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Dolutegravir resistance in sub-Saharan Africa: should resource-limited settings be concerned for future treatment?

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In sub-Saharan Africa (SSA) the burden of non-nucleoside reverse transcriptase inhibitor (NNRTI) HIV drug resistance (HIVDR) has been high over the years. Therefore, in 2018 the World Health Organization (WHO) recommended a regimen based on a integrase strand transfer inhibitor (INSTI), dolutegravir, as the default first-line antiretroviral therapy (ART) in countries in SSA. The scale-up of DTG-based regimens in SSA has gained significant momentum since 2018 and has continued to expand across multiple countries in recent years. However, whether or not the DTG robustness experienced in the developed world will also be achieved in SSA settings is still an important question. Evidence generated from *in vitro* and *in vivo* studies suggests that the emergence of DTG HIVDR is HIV-1 subtype dependent. These findings demonstrate that the extensive HIV-1 diversity in SSA can influence DTG effectiveness and the emergence of drug resistance. In addition, the programmatic approach to the transition to DTG adopted by many countries in the SSA region potentially exposes individuals to DTG functional monotherapy, which is associated with the emergence of DTG resistance. In this mini review, we describe the current trends of the effectiveness of DTG as reflected by viral suppression and DTG resistance. Furthermore, we explore how HIV-1 diversity and the programmatic approach in SSA could shape DTG effectiveness and DTG HIVDR in the region.

KEYWORDS

HIV drug resistance, dolutegravir, antiretroviral treatment, HIV-1 subtypes, viral suppression, sub-Saharan Africa, people living with HIV, mutations

1 Introduction

Sub-Saharan Africa (SSA) contains almost two-thirds of the people living with HIV (PLHIV) worldwide (1, 2). Several global efforts have been made to combat HIV infection, such as the scale up of antiretroviral therapy (ART) (3, 4). However, despite these efforts, HIV-1 drug resistance (HIVDR) has had a significant impact on HIV control in SSA, as demonstrated by the prevalence of non-nucleoside reverse transcriptase inhibitor (NNRTI)

resistance (5, 6). In 2018 the World Health Organization (WHO) recommended the use of dolutegravir (DTG), an integrase strand transfer inhibitor (INSTI) as the preferred drug in place of NNRTIs as the first-line ART regimen in the management of HIV in resource-limited settings (7). DTG is reported to have high potency, tolerability, and effectiveness in HIV-1 viral suppression; exhibits fewer drug interactions; and possesses a high genetic barrier to resistance (8–10).

Several groups have investigated the potential of the natural variability of the integrase gene in HIV-1 strains circulating in SSA for primary INSTI resistance prior to the introduction of DTG. Most of the studies reported a lack of major INSTI resistance mutations against DTG and a low frequency of accessory INSTI resistance mutations. For example, a study in Kenya reported a number of accessory DTG drug resistance mutations in polymorphic sites at a frequency of 20% among DTG pre-treatment PLHIV, and similar studies found frequencies of 5% in Tanzania and 4.3% in Ethiopia (11–13). Similarly, only accessory INSTI resistance mutations were reported at a low frequency in studies from Mozambique (14) and South Africa (15). However, one multicenter study involving PLHIV in Kenya, Nigeria, Uganda, Zambia, and South Africa reported a 2.4% prevalence of major DTG resistance mutations among INSTI- naive PLHIV (16). In addition, major resistance mutations were also detected in three Cameroonian studies at frequencies of 0.8%, 5.4%, and 1.4% in INSTI- naive populations (17–19). The low frequency of primary INSTI resistance mutations suggests that transitioning to DTG is likely to be effective in SSA.

However, studies emerging from SSA since the rollout of DTG are reporting the rapid selection of DTG resistance mutations at rates that were not observed in the INSTI -naive population previously. A study conducted a year after the DTG rollout in Malawi reported major DTG resistance mutations in 8 out of 27 (30%) samples of PLHIV in Malawi (20). Our group's recent national survey in Tanzania found a rapid selection of DTG drug resistance mutations within 18 months of transitioning to DTG (21). These emerging data suggest an impending danger to the success of the rollout of DTG in SSA. Evaluation of the pitfalls in the current approach is necessary to prevent the further spread of DTG drug resistance and ensure the long-term effectiveness of HIV treatment in SSA.

2 Treatment outcomes following dolutegravir based-regimens in SSA

Early evidence from SSA countries that have so far transitioned to DTG-based ART regimens indicates significant successes in early-time-point HIV viral suppression compared with NNRTI-based regimens among PLHIV (22–26). This is consistent with the fact that DTG-based ART has a better tolerability profile and can induce a rapid suppression of HIV viral load among PLHIV (8–10). Two large, randomized, landmark clinical trials, the ADVANCE and NAMSAL studies, assessed the effectiveness of DTG-based first-line ART among PLHIV in SSA and reported increased viral suppression in the DTG-based arms compared with the NNRTI-based arms (25, 26). Another study, conducted through the African Cohort Study (AFRICOS) in four countries (Kenya, Uganda, Tanzania, and Nigeria), that assessed the suppression rates following the use of tenofovir-lamivudine-dolutegravir (TLD) reported a viral suppression rate of 94.3% (22). A prospective cohort study in Lesotho (the DO-REAL study) reported that thethe country has achieved the third 95 of the 95-95-95 UNAIDS targets for the HIV cascade of care, with a viral suppression rate of >95% among PLHIV on DTG-based ART regimens (23). In a recent South African retrospective study that aimed to assess the outcomes of DTG-based ART involving two distinct cohorts of PLHIV from 2019 and 2022, it was reported that DTG was associated with improved clinical outcomes in terms of viral suppression (24). In Uganda, both clients initiating treatment and those switching from NNRTI- based to DTG-based regimens showed a high acceptability and a high viral suppression rate (94%) at 6 months following DTG use (27). These findings are promising and further validate the decision to transition to DTG-based ART in SSA.

Nonetheless, early selection of DTG resistance is increasingly being reported in virologic failures among PLHIV on DTG-based regimens in SSA. A study conducted in Uganda among PLHIV infected with non-B HIV-1 subtypes detected several major DTG resistance mutations— E138A/K, G140A/Q, S147G, Q148R/K, and G163R —in clients experiencing virological failure on a DTG-based regimen (28). Another study conducted in Malawi detected at least one major DTG resistance mutation (R263K, E138K, or S147G) in 8 of the 27 (30%) samples from clients on TLD experiencing virological failure (20). Similarly, a national representative survey conducted by our group in Tanzania in 2020 found instances of acquired DTG HIVDR where the major DTG resistance mutations Q148K, E138K, G118R, G140A, T66A, and R263K were detected in PLHIV on DTG-based ART and experiencing high viremia (21). Preliminary findings from a study of the EMEDT cohort that evaluated the prevalence of acquired DTG resistance and failure of viral suppression have shown a very low prevalence of DTG resistance in virally suppressed populations during the transition, but a high prevalence among those not suppressed, whereby DTG resistance was detected in 15% of the population (29). These recent findings suggest that in SSA settings, emergence of DTG resistance could be a threat to the effectiveness of the ART program in the region. Table 1 summarizes the relevant recent studies that have reported on acquired major and accessory DTG resistance mutations in SSA.

3 Role of HIV-1 natural diversity on DTG resistance in SSA

3.1 Major DTG drug resistance mutations and HIV-1 diversity

A number of studies have shown the role of viral HIV-1 subtype diversity on INSTI resistance (28, 35–38). SSA is characterized by the co-circulation of multiple group M HIV-1 subtypes

TABLE 1 Relevant studies reporting the acquired major and accessory INSTI resistance mutations in SSA.

Region	Country	Year	Participants	Prevalence of INSTI resistance	Major INSTI DRMs	Accessory INSTI DRMs	Predicted DTG susceptibility in individuals with INSTI resistance mutations	References
Eastern Africa	Tanzania	2020	Treatment experienced adults and children PLHIV	5.8%	Q148K, E138K, G118R, G140A, T66A, R263K	T97A, Q95K, E157K	High- level resistance (2/8) Intermediate resistance (2/8) Susceptible (4/8)	Kamori et al. (21)
Eastern Africa	Uganda	2019	Treatment experienced PLHIV (raltegravir experienced but DTG naive)	47.0%	E138A/K, G140A/Q, S147G, Q148R/K, G163R	T97A/T, L74I, G163R, E157Q, M50L, V151I	High- level resistance (2/11) Potential low- level resistance (8/11) Susceptible (1/11)	Ndashimye et al. (28)
South-Eastern Africa	Malawi	2020	Treatment experienced PLHIV	30.0%	R263K, E138K S147G		High- level resistance (1/8) Intermediate resistance (6/8) Low- level resistance (1/8)	Van Oosterhout et al. (20)
South-Eastern Africa	Malawi	2019	Treatment experienced PLHIV	14.3%	R263K, G118R		Intermediate resistance (2/2)	Schramm et al. (30)
Western Africa	Nigeria	2014–2018	Treatment experienced PLHIV but DTG naive	27.3%	Q148R, T66A, S147G, Y143C/H	L74M, T97A, F121Y,	Not detected	Oluniyi et al. (31)
Western Africa	Cameroon	1994–2010	Treatment experienced PLHIV but DTG naive	12.8%	T66A, Q148H, R263K, N155H	Q95K, T97A, G149A, E157Q, D232N	Intermediate resistance (1/37) Potential low- level resistance (1/37) Susceptible (35/37)	Mikasi et al. (32)
Western Africa	Nigeria	2021	Treatment experienced PLHIV	3.0%	T66A, G118R, E138K, R263K		High- level resistance (1/1)	Abdullahi et al. (33)
Southern Africa	Botswana	2015–2019	Treatment experienced PLHIV	32.0%	G138E/A/K/T, G140A, Q148R/K, G118R, S147G, N155H, T66A	D232N, E157Q, A128T	High- level resistance (6/12) Intermediate resistance (3/12) Potential low-l evel resistance (2/12) Susceptible (1/12)	Seatla et al. (34)

DRMs, drug resistance mutations; INSTI, integrase strand transfer inhibitor; PLHIV, people living with HIV; DTG, dolutegravir.

including A, C, D, G, H, J, K, CFR02_AG, and other inter-subtype recombinants (39, 40). In addition, HIV-1 groups O and N circulate in Central and Western Africa (41, 42). Following the WHO recommendation to introduce DTG in this relatively naive region with respect to INSTI drugs, a crucial question arises regarding the potential impact of HIV diversity on the susceptibility and selection of DTG resistance mutations. While *in vitro* studies suggest that DTG can be effective across HIV-1 subtypes, emerging evidence shows that natural HIV-1 variation can influence the genetic barrier to resistance and level of resistance conferred by mutations selected by DTG (43). Therefore, HIV-1 subtype diversity in SSA could play a crucial role in determining DTG effectiveness in the region.

Mutations across eight positions in the integrase region have been identified as being associated with DTG resistance, including T66K, E92Q, G118R, E138/K/A/T, G140S/A/C, Q148H/R/K, S153 F/Y, N155H, and R263K (44). However, only some, either individually or combined, are frequently reported in cases of DTG resistance in SSA (Table 1).

Selection of R263K was first observed *in vivo* in the SAILING clinical trial among individuals on a DTG-based regimen experiencing virologic failure (6). Since then, this mutation has been frequently selected among individuals on DTG (45). Viruses harboring R263K show moderate resistance to DTG but have significantly impaired integrase enzymatic function and viral replication (46). The deleterious effect of the mutation tends to differ between subtypes, and has been shown to be higher in subtype C than in subtype B (47, 48). Nevertheless, *in vivo* selection of R263K has been observed in subtypes C, D, CRF02_AG, and B (21, 49–53). Furthermore, some studies suggest that R263K mutations

may protect against further selection of resistance mutations within the integrase region (46, 54, 55) and in the reverse transcriptase region (48). However, studies from Malawi and Nigeria have demonstrated that this may not be the case. In the Nigeria study, one individual harbored a combination of T66A, G118R, and E138K, in addition to an R263K mutation (33). In the Malawi study, the R263K mutation co-occurred with two other major INSTI resistance mutations (E138K and S147G) and the accessory mutation E157Q in one individual. Overall, out of eight participants with major INSTI resistance mutations (all infected with subtype C), seven had selected the R263K mutation. Three of these participants also harbored the accessory mutation E157Q, while the remaining participants had no additional mutations in integrase, except for M50I in one individual (20). Importantly, all individuals in the Malawi study exhibited significantly high levels of viremia. In another study from Tanzania by our group, we identified four viremic individuals who were on TLD and harbored major INSTI resistance mutations; in one individual infected with subtype C, R263K was detected as a single INSTI resistance-associated mutation (21). Recently, R263K was reported in one drug-naive individual infected with subtype C in Ethiopia (52). However, exposure to DTG in this case was not ruled out. These emerging data suggest that although the R263K mutation was thought to be deleterious to the subtype C virus, it may still play an important role in DTG resistance in the SSA region.

G118R is another important resistance mutation selected by DTG and rarely by other INSTIs (56). It is associated with a 5–10 -fold reduced susceptibility to DTG. The mutation is associated with a significant reduction in both strand transfer and 3′ processing activities. Notably, strand transfer activity is relatively spared in subtype C compared to subtype B. The selection of a secondary mutation such as H51Y and/or E138K in addition to G118R was shown to affect the integrase enzymatic activity and DTG resistance profile in an HIV- 1 subtype-dependent manner (37). In one study, G118R substitution was selected alone and in combination with H51Y in tissue culture selection experiments in CRF02_AG and subtype C viruses but never in subtype B viruses (37). This further demonstrates the influence of polymorphisms on the integrase backbone between HIV subtypes in the selection of DTG resistance mutations. The study by our group identified two out of four individuals with major INSTI resistance mutations, harboring the G118R substitution as a single mutation in one case (infected with subtype A1) and in combination with T66I and E138K in another case (subtype A1C) (21). Interestingly, the latter combination of mutations was also observed in a South African individual failing DTG-based triple therapy (57). Rapid selection of G118R has also been reported in another recent study in SSA (30). These findings suggest that G118R could be an important DTG resistance pathway across non-B subtypes circulating in the SSA region.

The combination of E138K, G140A/S, and Q148K is another important pathway to DTG resistance usually observed in INSTI-exposed individuals. This combination results in a high level of resistance to DTG. Interestingly, in our study one individual (infected with subtype A1D) with no known prior exposure to INSTIs had selected this combination of mutations (21). This evidence supports the idea that the viral diversity in SSA may influence the patterns and possibly the frequency of DTG resistance in SSA. Phenotypic resistance information in the context of combinations of mutations in non-B backbones is needed to better understand DTG resistance in the SSA context.

3.2 Naturally arising INSTI resistance-associated mutations and HIV-1 diversity in SSA

Given that the genetic backbone can significantly influence the genetic barrier to a specific mutation, natural integrase variations have implications for the susceptibility or selection of resistance mutations to DTG. Integrase is a relatively conserved protein (58); however, significant variations among HIV subtypes exist. Five polymorphic integrase positions (M50, L74, T97, V151, and E157) have been associated with low-level resistance to first- and/or second-generation INSTIs or an increase in the level of resistance when they occur alongside other INSTI drug resistance mutations (59, 60). Specifically, mutations M50, L74, and T97, in combination with other mutations, have been associated with reduced susceptibility to DTG (59, 61). The frequency of naturally occurring variations in these polymorphic positions depends on the subtypes (Figure 1). Interestingly, there is wide sub-regional variation in the intra- and inter-subtype prevalence of these polymorphic mutations in SSA (Figure 1).

The M50I polymorphism can increase DTG resistance conferred by the R263K substitution in HIV subtype B (59). M50I has also been selected by DTG in *in vitro* passage experiments (59). The polymorphism is highly prevalent in non-B subtypes circulating in SSA. In HIV subtype A, M50I present in >50% of the sequences obtained from central Africa while occurring in about 10% of Subtype A sequences from Eastern Africa. Nearly one- third of subtype C sequences from Eastern and Southern Africa harbor this polymorphism. Much lower frequencies are observed in HIV subtypes D and CRF02-AG (Figure 1). Although selected by DTG, the M50I substitution does not seem to compensate for the viral fitness cost of R263K, at least in HIV subtype B (59). This could indicate that the selection is driven by the advantage in the resistance profile against DTG. Amid the rollout of the DTG, whether or not the high background prevalence of the M50I polymorphism in HIV subtypes circulating in SSA regions could influence the R263K resistance pathway against DTG needs to be investigated.

L74 residue is located in the catalytic core domain and is a part of the hydrophobic cluster near the active site of integrase (62). The role of L74 substitutions in DTG resistance remains inconclusive. The L74M substitution can be selected by DTG and increase resistance conferred by G118R or E138K mutations (26). On the other hand, L74F in combination with V75I has been reported to increase resistance levels of N155H or G140S together with levels of Q148H against DTG by several orders of magnitude (63). In contrast, L74I is weakly associated with selection by INSTI.

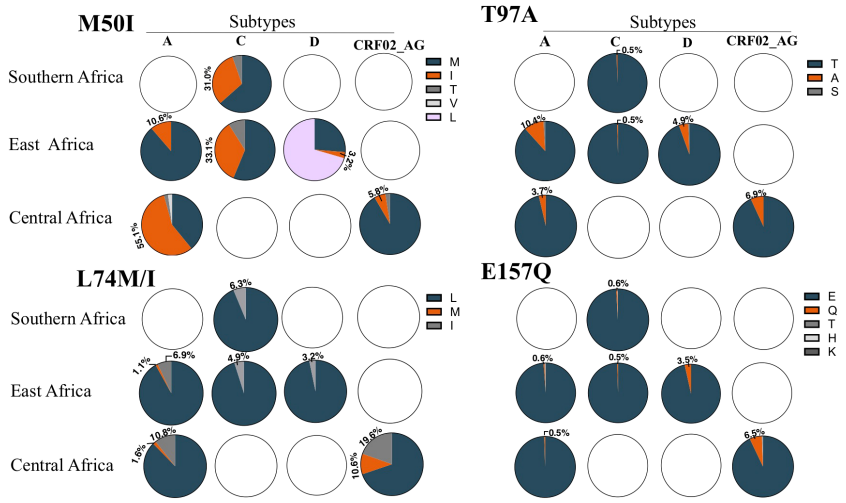

FIGURE 1
The frequency of polymorphisms in four integrase positions associated with DTG resistance. The figure depicts the frequency of polymorphisms analyzed from sequences deposited in the Stanford University HIV database. Only HIV subtypes with over 100 sequences per subtype per sub region are shown. The Western Africa region had fewer than 100 sequences per subtype and therefore was not included. Only the percentages of relevant substitutions are indicated. Blank pie charts indicate that <100 sequences were available for the particular HIV-1 subtype.

Analysis of sequences obtained in the SSA region indicate diversity in the prevalence of L74 polymorphisms. Subtype A and CRF02_AG from the Central African region tended to have a high prevalence of L74I and L74M polymorphisms, with CRF02_AG showing a combined prevalence of >20%. In contrast, a lower prevalence of L74M/I polymorphisms was observed in subtypes A, C, and D from the Eastern and Southern African region (Figure 1).

T97A is a relative common polymorphism enriched in non-B subtypes. In particular, sequences from Eastern African subtype A show a frequency of 10.4% compared to 3.7% in those obtained from Central Africa. A relatively high prevalence of 6.9% is also seen in CRF02_AG from Central Africa, while subtype C from Eastern and Southern Africa shows a prevalence of less than 1% (Figure 1). The polymorphism T97A has been shown to have no impact on the outcome of DTG treatment in INSTI- naive individuals (64). However, selection of this mutation has been associated with increased resistance to DTG in INSTI- experienced individuals with major INSTI resistance mutations at positions 140 and 148 (61, 65). On the other hand, co-occurrence of the T97A mutation with DTG- selected N155H and R263K resistance mutations improves neither the level of resistance nor replicative capacity (55). This suggest that T97A mutation may be important in conferring resistance to DTG only in certain resistance pathways. Although first generation INSTIs tend to select the 140 and 148 mutations, these mutations have also been selected by DTG *in vivo*, suggesting the potential relevance of T97A substitution in DTG resistance (13).

The E157Q substitution is also relatively common in INSTI-naive sequences from SSA. While a prevalence of less than 1% has been observed in subtypes A and C, in subtypes D (Eastern Africa) and CRF02_AG (Central Africa) a prevalence of 3.5% and 6.5%, respectively, has been reported. To date there is limited evidence of selection of this mutation by dolutegravir; however, its occurrence together with R263K has been associated with the compensation of viral fitness. The combination of these mutations has been reported *in vivo* in individuals infected with subtype C (20), suggesting that E157Q polymorphisms may have some role in conferring DTG resistance in the SSA context.

4 Role of SSA ART programs in the emergence of DTG resistance

The accelerated rollout of a fixed- dose combination of TLD in PEPFAR-funded ART programs throughout SSA has been commendably rapid and highly effective (29). The push is also aided by the donor's desire to phase out the acquisition of the NNRTI-based regimen, not only because of the clinical benefits, but also because of the lower cost of DTG-based fixed combination treatment (66). The transition involves the initiation of all treatment -naive individuals as well as switching all eligible individuals on a NNRTI-based first- line regimen to a DTG-based fixed combination regimen (67). The adopted strategy in many countries does not require confirmation of virological suppression prior to switching to DTG. This is in line with the initial studies that suggested the lack of need for viral load estimation and resistance genotyping during switching to a DTG-based fixed combination regimen (25, 26). Therefore, there may be a substantial number of clients experiencing virological failure that are being switched to TLD with undetected resistance to NRTIs. In this case such clients can be subjected to DTG functional monotherapy, which is associated with the selection of DTG resistance. Indeed, the emerging data since the rollout indicate that DTG resistance is more frequently selected in treatment- experienced than in ART-naive individuals (20, 21). In fact, all of the observed cases of DTG

resistance reported in our study from Tanzania (21), the Malawi study (20), the Uganda study (28), and the Nigerian study (33) were from treatment- experienced individuals that transitioned to TLD. The drug resistance profile in these individuals showed extensive NRTI resistance mutations that render the tenofovir and lamivudine backbone in the DTG fixed combination treatment less susceptible (20, 21, 28, 33).

DTG functional monotherapy together with infrastructural and other programmatic challenges, including suboptimal ART adherence, lack of adherence to viral load testing guidelines, poor retention in care, and limited viral load coverage, may significantly impair the effectiveness of DTG-based fixed combination treatments and promote the selection of DTG resistance in SSA (68–72). Therefore, in order to curb DTG resistance in SSA, it is essential to ensure that national ART programs in SSA carry out intensified monitoring of individuals on DTG-based fixed combination, conduct routine resistance surveillance, and consider the revision of existing ART guidelines to estimate viral loads prior to switching to DTG.

5 Conclusion and future perspectives

Recent data since the rollout of DTG provide good indications that DTG can steer the SSA region toward achieving the third 95 of the 95-95-95 UNAIDS targets for the HIV cascade of care. However, it is also becoming clear that SSA settings expose DTG to factors that threaten its effectiveness and promote the rapid selection of resistance mutations not previously encountered in other regions. The diversity of HIV-1 integrase in SSA could allow for the selection of DTG resistance mutations or combinations of mutations that are rarely observed in HIV subtype B. Therefore, measures to control the development of drug resistance are equally important in this era of DTG. Studies to understand DTG resistance in the context of SSA should be conducted to inform the ART programs in the region.

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Conflict of interest

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