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BRIEF REPORT

Dolutegravir Resistance in Malawi's National HIV Treatment Program

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Dolutegravir HIV drug resistance (HIVDR) data from Africa remain sparse. We reviewed HIVDR results of Malawians on dolutegravir-based antiretroviral therapy (November 2020–September 2021). Of 6462 eligible clients, 33 samples were submitted to South Africa, 27 were sequenced successfully, and 8 (30%) had dolutegravir HIVDR. Malawi urgently requires adequate HIVDR testing capacity.

Keywords. Africa; antiretroviral therapy; dolutegravir, HIV; Malawi; resistance.

Dolutegravir, a well-tolerated and highly effective antiretroviral drug, is recommended in first- and second-line antiretroviral therapy (ART) by the World Health Organization [1]. A major advantage of dolutegravir is its high genetic barrier to the development of HIV drug resistance (HIVDR) [2]. Dolutegravir resistance did not develop among ART-naïve participants in landmark trials [3, 4] and has been described in only a very few ART-naïve people with HIV (PWH) who started dolutegravir-based first-line ART [5]. However, dolutegravir resistance can develop, particularly in persons with previous exposure to older integrase inhibitors with low genetic barriers to resistance development or those with high-level resistance to the drugs used in the nucleoside backbone, resulting in dolutegravir monotherapy [6]. The risk of dolutegravir resistance may also be increased by infection with a non-B HIV subtype, high viral load (VL) and low CD4 cell count, insufficient adherence to ART, and drug interactions or malabsorption, which reduce dolutegravir drug levels [5]. These risk factors are common in Sub-Saharan Africa, but dolutegravir resistance data from the region are sparse. We therefore sought to describe dolutegravir resistance mutations in the routine setting of the Malawi HIV treatment program.

METHODS

Dolutegravir-based regimens were introduced in Malawi in 2019 [7]. Since then, a rapid transition from non-nucleoside reverse transcriptase inhibitor (NNRTI)–based (primarily efavirenz) to dolutegravir-based first-line ART has taken place. The Malawi treatment guidelines did not require documentation of viral suppression as a condition for transitioning to dolutegravir-based regimens, nor a change of the NRTI backbone if a VL result was available and elevated. Therefore, many clients may have switched while viremic and with undetected HIVDR. At the end of March 2021, >838 000 Malawians (96% of ~871 000 PWH alive on ART) in the national program were on dolutegravir-based regimens [8]. When individuals on dolutegravir-based regimens develop virological failure, defined as a second VL result of >1000 copies/mL after a period of 3 months of intensive adherence support, Malawi HIV guidelines require evidence of HIVDR before switching to a next-line ART regimen. Data from the Malawi Laboratory Information Management System from November 2020 through August 2021 indicate that 6462 samples from individuals on dolutegravir-based regimens had virological failure and were eligible for HIVDR testing. Applications for HIVDR testing need to be submitted to a national HIVDR committee [9], which determines eligibility for sample transportation to the National Health Laboratory Service, Johannesburg, South Africa. At this laboratory, RNA is extracted from dried blood spot (DBS) samples, which are stored at –80°C before testing. Two DBS samples (75 µL each) are added to 2 mL of lysis buffer for RNA extraction using NucliSENS easyMAG. HIVDR testing is performed using previously validated in-house protocols adapted from Zhou et al. and Van Laethem et al. [10, 11]. Partial pol gene sequences are assembled and edited using RECall (British Columbia's Centre for Excellence in HIV/AIDS Research). Sequences are loaded onto the Stanford HIVdb, version 9.0, genotypic resistance system (https://hivdb.stanford.edu/hivdb/by-sequences/) to generate resistance reports.

We reviewed all cases submitted to Malawi's HIVDR expert committee and approved for integrase gene sequencing as part of HIVDR testing from the time of dolutegravir rollout,

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including client characteristics, current health status, ART history, comorbidities and comedications, adherence measures, VL results, and genotyping results. In Malawi, integrase gene sequencing is only allowed for clients with current exposure to integrase inhibitors.

Patient Consent

The National Health Science Research Committee, Lilongwe, Malawi, waived individual informed consent and provided ethical approval for analysis and dissemination of the routinely collected, anonymized data presented.

RESULTS

Eighty-seven applications for HIVDR testing of clients on dolutegravir were received between November 12, 2020, and September 2, 2021. Of these, 34 were not accepted due to ineligibility (50% because of indications of current poor adherence), and 20 samples could not be transported to South Africa due to coronavirus disease 2019 (COVID-19)–related suspension of air flights. Samples from 33 PWH underwent resistance testing including integrase gene sequencing. Of these, 4 clients were on treatment at central hospitals, 21 at rural, mission, or district hospitals, and 8 at health centers. All clients had HIV subtype C. We identified dolutegravir resistance mutations from 8 of the 27 samples that were successfully sequenced (6 did not amplify). Clinical details and HIVDR testing results of the 8 cases are summarized in Tables 1 and 2. Seven were male (88%); the ages ranged from 15 to 46 years, and all were on a single-tablet generic combination of dolutegravir/tenofovir disoproxil fumarate/lamivudine. At initiation of that regimen, 1 patient was ART naïve, 4 had switched from non-dolutegravir-based first-line ART, and 3 from second-line ART. The median duration of viremia on dolutegravir was 12.5 months. Recommendations for next-line regimens were provided based on HIVDR test results and included tenofovir/lamivudine plus a double dose of dolutegravir and/or darunavir/ritonavir.

DISCUSSION

Our cases demonstrate the emergence of dolutegravir resistance among Malawians who are treated in routine settings of the national HIV treatment program. In a 2019 observational study with >1300 Malawians who had transitioned to a dolutegravir-based regimen and were assessed 6 months later, 98% achieved VL suppression. Of 6 clients with confirmed virological failure, 2 had dolutegravir resistance. These results documented early dolutegravir resistance development among clients who had transitioned to dolutegravir-based regimens from mainly NNRTI-based regimens [13]. While our finding of 8 cases with dolutegravir resistance mutations among 27 PWH (30%) with integrase sequencing results may suggest that dolutegravir resistance is common in the Malawi HIV program among individuals with virological failure, the true prevalence is currently unknown.

Of 6462 clients who were eligible for integrase sequencing from November 2020 through August 2021, only 33 samples were actually sent for HIVDR testing, indicating the practical and logistical challenges of HIVDR testing procedures, such as limited awareness of HIVDR testing indications and procedures, lack of local integrase sequencing capacity, insufficient funding, and air flight restrictions for sample transport during the COVID-19 pandemic. These restrictions may have limited switching to second-line treatment.

Several factors may increase the development of dolutegravir drug resistance in the Malawi HIV program. First, many individuals in Malawi who transitioned from NNRTI- to dolutegravir-based regimens did not have a VL result at the time of switching (as observed in 5 of our 8 cases) and continued the same NRTI backbone, creating the potential for functional dolutegravir monotherapy. Surprisingly, we did not observe intermediate/high-level resistance to tenofovir in most of our cases (Table 2). The NADIA study showed that in similar settings as ours, high VL suppression was achieved with dolutegravir regimens containing NRTIs with no activity as predicted by genotyping. Of concern and in line with our findings, 4 of 14 participants with viral rebound among 441 NADIA participants in the dolutegravir arm developed intermediate- or high-level dolutegravir resistance [14]. Second, considerable delays in the management of virological failure and HIVDR testing procedures were observed among our cases, leading to long-term viremia during dolutegravir exposure, which may facilitate progressive accumulation of dolutegravir resistance mutations. Five of our cases had detectable viremia for more than a year before HIVDR testing was done. These findings underline that enhanced VL testing is needed to improve virological failure management and prevent HIVDR development. Lastly, all samples that amplified, with and without dolutegravir resistance, demonstrated HIV subtype C, which is the predominant HIV strain among Malawian PWH [15], and non-B subtypes may increase the risk of dolutegravir resistance [5].

Client 1 was reportedly ART naïve and developed dolutegravir resistance on first-line dolutegravir/tenofovir disoproxil fumarate/lamivudine, which according to the literature is extremely rare [5]. The presence of NNRTI drug resistance mutations suggests transmitted NNRTI resistance or nondisclosure of previous exposure to ART. Investigators from the ADVANCE study in South Africa have proposed that virological failure on a dolutegravir-based regimen may be facilitated by baseline NNRTI resistance through an unknown mechanism [16].

We did not observe protease inhibitor (PI) resistance in any of the samples of the 8 cases. Five individuals had never been exposed to PIs, while the 3 who had been on PI-based second-line ART before switching to a dolutegravir-based regimen may

Table 1. Clinical Details of Patients With DTG Resistance Mutations

Client Number	Age[a]	Sex	Total Duration ART, mo	Current Regimen (Duration, mo)	On First- or Second-Line Regimen[b]	Previous ART Regimens[c]	Virological Status at Switch to DTG Regimen	Pill Count Adherence[d]	Self-Reported Adherence[e]	Adherence Assessment After IAC Sessions[f]	Viremia Duration on DTG Regimen, mo[g]	Clinical Status	Potential DTG Drug Interactions
1	46	M	29	TDF/3TC/DTG (29)	1st	None	NA	Optimal	Optimal	Good	4	CD4 = 61; HBsAg positive, weight loss	Unknown herbal medications
2	39	M	123	TDF/3TC/DTG (23)	1st	d4T/3TC/NVP, TDF/3TC/EFV	No VL available	Suboptimal	Optimal	Good	13	No CD4 count available; asymptomatic	None
3	38	M	121	TDF/3TC/DTG (23)	1st	d4T/3TC/NVP, TDF/3TC/EFV	No VL available	Optimal	Good	Good	13	No CD4 count available; asymptomatic	None
4	20	M	125	TDF/3TC/DTG (28)	1st	d4T/3TC/NVP, AZT/3TC/NVP	No VL available	Suboptimal	Optimal	No info	28	No CD4 count available; asymptomatic	None
5	41	M	30	TDF/3TC/DTG (24)	1st	TDF/3TC/EFV	No VL available	Optimal	Optimal	Good	20	No CD4 count available; asymptomatic	None
6	42	M	179	TDF/3TC/DTG (7)	2nd	d4T/3TC/NVP, TDF/3TC/EFV, AZT/3TC + ATV/r	No VL available	Suboptimal	Optimal	Good	3	CD4 = 70; weight loss; active EPTB	Rifampicin; DTG dose was doubled during TB treatment
7	15	F	26	TDF/3TC/DTG (9)	2nd	ABC/3TC + LPV/r	>300 000 copies/mL	Suboptimal	Suboptimal	No info	12	No CD4 count available; asymptomatic	None
8	46	M	126	TDF/3TC/DTG (8)	2nd	d4T/3TC/NVP, TDF/3TC/EFV AZT/3TC + ATV/r	>22 000 copies/mL	Suboptimal	Suboptimal	Suboptimal	8	CD4 = 180; asymptomatic	None

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; AZT, zidovudine; DTG, dolutegravir; EFV, efavirenz; IAC, intensive adherence counseling; LPV, lopinavir; NA, not applicable (the Malawi national HIV guidelines do not support VL testing at ART initiation); NVP, nevirapine; r, ritonavir; TDF, tenofovir; VL, viral load.

[a]Age is at the time of application for genotyping.

[b]Second line: switched from PI-based second-line regimen to TDF/3TC/DTG, as recommended in national guidelines.

[c]Including single-drug changes due to side effects and listed from oldest to most recent.

[d]Optimal = 95%–105% pill count adherence; suboptimal = any value outside the optimal range.

[e]Optimal = positive response to all 3 questions; suboptimal = negative response to any of 3 questions.

[f]Narrative description by submitting clinician.

[g]From the time of the first VL >1000 copies/mL result on the DTG-based regimen to the application date for HIVDR testing.

Table 2. Summary of Genotypic HIVDR Test Results

Client No.	VL Result at Time of HIVDR Testing Application	Details INSTI Resistance Mutations	Summary DTG Resistance [12]	Details RT Resistance Mutations	Summary NRTI Resistance	Summary NNRTI Resistance	Summary PI Resistance
1	46 100	Major: R263K; Accessory: M50I	Intermediate resistance; mutation score 30	NRTI: M184V/I; NNRTI: H221I	TDF, AZT susceptible; high-level resistance 3TC; low-level resistance ABC	Potential low-resistance EFV, low-resistance NVP	ATV, LPV, DRV susceptible
2	29 990	Major: R263K; Accessory: E157Q	Intermediate resistance; mutation score 40	NRTI: M41L, D67N, T69D, K70KN, V75M, M184V, T215F; NNRTI: K103N, V108I, G190A	Intermediate-resistance TDF; high-level resistance ABC, AZT, 3TC	High-level resistance NVP and EFV	ATV, LPV, DRV susceptible
3	369 000	Major: E138K, S147G, R263K; Accessory: A49G, Q95K, E157Q	High-level resistance; mutation score 60	NRTI: D67 deletion, T69G, K70R, L74I, M184V, T215V, K219E; NNRTI: A98G, V108I, G190S	Intermediate-resistance TDF; high-level resistance ABC, AZT, 3TC	High-level resistance NVP and EFV	ATV, LPV, DRV susceptible
4	53 943	Major: R263K; Accessory: E157Q	Intermediate resistance; mutation score 40	NRTI: M41L; D67N, T69G, K70R, M184V/I, T215Y, K219Q/E. NNRTI: V108I/V	Intermediate-resistance TDF; high-level resistance ABC, AZT, 3TC	High-level resistance NVP; intermediate-resistance EFV	ATV, LPV, DRV susceptible
5	444 921	Major: S147G; Accessory: H51Y	Low-level resistance; mutation score 20	NRTI: K70Q; M184V; NNRTI: Y188HL; P225H	High-level resistance 3TC; low-level resistance TDF; intermediate-resistance ABC; susceptible AZT	High-level resistance NVP and EFV	ATV, LPV, DRV susceptible
6	4 424 530	Major: R263K; Accessory: none	Intermediate resistance; mutation score 30	NRTI: M184V; NNRTI: none	High-level resistance 3TC; low-level resistance ABC; susceptible TDF, AZT	Susceptible EFV, NVP	ATV, LPV, DRV susceptible
7	248 541	Major: R263K; Accessory: none	Intermediate resistance; mutation score 30	NRTI: D67N, M184V, T215F, K219E; NNRTI: A98G, E138A, Y181V	Low-level resistance TDF; intermediate-level resistance ABC; high-level resistance AZT, 3TC	High-level resistance NVP; intermediate resistance EFV	ATV, LPV, DRV susceptible
8	88 500	Major: R263K; Accessory: none	Intermediate resistance; mutation score 30	Not determined	Not determined	Not determined	Not determined

All samples exhibited HIV subtype C.

Abbreviations: 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; ATV, atazanavir; AZT, zidovudine; DRV, darunavir; DTG, dolutegravir; EFV, efavirenz; HIVDR, HIV drug resistance; IAC, intensive adherence counseling; INSTI, integrase strand transfer inhibitor; LPV, lopinavir; NA, not applicable; NNRTI, non-nucleoside reverse inhibitor; NRTI, nucleoside reverse inhibitor; NVP, nevirapine; PI, protease inhibitor; r, ritonavir; TDF, tenofovir; VL, viral load.

have had undetected HIV minority variants with PI resistance mutations (archived resistance).

HIVDR testing can prevent unnecessary switching to alternative regimens in patients who are not adherent and have no significant HIVDR [17]. It also allows identification of patients with dolutegravir resistance who can benefit from a switch of regimen or from doubling the dolutegravir dose, which has been associated with successful outcomes in ART-experienced patients who harbored HIV with integrase inhibitor resistance mutations due to previous treatment with raltegravir or elvitegravir [18]. Study of the outcomes of such patients on their modified ART regimens is needed to gain better understanding of dolutegravir resistance mutations and clinical outcomes in African PWH. Local HIVDR capacity is also essential for regular surveillance of dolutegravir resistance development in the Malawi national program. Due to the required high-level expertise and the high costs, HIVDR testing capacity is currently very limited in Sub-Saharan Africa, and integrase sequencing is not yet available for individual clinical care within Malawi. The number of Malawi PWH who require HIVDR testing for their individual management, as current national guidelines require, is therefore much greater than the available laboratory capacity, and it is uncertain if genotyping for all clients failing dolutegravir-based regimens is feasible within the public health approach to ART. More research is needed to establish the exact role of genotyping in settings such as ours. There are many areas of uncertainty about management of patients with persistent viremia on dolutegravir-based regimens in settings where HIVDR capacity is limited or unavailable [19]. Knowledge gaps include the optimal duration of adherence support measures before consideration of HIVDR testing, ART switch decisions in the absence of HIVDR test results, and the best next-line/alternative regimens.

Six of the 33 samples failed to amplify before sequencing, which may be because DBS samples were used for transport to South Africa due to logistical and cost considerations. Another limitation of our survey is that the proportion of dolutegravir resistance we observed may not be extrapolated to the population of Malawians with virological failure on dolutegravir, because the number of individuals who underwent dolutegravir HIVDR testing was very small and likely overrepresented clients from large health facilities.

CONCLUSIONS

We have presented 8 cases with dolutegravir resistance from the Malawi HIV treatment program, where risk factors for dolutegravir resistance are prevalent. These findings advocate for the establishment of adequate HIVDR testing capacity in Malawi to support individual clinical management and regular dolutegravir HIVDR surveillance nationally.

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