; ‡, International Antiviral Society USA (IAS-USA) 2017 mutational list; §, co-receptor usage assessed with geno2pheno<sub>[coreceptor]</sub>.

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